British Journal of Pharmacology (1999) 127, 1751–1764
http://www.stockton-press.co.uk/bjp

REVIEW ARTICLE

The recombinant 5-HT_{1A} receptor: G protein coupling and signalling pathways

*,[1]John R. Raymond, [1]Yurii V. Mukhin, [2,3]Thomas W. Gettys & [1]Maria N. Garnovskaya

[1]Division of Nephrology, Department of Medicine, Medical University of South Carolina and the Ralph H. Johnson Veterans Affairs Medical Center, Charleston, South Carolina 29425, U.S.A.; [2]Division of Gastroenterology, Department of Medicine, Medical University of South Carolina, Charleston, South Carolina 29425, U.S.A. and [3]Department of Biochemistry, Medical University of South Carolina, Charleston, South Carolina 29425, U.S.A.

The 5-hydroxytryptamine 5-HT_{1A} receptor was one of the first G protein coupled receptors whose cDNA and gene were isolated by molecular cloning methods. Transfection of the cDNA of this receptor into cells previously bearing no 5-HT receptors has resulted in the acquisition of large amounts of information regarding potential signal transduction pathways linked to the receptor, correlations of receptor structure to its various functions, and pharmacological properties of the receptor. Transfection studies with the 5-HT_{1A} receptor have generated critical new information that might otherwise have been elusive. This information notably includes the discovery of unsuspected novel signalling linkages, the elucidation of the mechanisms of receptor desensitization, the refinement of models of the receptor pharmacophore, and the development of silent receptor antagonists, among others. The current review summarizes the most important studies of the recombinant 5-HT_{1A} receptor in the decade since the identificiation of its cDNA.

Keywords: 5-Hydroxytryptamine; transfection; G protein; adenylyl cyclase; phospholipase; calcium; efficacy; potency

Abbreviations: cyclic AMP, 3′,5′-cyclic adenosine monophosphate; CFTR, cystic fibrosis transmembrane regulator; DAG, diacylglycerol; Erk, extracellular signal-regulated kinase; G protein, guanine nucleotide regulatory binding protein; G_i, G protein that inhibits adenylyl cyclase; GIRK, G protein-gated inwardly rectified K^+ channel; G_o, G protein that serves functions other than to regulate adenylyl cyclase; G_q, G protein that activates phospholipase C; Grb2, protein that serves as a molecular adapter; GRK, G protein-coupled receptor kinase; GTP, guanosine triphosphate; GTPγS, nonhydrolysable GTP analogue; G_S, G protein that stimulates adenylyl cyclase; 8-OH-DPAT, 8-hydroxy-2-(di-n-propylamino)-tetralin; 5-HT, 5-hydroxytryptamine; serotonin; i2 loop, putative second intracellular loop of the 5-HT_{1A} receptor protein; i3 loop, putative third intracellular loop of the 5-HT_{1A} receptor protein; IκBα, inhibitor of NF-κB; IP_3, inositol triphosphate; Mek, mitogen and extracellular signal regulated kinase, which phosphorylates and activates Erk; NF-κB, nuclear factor-κB; PC-PLC, phosphatidylcholine-specific phospholipase C; PI-PLC, phosphatidylinositol-specific phospholipase C; PKA, cyclic AMP-dependent protein kinase; PKC, protein kinase C; PLA_2, phospholipase A_2; PLC, phospholipase C; Raf; a kinase that is activated by Ras, and that phosphorylates and activates MEK; Ras, a monomeric low molecular weight G protein that activates Raf; RGS proteins, regulators of G protein signalling; ROI, reactive oxygen intermediates; R-SAT, receptor selection and amplification technology; Shc, a protein that serves as a docking platform; Src, non-receptor tyrosine kinase; WAY-100635, N-[2-[4-(2-methoxyphenyl)1-piperaziny-l]ethyl)-n-(2-pyridiynl)cyclohexanecarboxamide trihydrochloride

Introduction

Serotonin (5-HT, 5-hydroxytryptamine) was discovered in 1948 by Rapport *et al*. as a potent vasotonic factor. For several decades, many of the effects of 5-HT were attributed to two major subtypes of 5-HT receptors (Gaddum & Picarelli, 1957). With the development in the 1980s of specific ligands for the various subtypes of 5-HT receptors came the realization that there must be more than two subtypes of 5-HT receptor. Molecular cloning studies over the last decade have confirmed the existence of at least 14 subtypes of 5-HT receptors, each encoded by a distinct gene. Most of those receptors belong to a large family of receptors that transduce signals through G proteins.

One of the best-characterized 5-HT receptors is the 5-HT_{1A} receptor (Pedigo *et al*., 1981). This receptor is characterized pharmacologically (like all 5-HT_1 receptors) by its high affinity for 5-HT. It also has a uniquely high affinity for 8-OH-DPAT, and for second generation, arylpiperazine anxiolytic agents such as buspirone, gepirone and ipsapirone. Physiological, clinical, and pharmacological studies have documented potential roles for the 5-HT_{1A} receptor in neuroendocrine function and thermoregulation (Balcells-Olivero *et al*., 1998; Seletti *et al*., 1995), vasoreactive headaches (Leone *et al*., 1998), sexual behaviour (Maswood *et al*., 1998), food intake (Gilbert *et al*., 1988; Yamada *et al*., 1998), tooth-germ morphogenesis (Moisewitsch *et al*., 1998), memory (Edagawa *et al*., 1998), immune function (Iken *et al*., 1995), aggression (Miczek *et al*., 1998), depression (Blier *et al*., 1997; Shiah *et al*., 1998) , and anxiety (Parks *et al*., 1998; Ramboz *et al*., 1998).

The 5-HT_{1A} receptor was one of the first G protein-coupled receptors for which the cDNA and gene were cloned (Albert *et al*., 1990; Chanda *et al*., 1993; Fargin *et al*., 1989; Fujiwara *et al*., 1990; Kobilka *et al*., 1987; Stam *et al*., 1992). This gene is intronless, and its message is expressed mainly in the brain, spleen, and neonatal kidney (Fargin *et al*., 1989; Kobilka *et al*., 1987). A number of interesting observations have been derived

* Author for correspondence at: Room 829 CSB, Medical University of South Carolina, 171 Ashley Avenue, Charleston, S.C., 29425-2227, U.S.A. E-mail: raymondj@musc.edu

from the primary nucleic and amino acid sequences of the 5-HT$_{1A}$ receptor. The rat and human 5-HT$_{1A}$ receptor nucleic acid sequences are 88% homologous with each other (Albert *et al*., 1990; Fargin *et al*., 1989; Fujiwara *et al*., 1990; Kobilka *et al*., 1987; Stam *et al*., 1992), whereas those sequences possess significantly less homology with other members of the family of G protein-coupled 5-HT receptors such as the 5-HT$_{2A}$ (19%) and 5-HT$_{2C}$ (18%) receptors and the 5-HT$_{1D}$ receptors (43%) (Fujiwara *et al*., 1993). The potential importance of the 5-HT$_{1A}$ receptor has been underscored by the recent cloning of putative homologues in non-mammalian species such as *Xenopus laevis* (Maracci *et al*., 1997) and *Fugu rubripes* (Yamaguchi & Brenner, 1997).

The encoded human protein, composed of 422 amino acids is characterized by a core molecular weight of $\approx$46,000, and an isoelectric point of 8.8. Hydropathicity analysis reveals that the 5-HT$_{1A}$ receptor contains seven hydrophobic stretches that could possibly form membrane-spanning α-helices. By analogy with the β_2-adrenoceptor, and because of the presence of three consensus sequences for *N*-linked glycosylation on the amino terminus, the receptor is probably oriented in the plasma membrane with the amino terminus facing the extracellular domain. Hydrophilic sequences that form three intracellular and three extracellular loops connect the seven hydrophobic transmembrane regions. In the putative second extracellular domain the 5-HT$_{1A}$ receptor possesses a cysteine residue (Cys^{186}) which may form a disulfide bond with Cys^{109}, which is located at the limit between the first extracellular loop and the third transmembrane domain (Figure 1). By analogy with what has been shown for the β_2-adrenoceptor (Dohlman *et al*., 1990; Fraser *et al*, 1989), this disulfide bond may stabilize the receptor conformation and explain in part, why reducing agents affect 5-HT$_{1A}$ receptor binding properties (Emerit *et al*., 1991; Gozlan *et al*., 1988). It should be noted that the original sequence of the human receptor (Kobilka *et al*., 1987) was modified slightly by Chanda *et al*. (1993), because the initial publication of Kobilka *et al*. (1987) appears to have contained a sequencing error near the junction of the second intracellular loop and the fourth transmembrane domain.

The 5-HT$_{1A}$ receptor is quite interesting in that it has been implicated in numerous signalling pathways in physiologically relevant model systems. In nearly every case, the signals have been shown to be exquisitely sensitive to pertussis toxin, exclusively implicating G$_{i/o}$ protein in signals initiated by the 5-HT$_{1A}$ receptor in physiological settings. In neurons, the major signals emanating from the 5-HT$_{1A}$ receptor are inhibition of adenylyl cyclase (De Vivo & Maayani, 1986; Weiss *et al*., 1986), and opening of K^+ channels (Andrade & Nicoll, 1986; Colino & Halliwell, 1987; Zgombick *et al*., 1989). The 5-HT$_{1A}$ receptor has also been demonstrated to inhibit a Ca^{2+} current (Pennington & Kelly, 1990), to stimulate adenylyl cyclase (Markstein *et al*., 1986; Shenker *et al*., 1985), and to inhibit phospholipase C activation (Claustre *et al*., 1988) in various neuronal preparations. In non-neuronal rat ventral prostate cells, endogenous 5-HT$_{1A}$ receptors inhibit adenylyl cyclase and stimulate nitric oxide synthase (Carmena *et al*., 1998).

The realization that the 5-HT$_{1A}$ receptor couples to multiple signalling pathways in cells and tissues in which it is normally expressed reflects a relatively new understanding of the potential promiscuity of receptor-G protein signalling pathways. Nevertheless, it has long been suspected that single receptor subtypes might be linked to various second messengers in a single cell system (Limbird, 1988). No matter how carefully constructed the experiments, however, there is always the consideration in complex tissues and organs that the effects may be mediated by more than one receptor subtype, which are pharmacologically similar but functionally distinct. These considerations can be eliminated by inserting a receptor through transfection methods into a cellular model which previously lacked that or related receptors. Transfected cells have the advantage of expressing single well-defined receptor subtypes. Non-transfected or dummy-transfected cells serve as excellent controls. The purpose of this review is to summarize information that has been obtained by transfection of the recombinant 5-HT$_{1A}$ receptor into various cell models. We will focus first upon issues relevant to signal transduction and then upon other functions of the 5-HT$_{1A}$ receptor.

Signalling linkages

Coupling of the 5-HT$_{1A}$ receptor to many of the signalling pathways described in tissues has been recapitulated in various transfected cell lines, with two notable exceptions. First, positive coupling of the 5-HT$_{1A}$ receptor to adenylyl cyclase has not been documented in any transfected cell line to date. Various manoeuvres in CHO cells, including prolonged treatment with pertussis toxin and/or co-stimulation with G$_{s\alpha}$-coupled receptors failed to reveal a positive regulation of adenylyl cyclase or cellular cyclic AMP levels. Further, no direct coupling between the 5-HT$_{1A}$ receptor and G$_{s\alpha}$ has been detected with G protein photoaffinity labelling, cholera toxin catalyzed ADP-ribosylation reactions, or co-immunoprecipitation experiments (Raymond *et al*., 1993b; and unpublished observations). Second, coupling to nitric oxide has not been described in transfected, cultured cells, but the discovery of this pathway in rat ventral prostate was only recently described (Carmena *et al*., 1998).

Inhibition of adenylyl cyclase

The most consistent coupling of the recombinant 5-HT$_{1A}$ receptor is to the inhibition of adenylyl cyclase through pertussis toxin-sensitive G proteins. The coupling to the inhibition of adenylyl cyclase appears to be universally expressed (Banerjee *et al*., 1993; Fargin *et al*., 1989; Liu & Albert, 1991; Varrault *et al*., 1992b), and is extremely efficient in that the efficacy of coupling is maximal at low physiologically relevant levels of receptor expression. The efficiency of coupling is probably due to abundant receptor reserve. Langlois *et al*. (1996) demonstrated that 5-HT$_{1A}$ receptors transfected into polarized epithelial LLC-PK$_1$ cells were expressed on both basolateral and apical membranes. Receptors on both surfaces were able to inhibit cyclic AMP accumulation. Thus, the recombinant 5-HT$_{1A}$ receptor has been shown to consistently inhibit adenylyl cyclase in multiple cells, and also on two key membrane domains in a polarized cell line.

Despite its obvious importance, the mechanism of the inhibition of adenylyl cyclase by the 5-HT$_{1A}$ receptor remains largely unexplored. The family of adenylyl cyclases consists of at least nine distinct members, each possessing a diverse repertoire of regulatory inputs (Hurley, 1999; Mons *et al*., 1998; Taussig & Zimmerman, 1998). Because the 5-HT$_{1A}$ receptor can activate a multitude of signalling pathways (described below), it could regulate adenylyl cyclase in a number of ways depending both upon the specific signals generated by receptor occupancy, and upon the types and amounts of adenylyl cyclases expressed in the cells or tissues of interest. For example, G$_{i\alpha}$ subunits bind to and inhibit types 5 and 6 adenylyl cyclase. This would seem to be a rather

straightforward means of regulation of adenylyl cyclase by the 5-HT$_{1A}$ receptor since this receptor almost universally couples to $G_{i\alpha}$ subunits. However, $G_{\beta\gamma}$ subunits can conditionally stimulate type 2 adenylyl cyclase when activated $G_{s\alpha}$ is present. Elevations of intracellular Ca^{2+} also can inhibit types 5 and 6 adenylyl cyclase. In contrast, activation of Ca^{2+}/calmodulin can stimulate types 1, 3, and 8 of adenylyl cyclase. Protein kinase C can stimulate types 2 and 7 adenylyl cyclase, whereas Ca^{2+}/calmodulin-dependent protein kinase II can inhibit type 3 adenylyl cyclase (Hurley, 1999; Mons *et al*., 1998; Taussig & Zimmerman, 1998). Moreover, the 5-HT$_{1A}$ receptor could regulate cellular levels of cyclic AMP downstream of adenylyl cyclase by inhibiting its destruction by phosphodiesterases or other mechanism (Wang *et al*., 1999). Thus, what on the surface appears to be a rather straightforward regulation of adenylyl cyclase by the 5-HT$_{1A}$ receptor may in fact be a complex summation of a number of distinct regulatory signals. Transfected cell systems hold promise for exploring this issue

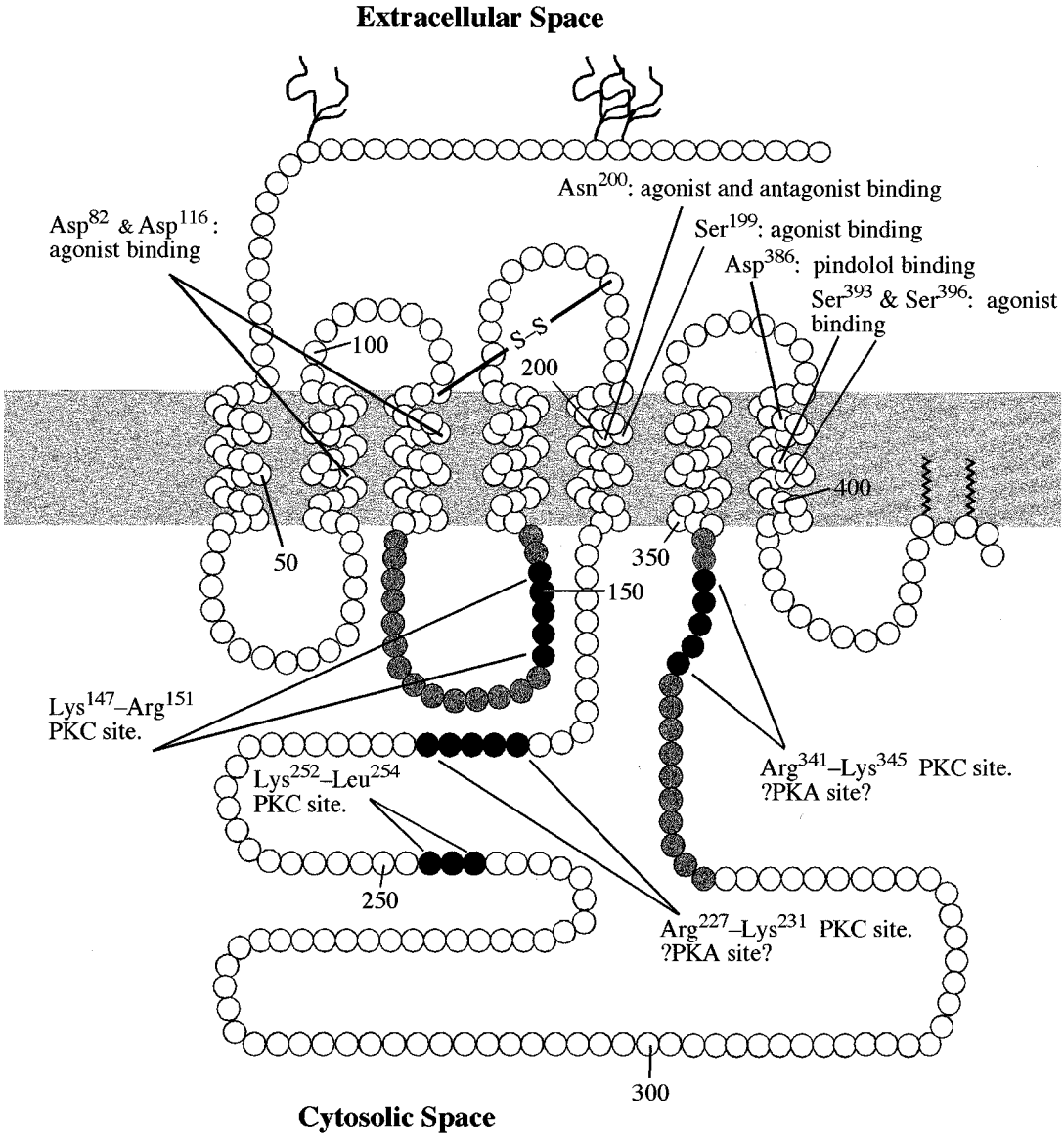

Figure 1 Two-dimensional topographical representation of the human 5-HT$_{1A}$ receptor. Each circle represents an amino acid. Numbering of the amino acids commences from the initiator methionine, and every 50 amino acids are marked for convenience. The amino terminus faces the extracellular space, and the carboxyl terminus faces the cytoplasm. Seven transmembrane stretches, each composed of $\approx 20-26$ hydrophobic amino acids, are connected by three extracellular loops (termed e1, e2, and e3) and three intracellular loops (termed i1, i2, and i3). There are three potential sites of *N*-linked glycosylation on the amino terminus (depicted as branching trees). A disulfide bond between Cys^{109} and Cys^{187} putatively links the e1 and e2 loops. Transmembrane (TM) domains contain residues important for ligand binding. Asp^{82} (in TM2) and/or Asp^{116} (in TM3) are important for binding of 5-HT, perhaps by serving as a counterion for the amine group. Ser199 (in TM5) also is important for binding of 5-HT. Asn^{200} (in TM5) is important both for agonist and antagonist binding. Asp^{386} (in TM7) is critical for selective binding to β-adrenoceptor blocker like pindolol. Ser^{393} and Ser^{396} (also in TM7) are important for agonist binding. The intracellular domains contain sites that are important for contacting G proteins particularly the entire i2 loop and the carboxy terminal end of the i3 loop (gray circles). Sites putatively involved in phosphorylation by PKC and PKC are depicted as black circles. Specific PKC phosphorylation sites are as follows: Lys^{147}-Arg-Thr-Pro-Arg^{151} (i2); Arg^{227}-Lys-Thr-Val-Lys^{231} (i3); Lys^{252}-Ser-Leu^{254} (i3); and Arg^{341}-Lys-Thr-Val-Lys^{345} (i3). There are two putative PKA sites: Arg^{227}-Lys-Thr^{229} and Arg^{341}-Lys-Thr^{343}. Underlined residues are putative phosphate acceptor sites. The carboxy terminal tail is putatively anchored to the interior face of the plasma membrane by one or two palmitoyl anchors bound to Cys^{418} and/or Cys^{420}.

because they offer the ability to alter the amount and types of adenylyl cyclase present within the host cell.

Coupling to phospholipases

Activation of phosphatidylinositol-specific phospholipase C (PLC) results in the generation of two key second messengers. The first is inositol trisphosphate (IP_3), which regulates intracellular Ca^{2+} release (Berridge *et al*., 1998). The second is diacylglycerol (DAG), which binds to and activates protein kinase C (PKC). PLC can be activated by receptors that couple to both pertussis toxin-sensitive and -insensitive G proteins. Activation of PLC by G_q-coupled 5-HT$_2$ receptors appears to be almost universal. Although 5-HT$_{1A}$ receptors can clearly activate PLC, this effect is highly host cell-specific. Fargin *et al*. (1989) first demonstrated that the 5-HT$_{1A}$ receptor could activate PI-PLC in HeLa cells. This coupling was shown to be as effective as that induced by endogenous histamine H_1-like receptors (Raymond *et al*., 1989). However, this coupling was not as efficient as coupling to the inhibition of adenylyl cyclase in that the EC_{50} of PLC stimulation occurred at significantly higher ligand concentrations, and because virtually no receptor reserve was apparent. This coupling may be physiologically relevant in that endogenous 5-HT$_{1A}$ receptors in human Jurkat (T cell-like) cells activates PLC (Aune *et al*., 1993). Liu & Albert (1991) were the first to demonstrate that activation of PI-PLC by the 5-HT$_{1A}$ receptor is cell-specific. They showed that the 5-HT$_{1A}$ receptor expressed in Ltk$^-$ fibroblasts activates PI-PLC because 5-HT increased phosphoinositide hydrolysis and levels of intracellular Ca^{2+}. In BALB/c-3T3 cells and in Ltk$^-$ fibroblasts, 5-HT$_{1A}$ receptor-mediated increases in intracellular Ca^{2+} derive from the release of intracellular Ca^{2+}, rather than from influx of extracellular Ca^{2+} (Abdel-Baset, 1992). In contrast, when the 5-HT$_{1A}$ receptor was expressed in GH_4C_1 pituitary cells, no evidence of PI-PLC activity was detected (Liu & Albert, 1991). Interestingly when the human 5-HT$_{1A}$ receptor was expressed in *Xenopus* oocytes, it was shown to activate PLC (Ni *et al*., 1997). In aggregate, the work of several laboratories has shown that the 5-HT$_{1A}$ receptor couples to PI-PLC in HeLa cells (Boddeke *et al*., 1992; Harrington *et al*., 1994; Middleton *et al*., 1990), in Ltk$^-$ cells (Liu & Albert, 1991), in *Xenopus* oocytes (Ni *et al*., 1997), and in BALB/c-3T3 cells (Abdel-Baset *et al*., 1992), but only weakly or not at all in CHO cells (Cowen *et al*., 1997; Newman-Tancredi *et al*., 1992; Raymond *et al*., 1992), Cos-7 cells (Fargin *et al*., 1989), NIH-3T3 cells (Varrault *et al*., 1992a), or GH_4C_1 cells (Liu & Albert, 1991).

Activation of protein kinase C (PKC) occurs in HeLa cells (Middleton *et al*., 1990; Raymond *et al*., 1989), and depends upon phospholipase C activation (Fargin *et al*., 1989). This effect is probably mediated by G protein $\beta\gamma$ subunits, and almost certainly depends upon the expression of $\beta\gamma$-regulatable PLC. The coupling of the 5-HT$_{1A}$ receptor to PKC is as efficacious as that induced by the endogenous histamine receptor expressed in HeLa cells. The activation of PKC by the 5-HT$_{1A}$ receptor is relevant in that it increases Na^+-dependent phosphate uptake to a level similar to that induced by the endogenous histamine receptor (Raymond *et al*., 1991). Thus, in HeLa cells, the 5-HT$_{1A}$ receptor regulates an endogenous transport process *via* PKC activation. However, like the activation phosphoinositide hydrolysis in HeLa cells, the coupling is much less efficient than is the coupling to the inhibition of adenylyl cyclase. Activation of PKC and Na^+-dependent phosphate uptake is more efficacious in cells expressing $\approx$3 pmol of receptors mg^{-1} protein than in cells expressing $\approx$500 fmol of receptors mg^{-1} protein (Raymond *et al*., 1989).

The recombinant 5-HT$_{1A}$ receptor has also been shown to activate PLA_2 in HeLa cells (Harrington *et al*., 1994), and to augment Ca^{2+}-induced arachidonic acid metabolism in CHO cells (Raymond *et al*., 1992). Cowen *et al*. (1997) showed the 5-HT$_{1A}$ receptor activates phosphatidylcholine-specific phospholipase C (PC-PLC) in CHO cells. However, details of those linkages remain to be elucidated.

Regulation of channels

The recombinant 5-HT$_{1A}$ receptor has been shown to regulate the function of several distinct types of channels, including inwardly rectified K^+ channels, high conductance anion channels, CFTR Cl^- channels, and Ca^{2+} channels. G protein-gated inwardly rectified K^+ (GIRK) channels mediate hyperpolarizing postsynaptic potentials in the nervous system and in the heart during activation of $G_{i/oz}$-coupled receptors, including the 5-HT$_{1A}$ receptor (Andrade & Nicoll, 1986; Colino & Halliwell, 1987; Zgombick *et al*., 1989). The regulation of GIRK channels by receptors relies upon the interaction of G protein $\beta\gamma$ subunits (released by receptor activation) with regulatory sites on the channels (Doupnik *et al*., 1996). Karschin *et al*. (1991) used a highly efficient recombinant *vaccinia* virus vector system to express the 5-HT$_{1A}$ receptor in primary cultures of rat atrial myoctyes, and documented that the 5-HT$_{1A}$ receptor could stimulate an endogenous atrial inward rectifier K^+ current. Those studies were expanded by co-injecting rat atrial RNA with 5-HT$_{1A}$ receptor RNA into *Xenopus* oocytes (Dascal *et al*., 1993), which resulted in the expression of a G protein-activated K^+ channel that could be activated by the 5-HT$_{1A}$ receptor. Moreover, the 5-HT$_{1A}$ receptor was also able to activate GIRK1 channels when receptor and channel RNAs were co-injected into *Xenopus* oocytes (Doupnik *et al*., 1997).

One curious observation has been that the kinetical characteristics of GIRK regulation by receptors are markedly different in transfected cells *vs* in cells that natively express the GIRK channels and receptors. In neurons and atrial myocytes, the time courses for receptor-mediated GIRK current deactivation are 20–40 times faster than are those observed in systems in which cloned receptors and GIRK channels have been co-expressed heterologously (Andrade & Nicoll, 1986; Colino & Halliwell, 1987; Dascal *et al*., 1993; Karschin *et al*., 1991; Zgombick *et al*., 1989). That finding suggested that additional components might be required to confer the rapid kinetical properties of the native transduction pathway. Doupnik *et al*. (1997) studied the effects of co-expression of the 5-HT$_{1A}$ receptor, GIRK1, and various 'regulators of G protein signalling' (RGS proteins) in *Xenopus* oocytes. They found that they could restore rapid activation and deactivation to GIRK current waveforms evoked by activation of 5-HT$_{1A}$ receptors by co-expression of RGS1, RGS3, or RGS4, but not by RGS2. This work provided evidence for functional regulation of 5-HT$_{1A}$ receptor-mediated GIRK activation by RGS1, RGS3, and RGS4.

The 5-HT$_{1A}$ receptor has been shown to regulate several other channels in transfected cells. Uezono *et al*. (1993) showed that the 5-HT$_{1A}$ receptor expressed in *Xenopus* oocytes could augment the activation of CFTR Cl^- channels induced by β_2-adrenoceptors. This effect was indirect in that the conditional activation of CFTR by the 5-HT$_{1A}$ receptor was enhanced by co-expression of adenylyl cyclase type II and $G_{s\alpha}$, and likely proceeded *via* G protein $\beta\gamma$ subunits. Ni *et al*. (1997)

demonstrated that the 5-HT$_{1A}$ receptor expressed in *Xenopus* oocytes could also stimulate an oscillatory Ca^{2+}-activated Cl^- current. Mangel *et al.* (1993) showed that the 5-HT$_{1A}$ receptor inhibited a high conductance anion channel in CHO cells through either $G_{i\alpha 2}$ or $G_{i\alpha 3}$. The potential significance of the inhibition of high-conductance anion channels by the 5-HT$_{1A}$ receptor is not known, but those channels are thought to be important in cell volume regulation and the maintenance of membrane potential. Liu & Albert (1991) demonstrated that the rat 5-HT$_{1A}$ receptor inhibited Bay K8644-mediated Ca^{2+} influx in GH_4C_1 cells, and that this effect required expression of $G_{o\alpha}$ (Liu *et al.*, 1994). The ability of the 5-HT$_{1A}$ receptor to inhibit Ca^{2+} influx has been confirmed in a preliminary manner in several putative neuronal cell lines (NCB-20, F11, and HN2) (Singh *et al.*, 1996b). Thus, the 5-HT$_{1A}$ receptor can stimulate or inhibit multiple distinct ion channels in transfected cells.

Coupling to active ion transport processes

In addition to regulating channels, the 5-HT$_{1A}$ receptor has been shown to activate several active ion transport processes. In HeLa cells, the receptor stimulates Na^+-dependent phosphate uptake through a PKC-mediated pathway (Raymond *et al.*, 1989; 1990), and Na^+/K^+-ATPase through a Ca^{2+}-mediated pathway (Middleton *et al.*, 1990). Both pathways probably depend upon $G_{\beta\gamma}$-mediated stimulation of PLC. In CHO cells, the 5-HT$_{1A}$ receptor activates Na^+/H^+ exchange (Garnovskaya *et al.*, 1997; 1998) through a pathway that requires $G_{i\alpha}$, Src tyrosine kinase, and PI3-K, but not Mek, Ras, or Raf. The coupling to Na^+-dependent phosphate uptake suggests a potential role for the 5-HT$_{1A}$ receptor in regulating cellular energy processing, whereas the coupling to and Na^+/K^+-ATPase and Na^+/H^+ exchange suggests potential roles in cell volume regulation.

Coupling to G proteins

The ability of the 5-HT$_{1A}$ receptor to couple to various G protein subunits has been carefully studied using several different expression systems. Manning's group (Barr *et al.*, 1997; Butkerait *et al.*, 1995) used *Spodoptera frugiperda* (Sf9) cells for co-expression of the human 5-HT$_{1A}$ receptor with mammalian G protein subunits. They assessed receptor/G protein coupling by [^{35}S]-GTPγS binding and by guanine nucleotide-sensitive agonist binding assays. Co-expression of the receptor with members of the α_i group (but not others) together with various combinations of β_1 and γ subunits increased the affinity for agonists. Using a similar system, Mulheron *et al.* (1994) documented that the 5-HT$_{1A}$ receptor could also functionally couple to an endogenous G_o-like G protein in Sf9 cells. When the receptor was co-expressed with β_1 and γ_2, relatively equivalent coupling to α_{i1}, α_{i2}, α_{i3}, α_o and α_z was seen, whereas there was essentially no detectable coupling to α_{12}, α_{13}, α_s and α_q. When β_1 and α_{i1} were co-expressed with various γ-subunits, the following rank order of affinity was established: $\gamma_2 \approx \gamma_3 \approx \gamma_5 > \gamma_1$ (Barr *et al.*, 1997; Butkerait *et al.*, 1995).

Other groups have detected measurable differences in the affinity of 5-HT$_{1A}$ receptor for $G_{i/o/z}$ family α-subunits in various transfected cell systems. Clawges *et al.* (1997) added purified G protein subunits to Sf9 cell membranes expressing 5-HT$_{1A}$ receptors, and established that α_{i1}, α_{i2}, α_{i3}, and α_o were able to shift the receptors to a high-affinity state in the presence of either brain or retinal β_1/γ. α-Transducin ($G_{t\alpha}$) subunits were inactive regardless of which β_1/γ preparation was used. A significantly higher affinity for agonist was observed for the 5-HT$_{1A}$ receptor in the presence of α_{i3} compared with either α_{i2} or α_o. Bertin *et al.* (1992) expressed 5-HT$_{1A}$ receptors in *E. coli* to determine a relative rank order of affinity for this receptor to reconstituted purified mammalian G protein α-subunits of $G_{i\alpha 3} > G_{i\alpha 2} > G_{i\alpha 1} \gg G_{o\alpha} \gg G_{s\alpha}$. Another group (Garnovskaya *et al.*, 1997; Raymond *et al.*, 1993b) demonstrated agonist-promotable physical coupling of the 5-HT$_{1A}$ receptor to G proteins in HeLa and CHO cells using high affinity agonist binding and co-immunoprecipitation assays. Agonist treatment induced coupling of the 5-HT$_{1A}$ receptors to G proteins with an apparent rank order of $G_{i\alpha 3} > G_{i\alpha 2} \approx G_{i\alpha 1} \approx G_{o\alpha} > G_{z\alpha} \gg G_{s\alpha}$. Thus, despite using markedly different transfections systems (bacteria, insect and mammalian cells) there is reasonable consensus that the 5-HT$_{1A}$ receptor functionally couples nearly exclusively through α-subunits of $G_{i/o/z}$ proteins, with a likely rank order of $G_{i\alpha 3} > G_{i\alpha 2} \geqslant G_{i\alpha 1} \geqslant G_{o\alpha} > G_{z\alpha}$. There is currently little evidence that the 5-HT$_{1A}$ receptor can couple to $G_{t\alpha}$, $G_{s\alpha}$, $G_{q\alpha}$, $G_{12\alpha}$ or $G_{13\alpha}$.

Some groups have taken these studies a step further by trying to link specific G protein subunits with signals that occur downstream of the G proteins. Fargin *et al.* (1991) used an immuno-neutralization method to link $G_{i\alpha 3}$ to the inhibition of adenylyl cyclase and to the activation of PI-PLC in HeLa cells. Raymond *et al.* (1993b) used a similar method in CHO and HeLa cells to demonstrate that 5-HT$_{1A}$ receptor-mediated inhibition of adenylyl cyclase can be mediated by either $G_{i\alpha 3}$ or $G_{i\alpha 2}$, and probably $G_{i\alpha 1}$. These findings are not surprising in light of the work of Wong *et al.* (1992) that demonstrated that $G_{i\alpha 1}$, $G_{i\alpha 2}$, $G_{i\alpha 3}$, and $G_{z\alpha}$, can all inhibit cyclic AMP accumulation in mammalian cells. Gettys *et al.* (1994) used selective photoaffinity labelling of $G_{i\alpha}$ subunits in CHO cells to demonstrate a close correlation between the ability of a panel of 5-HT$_{1A}$ receptor agonists to activate $G_{i\alpha 2}$ and to inhibit adenylyl cyclase, whereas there was much less correlation between $G_{i\alpha 3}$ and the inhibition of adenylyl cyclase. Immunoneutralization studies suggested that $G_{i\alpha 3}$ and $G_{i\alpha 2}$ were about equally important for the inhibition of adenylyl cyclase in HeLa cells, whereas $G_{i\alpha 2}$ was more important in CHO cells. The differences in the relative importance of $G_{i\alpha 2}$ and $G_{i\alpha 3}$ may have been affected by the relative abundance of the G protein α-subunits in HeLa and CHO cells. In HeLa cells, $G_{i\alpha 3}$ is much more highly expressed than $G_{i\alpha 2}$ (which is almost undetectable by immunoblot), whereas in CHO cells, there is $\approx$9 fold more $G_{i\alpha 2}$ than $G_{i\alpha 3}$ (Raymond *et al.*, 1993b).

Liu *et al.* (1994) used an elegant antisense approach to show that $G_{i\alpha 2}$ was primarily responsible for 5-HT$_{1A}$ receptor-mediated inhibition of adenylyl cyclase in GH_4C_1 cells, whereas $G_{o\alpha}$ was responsible for the inhibition of Ca^{2+} channels. Using the same system, they have also shown that $G_{i\alpha 2}$ is responsible for 5-HT$_{1A}$ receptor-mediated increases in intracellular Ca^{2+} in Ltk^- cells (Albert *et al.*, 1996). Garnovskaya *et al.* (1997) showed that either $G_{i\alpha 2}$ or $G_{i\alpha 3}$ could couple the 5-HT$_{1A}$ receptor to the activation of Na^+/H^+ exchange in CHO cells, whilst $G_{i\alpha 1}$, $G_{o\alpha}$, and $G_{z\alpha}$ could not. Langlois *et al.* (1996) presented evidence to suggest that 5-HT$_{1A}$ receptors inhibit adenylyl cyclase *via* $G_{i\alpha 3}$ on the apical cell surface, and *via* $G_{i\alpha 2}$ on the basolateral surface of polarized epithelial LLC-PK$_1$ cells. In aggregate, these studies link the effects of the 5-HT$_{1A}$ receptor to the inhibition of adenylyl cyclase, activation of Na^+/H^+ exchange, and activation of PLC through $G_{i\alpha 2}$ or $G_{i\alpha 3}$. The inhibition of Ca^{2+} channels appears to require $G_{o\alpha}$.

DNA synthesis, growth, and transformation

5-HT receptors coupled to pertussis toxin-sensitive G proteins have previously been implicated as growth stimulatory (Ishizuka *et al*., 1992; Seuwen *et al*., 1988). Furthermore, in glial cells, endogenous 5-HT$_{1A}$ receptors stimulate secretion of the S100 protein, which has been shown to promote the growth of serotoninergic neurons (Lauder, 1993; Whitaker-Azmitia, 1991). However, a direct link between the 5-HT$_{1A}$ receptor and growth cascades has not been demonstrated in cells in which the receptor is expressed naturally. Therefore, three different transfected cell lines have been used to study the links of 5-HT$_{1A}$ receptors to DNA synthesis, growth and cellular transformation. Abdel-Baset *et al*. (1992) transfected BALB/c-3T3 fibroblasts with the rat 5-HT$_{1A}$ receptor, and showed that 5-HT induced enhanced incorporation of [^{3}H]-thymidine into DNA in a clone that expressed 600 fmol mg^{-1} of protein of [^{3}H]-8-OH-DPAT. Long-term treatment of cell cultures resulted in phenotypical transformation and foci formation in transfected, but not in non-transfected cells. Those effects were sensitive to pertussis toxin, and were attributed to the actions of PI-PLC. Another group contemporaneously showed that the 5-HT$_{1A}$ receptor expressed in NIH-3T3 cells (at 40–500 fmol mg^{-1} of protein) induced focus formation, weakly stimulated DNA synthesis, and potentiated increases in DNA synthesis initiated by epidermal growth factor (Varrault *et al*., 1992a). Those effects were also sensitive to pertussis toxin, and did not appear to involve PI-PLC or lowering of intracellular cyclic AMP levels. Lam *et al*. (1996) showed that the 5-HT$_{1A}$ receptor expressed in Rat-1 fibroblasts increased proliferation as measured by the R-SAT method (Messier *et al*., 1995). Thus, the 5-HT$_{1A}$ receptor can stimulate proliferation and/or transformation in several transfected cell types as measured by different methods.

Regulation of transcriptional cascades

The 5-HT$_{1A}$ receptor has been linked to two specific signalling pathways involved in the regulation of transcription, namely activation of Erk (extracellular signal-regulated kinase) family mitogen-activated protein kinases, and the transcriptional regulatory factor, NF-κB (nuclear factor-κB). Cowen *et al*. (1997) used transfected CHO cells to document that 5-HT$_{1A}$ receptor agonists activate a signalling pathway that stimulates NF-κB, probably through accelerating the degradation of an inhibitor of NF-κB called IκBα. Phosphorylation of IκBα causes dissociation from NF-κB, resulting in nuclear translocation of NFκ-B, and accelerated degradation of IκBα *via* ubiquitination (Berg & Baldwin, 1993; Brown *et al*., 1993). Cowen *et al*. (1997) also showed that the degradation of IκBα was sensitive to inhibition of phosphatidylcholine-specific phospholipase C (PC-PLC).

Several groups have documented that the 5-HT$_{1A}$ receptor activates Erk kinases in CHO cells, and have identified a number of signalling molecules involved in that pathway. Like other G protein-coupled receptors (Luttrell *et al*., 1997; Marshall, 1995), the 5-HT$_{1A}$ receptor activates Erk through a complex pathway that involves many of the same molecules used by growth factor receptor tyrosine kinases. The activation of Erk by the 5-HT$_{1A}$ receptor is initiated by $\beta\gamma$ subunits released from pertussis toxin-sensitive G proteins. This results in tyrosine phosphorylation of Shc, a protein that serves as a docking platform. Shc phosphorylation results in the recruitment of a lipid kinase, and an adapter protein, Grb2 to the signalling complex. Grb2, in turn, binds to a Ras activator protein called Sos. Ras activation leads to sequential activation of Raf, which phosphorylates and activates MEK (mitogen and extracellular signal regulated kinase), which phosphorylates and activates Erk (Garnovskaya *et al*., 1996). The precise role of PI3-K in propagating the Erk signal is not known, but it is clearly necessary for 5-HT$_{1A}$ receptor-mediated Erk activation, and most likely operates at the level of Shc and Grb2 (Cowen *et al*., 1996; Garnovskaya *et al*., 1996; 1998). More recent studies have also implicated a Ca^{2+}/calmodulin-dependent endocytosis step between Ras and Raf (Della Rocca *et al*., 1999), and reactive oxygen intermediates upstream of Src in 5-HT$_{1A}$ receptor-mediated ERK activation in CHO cells (Mukhin, Raymond and Garnovskaya, unpublished observation). An NAD(P)H oxidase enzyme probably produces the reactive oxygen species.

Cowen *et al*. (1996) hypothesized a role for phosphatidylcholine-specific PLC (PC-PLC) in 5-HT$_{1A}$ receptor-mediated Erk activation based on their observations that 8-OH-DPAT elicited release of radioactivity from cells pre-loaded with [^{3}H]-choline. This effect and Erk activation were both attenuated by a PC-PLC inhibitor (tricyclodecan-9-yl-xanthogenate, D609). If PC-PLC is required for 5-HT$_{1A}$ receptor-mediated Erk activation in CHO cells, it most likely functions at or upstream of Raf based on several studies in other systems. Introduction of bacterial PC-PLC activates Raf, and D609 blocks Raf activation induced by epidermal growth factor and serum in NIH-3T3 cells. Dominant negative Raf constructs block replication, and this block is not overcome by introduction of bacterial PC-PLC (Cai *et al*., 1992; 1993). Moreover, dominant negative Ras attenuates the activation of PC-PLC in NIH-3T3 cells (Cai *et al*., 1993). Based on these studies and their own work, Cowen *et al*. (1996) hypothesized that PC-PLC augments the activation of Raf that is induced by Ras. If this were so, it would appear that the 5-HT$_{1A}$ receptor in CHO cells activates Erk through the activation of separate, yet converging lipid signalling pathways. The first involves PI3-K and intersects the Erk pathway upstream of Ras, whereas the second involves PC-PLC and also intersects the Erk pathway upstream of Ras.

Desensitization and phosphorylation

Desensitization is a process through which signalling pathways become progressively less able to mount a signal after repeated exposures to a stimulus. One major mechanism through which desensitization of G protein-coupled receptors occurs is kinase-directed phosphorylation of the receptors. Thus far, three distinct protein kinases have been implicated in the desensitization and phosphorylation of the 5-HT$_{1A}$ receptor, namely PKC, PKA, and GRK (G protein-coupled receptor kinase). There are four putative PKC sites and two putative PKA sites in the human 5-HT$_{1A}$ receptor (see Figure 1 and its legend). Stimulation of PKC by application of phorbol esters induces a rapid phosphorylation of the receptor at a stoichiometry of two phosphates per receptor (Raymond, 1991). This phosphorylation is associated with desensitization of several signals in various cell lines. In CHO cells, PKC-mediated desensitization of the inhibition of adenylyl cyclase by the 5-HT$_{1A}$ receptor is manifested only by a change in potency of agonist, and is not associated with a significant change in efficacy (Raymond, 1991), whereas in P11 rat pituitary cells, PKC-mediated desensitization is manifested by a change in both and efficacy of 8-OH-DPAT to cause inhibition of adenylyl cyclase (Hensler *et al*., 1996). In contrast, phorbol ester pretreatment of Ltk$^-$ fibroblasts and

GH_4C_1 cells transfected with the 5-HT$_{1A}$ receptor has no detectable effects on the inhibition of adenylyl cyclase (Lembo *et al*., 1995; 1997; Liu & Albert, 1991). In Ltk$^-$ fibroblasts, activation of PKC with phorbol ester results in desensitization of 5-HT$_{1A}$ receptor-mediated PLC activity as measured by increased intracellular Ca^{2+} and hydrolysis of phosphoinositides. The effects of phorbol ester on Ca^{2+} mobilization were profound, essentially eliminating subsequent responses to 5-HT. Desensitization could be reversed by mutation of three putative PKC sites in the i3 loop of the 5-HT$_{1A}$ receptor (Lembo *et al*., 1995), providing strong support for a functional link between phosphorylation and desensitization of the receptor. Harrington *et al*. (1994) provided further evidence for PKC-mediated desensitization of the 5-HT$_{1A}$ receptor. They showed that pretreatment with phorbol ester of 5-HT$_{1A}$-receptor transfected HeLa cells rapidly uncoupled the receptor from G proteins, and that inhibitors of PKC could block this effect. Thus, the evidence is fairly clear that PKC can induce desensitization and phosphorylation of the 5-HT$_{1A}$ receptor in transfected cells, although the functional consequences of the interaction varies depending on the cell type and signalling pathways being measured.

There is also strong evidence that activation of PKA can lead to phosphorylation and desensitization of the 5-HT$_{1A}$ receptor in multiple cell types. In CHO cells, PKA stimulation leads to phosphorylation of the receptor with a stoichiometry

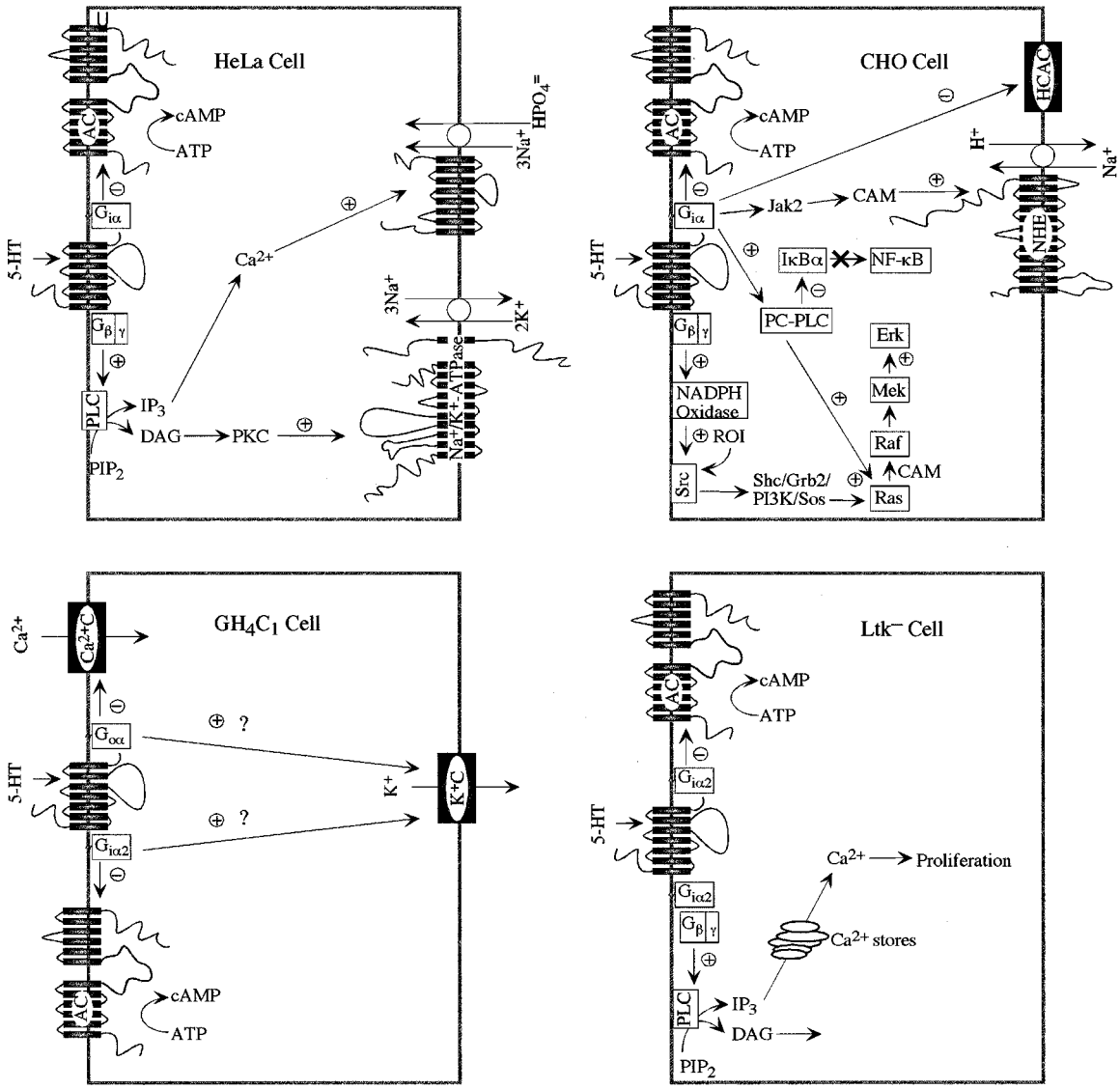

Figure 2 Second messenger and effector linkages of the 5-HT$_{1A}$ receptor in transfected cell systems. There are differences in the specific linkages depending upon the host cell in which the receptor has been expressed. Depicted are 5-HT$_{1A}$ receptor linkages with the inhibition of adenylyl cyclase (AC), stimulation of phosphatidylinositol 4,5-bisphosphate (PIP_2) hydrolysis, increased levels of intracellular Ca^{2+}, and activtion of phospholipases A_2 (PLA_2) and C (PLC). Phosphatidylinositol-specific PLC and phosphatidylcholine-specific PLC are abbreviated as (PI-PLC and PC-PLC). The receptor also couples to protein kinase C (PKC), activation of K^+ channels, inhibition of high conductance anion channels (HCAC) and Ca^{2+} channels, and activation of Erk extracellular signal regulated protein kinase. Erk activation proceeds from Src tyrosine kinase to a docking platform (Shc), where PI-3K (PI-3′ kinase) Sos (a G protein activator) and Grb2 (an adapter) aggregate. Sos activates the small monomeric G, Ras. This activates a sequential phosphorylation cascade involving Raf, Mek (mitogen and extracellular signal regulated protein kinase), and Erk. Erk activation also requires calmodulin (CAM) and reactive oxygen intermediates (ROI) generated by an NAD(P)H oxidase. The receptor couples to active transporters in CHO cells, including the Na^+/H^+-exchanger, Na^+/K^+-ATPase and Na^+/PO_4 cotransporter. All linkages described are sensitive to pertussis toxin, and involve either G_i or G_o proteins. α, β, and γ represent subunits of G proteins. Other abbreviations used are IP_3 (inositol 1,4,5-trisphosphate), and DAG (diacylglycerol).

of one phosphate per receptor. Pharmacological stimulation of PKA also augments PKC-induced phosphorylation and desensitization of the inhibition of adenylyl cyclase by the 5-HT$_{1A}$ receptor (Raymond & Olsen, 1994). Those results confirmed the observations of Liu & Albert (1991), who first showed that activators of PKA enhanced the phorbol ester-induced desensitization of 5-HT$_{1A}$ receptor coupling to PLC in Ltk$^-$ fibroblasts. In HeLa cells, Harrington *et al.* (1994) documented rapid PKA-induced reduction in high affinity agonist binding as well as a total attenuation of the ability of the 5-HT$_{1A}$ receptor to inhibit adenylyl cyclase.

The 5-HT$_{1A}$ receptor can mediate its own desensitization. Prolonged treatment with 5-HT leads to downregulation and desensitization of 5-HT$_{1A}$ receptors in Swiss 3T3 cells (van Huizen *et al.*, 1993). In HeLa cells, treatment with 8-OH-DPAT leads to rapid uncoupling of the receptor from G proteins and from the inhibition of adenylyl cyclase. These effects appear to depend upon PKC, PLA$_2$, and Ca^{2+} (Harrington *et al.*, 1994). In P11 cells, pretreatment with the agonist 5-carboxamidotryptamine results in a rightward shift of the concentration-response curve of 8-OH-DPAT-inhibited adenylyl cyclase activity (Hensler *et al.*, 1996). In Sf9 insect cells, pretreatment with 5-HT leads to rapid phosphorylation of the 5-HT$_{1A}$ receptor on serine and threonine residues, and to uncoupling of the receptor from G proteins and from the inhibition of adenylyl cyclase (Nebigil *et al.*, 1995). This effect could be blocked by heparin, but not by inhibitors of PKC, and was thus attributed to the actions of a GRK. There are 17 serine and threonine residues on the intracellular loops that might serve as potential GRK phosphorylation sites.

Correlation of receptor structure and function

Despite being one of the first G protein coupled receptors to be cloned, relatively few detailed structure-function studies have been performed on the 5-HT$_{1A}$ receptor. One of the major distinctive pharmacological characteristics of the 5-HT$_{1A}$ receptor is a high affinity for classical β-adrenoceptor blockers such as pindolol. Guan *et al.* (1992) delineated a single residue critical in the binding of β-adrenoceptor blockers to the 5-HT$_{1A}$ receptor. They mutated Asn^{386} in the seventh transmembrane domain of the human 5-HT$_{1A}$ receptor, based on the observation that this residue is uniquely conserved in all 5-HT$_{1A}$ and β-adrenoceptors of different species. Mutation of this residue to valine caused a selective decrease in the affinity of pindolol and similar ligands for the mutant Asn^{386}→Val receptor, whilst producing insignificant changes in the binding of other 5-HT$_{1A}$ receptor ligands. Thus, Asn^{386} is critical for binding to β-adrenoceptor blockers like pindolol, but not to 5-HT. Kuipers *et al.* (1997) examined the effects of the same mutation on a range of aryloxypropanolamine enantiomers and further suggested that Asn^{386} functions as a chiral discriminator in that the Asn^{386}→Val mutation more significantly lowered the affinities of the S-enantiomers in a ligand binding assay.

Ho *et al.* (1992b) used a *vaccinia* infection-transfection method to transiently express wild-type and mutant 5-HT$_{1A}$ receptors into COS-7 cells in order to study the effects of various point mutations in putative transmembrane regions on receptor ligand binding. Three substitutions, Asp^{82}→Asn, Asp^{116}→Asn, and Ser^{199}→Ala, resulted in a 60–100 fold decreased affinity of 5-HT for the receptor, but had no effect on the affinity of the antagonist, pindolol. The binding of 5-HT to a fourth mutant, Thr^{200}→Ala, was not measurable. Nevertheless, 5-HT induced GTPase activities for all of the mutant receptors studied. These findings indicate that Asp^{82}, Asp^{116}, and Ser^{199} play important roles in the binding of 5-HT, but have little effect on pindolol binding. Thr^{200} is important in binding to both 5-HT and to pindolol. By analogy with the β-adrenoceptor, Asp^{82} and/or Asp^{116} are likely to act as a counterion for the amine group of 5-HT (Strader *et al.*, 1989). While the first acidic residue is conserved in all cloned G protein-coupled receptors reported thus far, the second one is present in only the receptors which bind the bioamines epinephrine, norepinephrine, dopamine, acetylcholine and serotonin. These findings have been nicely integrated into a model of the 5-HT$_{1A}$ receptor pharmacophore by Kuipers *et al.* (1994).

Varrault *et al.* (1994) used a different approach to study potential G protein contact sites within the 5-HT$_{1A}$ receptor. They constructed synthetic peptides derived from the second (i2) and third (i3) intracellular loops of the human 5-HT$_{1A}$ receptor, and assessed the ability of those peptides to modulate the binding of a nonhydrolysable GTP analogue to G$_{i/o}$, and to inhibit adenylyl cyclase. A peptide consisting of the entire i2 loop (Asp^{133}-Arg^{153}) and a heptadecapeptide from the carboxyl terminal region of the i3 loop (Ala^{331}-Leu^{347}), but not a nonapeptide from the carboxyl terminal region of the i3 loop (Ala^{336}-Val^{344}), inhibited forskolin-stimulated adenylyl cyclase activity in membranes derived from NIH-3T3 cells, S49 cells, and rat hippocampus, and increased GTPγS binding to purified bovine brain G$_{i/o}$ proteins. Thus, those studies identified the entire i2 loop and a carboxyl terminal heptadecapeptide of the i3 loop of the human 5-HT$_{1A}$ receptor as key G protein regulatory sites. These sequences contain key threonine residues shown to be involved in receptor desensitization and signal transduction by Lembo *et al.* (1995; 1997). More recently, Albert *et al.* (1998) presented evidence that supported a key role for Thr^{149} in the i2 loop of the rat receptor in coupling specifically to G$_{\beta\gamma}$-mediated signals. These studies support a model in which the i2 loop and portions of the i3 loop form amphipathic α-helices aligned such that a hydrophobic G protein interaction site is formed, as described by Albert *et al.* (1998).

Lembo *et al.* (1995; 1997) used site-directed mutagenesis to examine the roles of intracellular threonine and serine residues in the i2 and i3 loops of the rat 5-HT$_{1A}$ receptor. They found that four specific residues played roles in signal initiation, and also in PKC-mediated desensitization of the receptor. A detailed mutagenesis study of the PKC sites on the receptor was needed because PKC-mediated phosphorylation reactions could affect the function of the receptor (Raymond, 1991), G$_i$ proteins (Drummond, 1985; Daniel-Issakani *et al.*, 1989; Bushfield *et al.*, 1990; Yatomi *et al.*, 1992; Strassheim & Malbon, 1994) or PLC (Ryu *et al.*, 1990; Ali *et al.*, 1997), any of which effects could lead to desensitization of this pathway. By constructing and expressing several mutant receptors with conservative point mutations, they showed that two threonines and one serine residue located within consensus PKC phosphorylation sequences in the i3 loop were needed to confer PKC-mediated desensitization of Ca^{2+} mobilization in Ltk$^-$ fibroblasts. When the individual point mutations (Thr^{229}→Ala, S^{253}→Gly, and Thre^{343}→A) were tested, there was no difference in desensitization when compared with non-mutated receptors. In contrast a double mutant (Thr^{229}→Ala/S^{253}→Gly) and a triple mutant (Thr^{229}→Ala/S^{253}→Gly/Thre^{343}→Ala) became progressively more resistant to PKC-mediated desensitization. Those results suggest that there is a good correlation between the presence of those three residues and PKC-mediated desensitization of the 5-HT$_{1A}$ receptor.

Lembo *et al*. (1997) also assessed the role of Thr^{149} in signal transduction by expressing a mutant rat 5-HT$_{1A}$ receptor (Thr^{149}→Ala) in Ltk$^-$ fibroblasts and in GH_4C_1 cells. They found that the mutant receptor lost its ability to elevate intracellular Ca^{2+} in Ltk$^-$ cells, and also was unable to inhibit opening of BayK8644 sensitive Ca^{2+} channels in GH_4C_1 cells. In contrast, the Thr^{149}→Ala mutation only partially uncoupled the receptor from adenylyl cyclase inhibition. Interestingly, the Thr^{229}→Ala mutation also partially uncoupled the receptor from the inhibition of adenylyl cyclase, and reduced the peak increase in intracellular Ca^{2+} in Ltk$^-$ cells (Lembo *et al*., 1995). Thus, the work of Lembo *et al*. (1995; 1997) suggests those three residues in the i3 loop of the 5-HT$_{1A}$ receptor (Thr^{229}, S^{253}, and Thre^{343}) mediated PKC-induced desensitization of the rat receptor. Additionally, Thr^{229} is important for efficient coupling to both inhibition of adenylyl cyclase and elevations of intracellular Ca^{2+}. Thr^{149} in the i2 loop is highly critical in both elevations of intracellular Ca^{2+} and blockade of Ca^{2+} channels by the receptor, and plays a smaller, but significant role in the inhibition of adenylyl cyclase.

Allelic variants of the 5-HT$_{1A}$ receptor

The possibility that the 5-HT$_{1A}$ receptor might possess allelic variants was supported by the cloning of the rat receptor by two different groups (Albert *et al*., 1990; Fujiwara *et al*., 1990) whose published nucleic acid sequences differed only by two nucleotides. One of the variants was silent at the protein level, whereas the other resulted in a predicted change in a single amino acid in the rat 5-HT$_{1A}$ receptor (Fujiwara *et al*., 1993). Several groups have subsequently described variations in the 5′-untranslated and the coding regions of the human 5-HT$_{1A}$ receptor gene. Some of the nucleic acid changes in the coding block do not result in amino acid changes, whereas several others result in single amino acid changes; these include Pro^{16}→Leu, Ile^{28}→Val, Gly^{22}→Ser, Arg^{219}→Leu; Gly^{272}→Asp; Asn^{417}→Lys (Erdmann *et al*., 1995; Harada *et al*., 1996; Kawanishi *et al*., 1998; Lam *et al*., 1996; Nakhai *et al*., 1995). The existence of these variant sequences raises the interesting possibility that there may be functional differences that could lead to disease manifestations. Although a detailed biochemical analysis of each of the variants has not yet been performed, Brüss *et al*. (1995) studied the Ile^{28}→Val variant in transfected COS-7 cells. They demonstrated that this receptor variant had ligand binding properties that were nearly identical to those of the wild-type receptor (Brüss *et al*., 1995). In addition, to this point, no definitive links in populations between the allelic variants have been made to alcoholism, schizophrenia, bipolar affective disorder, or Tourette's syndrome.

Ligand pharmacology

Cells transfected with the 5-HT$_{1A}$ receptor could provide a ready source for the study of this receptor absent of any input from other 5-HT receptors. However, the usefulness of pharmacological information obtained from transfected cell systems required some measure of validation. Several groups have directly compared the ligand binding and second messenger coupling parameters of transfected 5-HT$_{1A}$ receptors with those naturally expressed in tissues. They have concluded that transfected cell systems are valid for studying the pharmacology of the 5-HT$_{1A}$ receptor (Pauwels *et al*., 1993; Pou *et al*., 1997). Transfected cell lines have been used to characterize radioligands (Sundaram *et al*., 1992; 1995) and identify and characterize partial agonists (Arthur *et al*., 1993; Assie *et al*., 1997; Pauwels *et al*., 1993; 1997) and inverse agonists (Barr & Manning, 1997; Newman-Tancredi *et al*., 1998) of the 5-HT$_{1A}$ receptor. They have also been used in the development of silent antagonists of the 5-HT$_{1A}$ receptor, which has been a particularly nettlesome task because most putative silent antagonists actually turned out to be partial agonists (Routledge, 1996). Finally, WAY-100635, (N-[2-[4-(2-methoxyphenyl)1-piperazinyl]ethyl)-n-(2-pyridiynl) cyclohexanecarboxamide trihydrochloride) was characterized as a silent antagonist in a number of *in vivo* and *in vitro* models (Fletcher *et al*., 1996). Critically, the properties of WAY-100635 as a true silent antagonist of the 5-HT$_{1A}$ receptor were confirmed in a transfected CHO cell system using GTPγS binding (Newman-Tancredi *et al*., 1996) and measurements of the metabolic production of protons (Dunlop *et al*., 1998).

Transfected cells have also been used to characterize the interactions of novel ligands with the 5-HT$_{1A}$ receptor. For example, transfected cells were instrumental in delineating the specific interactions of modified oleamide, an endogenous sleep-associated fatty acid primary amide, with 5-HT$_{1A}$ and 5-HT$_{2A}$ receptors (Boger *et al*., 1998). Transfected cells were also used to disprove an interaction between 5-HT-moduline (Leu-Ser-Ala-Leu) and the 5-HT$_{1A}$ receptor and to confirm its interaction specifically with a very high apparent affinity and in a non-competitive manner with 5-HT$_{1B}$ receptors (Rousselle *et al*., 1998).

Coupling efficiency

Transfected cell systems have been used to compare the efficiency of coupling of the 5-HT$_{1A}$ receptor to various second messengers and G proteins. For example, the 5-HT$_{1A}$ receptor expressed in HeLa cells both inhibits adenylyl cyclase and activates PLC (Fargin *et al*., 1989). However, the receptor has been shown to differentially modulate those activities. The 5-HT$_{1A}$ receptor couples very efficiently to the inhibition of adenylyl cyclase because changing receptor expression levels from <20 fmol mg^{-1} protein to ≈ 3 pmol mg^{-1} of protein had no effect on the potency or efficacy of agonists to inhibit cyclic AMP accumulation (Boddeke *et al*., 1992; Fenrick *et al*., 1996). This tight coupling applied over a variety of full and partial agonists (Schoeffter *et al*., 1996). In contrast, coupling of the 5-HT$_{1A}$ receptor to PLC as measured by Ca^{2+} mobilization and phosphoinositide hydrolysis was much less efficient in that the potency, and especially the efficacy, of various agonists was considerably less than for the inhibition of adenylyl cyclase. Moreover, both efficacy and potency of coupling of the 5-HT$_{1A}$ receptor to PLC were improved at higher levels of receptor expression (Boddeke *et al*., 1992; Fargin *et al*., 1989; Fenrick *et al*., 1996; Raymond *et al*., 1989; Schoeffter *et al*., 1997).

Transfected cells have been used to study other aspects of 5-HT$_{1A}$ receptor coupling efficiency, including variables that affect ligand efficacy and potency. One such variable is receptor/G protein stoichiometry. Newman-Tancredi *et al*. (1997) studied the effects of altering receptor : G protein ratios in CHO cells by examining [^{35}S]-GTPγS binding in transfected lines bearing various numbers of 5-HT$_{1A}$ receptors. Their data suggested that increasing receptor/G-protein ratio (i) augments the potency of full agonists, (ii) increases the efficacy of partial agonists and (iii) increases the negative efficacy of inverse agonists at recombinant human 5-HT$_{1A}$ receptors. Those conclusions were similar to those of Boddeke *et al*. (1992),

who showed that increasing receptor numbers were associated with higher efficacy of partial agonists to stimulate PLC in HeLa cells. The levels of G proteins were constant among the cell lines in those two studies, whereas receptor levels varied. Raymond *et al*. (1992) altered the ratios of 5-HT$_{1A}$ receptors to G proteins by treating CHO cells with varying amounts of pertussis toxin to neutralize fractions of endogenous G proteins. They showed that increasing amounts of pertussis toxin resulted first in a rightward shift of the IC_{50} of 5-HT for the inhibition of adenylyl cyclase (reduction in potency) followed by a reduction in the efficacy. Those results are consistent with either the classical or operational models of receptor/effector coupling (Black *et al*., 1985; Kenakin & Morgan, 1989), both of which models assume unconstrained interactions of the various signalling molecules. In contrast, Varrault *et al*. (1992b) used transfected NIH-3T3 cells to show that increasing 5-HT$_{1A}$ receptor/G protein stoichiometry mainly increased the efficacies, but not the potencies of various full and partial agonists to inhibit adenylyl cyclase. In a few cases, alterations in efficacy were seen, but these were less than predicted, and varied depending upon the specific ligands being studied (Varrault & Bockaert, 1992). These latter results are consistent with a constrained model of receptor/G protein interaction in which the ability to interact efficiently is damped by functional membrane compartmentalization. The findings are significant in aggregate as they suggest that cell-specific constraints may alter the effects of 5-HT$_{1A}$ receptor/G protein coupling on ligand efficacy in various second messenger pathways.

Another variable that could affect ligand efficacy and potency at the 5-HT$_{1A}$ receptor is the possibility that certain ligands induce conformational changes in the receptor that are more or less favourable for G protein activation. For example, partial agonists could be less efficacious than full agonists because they are unable to induce the optimal conformational change in the receptor that regulates contact with G proteins. Gettys *et al*. (1994) confirmed that full agonists of the 5-HT$_{1A}$ receptor were more efficacious in activating $G_{i\alpha}$ subunits in CHO cells than were partial agonists. They further tested the hypothesis that partial agonists might induce relatively selective activation of specific $G_{i\alpha}$ subunits when compared with full agonists. They provided evidence in CHO cells that $G_{i\alpha 3}$ was activated with a rank order of efficacy of 8-OH-DPAT $\approx$ 5-HT > ipsapirone $\approx$ rauwolscine, whereas $G_{i\alpha 2}$ was activated with a rank order of efficacy of 5-HT > 8-OH-DPAT > rauwolscine > ipsapirone. Thus, both receptor/G protein stoichiometry and the availability of specific isotypes of $G_{i\alpha}$ subunits might affect the coupling efficiency of the 5-HT$_{1A}$ receptor to G proteins and specific second messengers.

Transcriptional regulation of the 5-HT$_{1A}$ receptor

Variables that regulate the absolute levels and the stability of receptor mRNA probably lead to critical regulation of receptor protein expression and function. For the 5-HT$_{1A}$ receptor, this point was experimentally tested by Konigs *et al*. (1995), who showed that levels of receptor mRNA (as measured by Northern blot and *in situ* hybridization) correlated very well with receptor protein expression (as measured by radioligand binding) in clones of Swiss 3T3 cells transfected with the human 5-HT$_{1A}$ receptor. The levels of expression of the 5-HT$_{1A}$ receptor can be altered *in vivo* by several distinct stimuli. For example, glucocorticoids have been shown to negatively regulate 5-HT$_{1A}$ receptor mRNA expression within the hippocampus (Chalmers *et al*., 1994). The selective 5-HT$_{1A}$ receptor agonist 8-OH-DPAT differentially regulates the levels of 5-HT$_{1A}$ receptor mRNA in the dorsal and median raphe nuclei (Razani *et al*., 1997). Hyperammonaemia increased 5-HT$_{1A}$ receptor mRNA and protein expression in rat hippocampus and transfected HN2 cells (Alexander *et al*., 1995). Stress induced by serum deprivation increases 5-HT$_{1A}$ receptor protein and message levels in transfected HN2 and NCB-20 cells (Singh *et al*., 1996b). The recombinant 5-HT$_{1A}$ receptor also has been shown to modulate the levels of gangliosides when expressed in HN2 hippocampal, dorsal root ganglion-derived F-11 cells and NCB-20 brain cells (Singh *et al*., 1996a). This may represent a cellular defense response in that 5-HT$_{1A}$ receptors protected primary cultures of neurons from chick embryo telencephalons from apoptotic cell death, possibly by stimulating production of NGF (Ahlmeyer & Kriegelstein, 1997). Despite the potential importance of the regulation of 5-HT$_{1A}$ receptor message and protein levels, almost nothing is known about the molecular mechanisms through which those various stimuli regulate the expression of the 5-HT$_{1A}$ receptor. In that regard, Charest *et al*. (1993) have identified a septal cell line that can be induced to express 5-HT$_{1A}$ receptors by retinoic acid treatment. Other non-neuronal cell lines (Jurkat cells) have also been suggested to express the 5-HT$_{1A}$ receptor (Aune *et al*., 1993; 1994). These cell lines might prove to be useful for analysis of promoter elements of the 5-HT$_{1A}$ receptor gene that regulate its expression in the central nervous system.

Conclusions

The isolation of the gene and cDNA for the 5-HT$_{1A}$ receptor has resulted in an explosion of information regarding the characteristics and functions of this receptor in heterologous expression systems. This information has translated important into insights in some cases. In other cases, it has mainly given us a glimpse of what may be possible in physiological settings. The challenge of the next few years will be to separate what is only theoretically possible from what actually happens in cells in which the receptor is naturally expressed.

The authors were supported by grants from the Department of Veterans Affairs (Merit Awards to M.N. Garnovskaya and J.R. Raymond) the National Institutes of Health (DK52448 and DK53981 to J.R. Raymond and T.W. Gettys), the U.S. Department of Agriculture (NRICGP/USDA 9800699 to T.W. Gettys), a laboratory endowment jointly supported by the M.U.S.C. Division of Nephrology and Dialysis Clinics, Incorporated (J.R. Raymond), an American Heart Association Fellowship Award (Y.V. Mukhim), and a M.U.S.C. University Research Foundation award (M.N. Garnovskaya).

References

ABDEL-BASET, H., BOZOVICK, V., SZYF, M. & ALBERT, P.R. (1992). Conditional transformation mediated via a pertussis toxin-sensitive receptor signalling pathway. *Mol. Endo.*, **6,** 730 – 740.

AHLEYMEYER, B. & KRIEGLSTEIN, J. (1997). Stimulation of 5-HT$_{1A}$ receptor inhibits apoptosis induced by serum deprivation in cultured neurons from chick embryo. *Brain Res.*, **777,** 179 – 186.

ALBERT, P.R., LEMBO, P., STORRING, J.M., CHAREST, A. & SAUCIER, C. (1996). The 5-HT1A receptor: Signaling, desensitization and gene transcription. *Neuropsychopharmacology,* **14,** 19–25.

ALBERT, P.R., MORRIS, S.J., GHAHREMANI, M.H., STORRING, J.M. & LEMBO, P.M. (1998). A putative a-helical $G_{\beta\gamma}$-coupling domain in the second intracellular loop of the 5-HT$_{1A}$ receptor. *Ann. N.Y. Acad. Sci.,* **861,** 146–161.

ALBERT, P.R., ZHOU, Q.-Y., VANTOL, H.H.M., BUNZOW, J.R. & CIVELLI, O. (1990). Cloning, functional expression, and mRNA distribution of the rat 5-hydroxytryptamine$_{1A}$ receptor gene. *J. Biol. Chem.,* **265,** 5825–5832.

ALEXANDER, J.J., BANERJEE, P., DAWSON, G. & TONSGARD, J.H. (1995). Hyperammonemia increases serotonin 1A receptor expression in both rat hippocampus and a transfected hippocampal cell line, HN2-5. *J. Neurosci. Res.,* **41,** 105–110.

ALI, H., FISHER, I., HARIBABU, B., RICHARDSON, R.M. & SNYDERMAN, R. (1997). Role of phospholipase Cb3 phosphorylation in the desensitization of cellular responses to platelet-activating factor. *J. Biol. Chem.,* **272,** 11706–11709.

ANDRADE, R. & NICOLL, R.A. (1986). Pharmacological distribution of serotonin receptors on single pyramidal neurones of the rat hippocampus recorded *in vitro*. *J. Physiol.,* **394,** 99–124.

ARTHUR, J.M., CASAÑAS, S.J. & RAYMOND, J.R. (1993). Partial agonist properties of rauwolscine and yohimbine for the inhibition of adenylyl cyclase by recombinant human 5-HT$_{1A}$ receptors. *Biochem. Pharmacol.,* **45,** 2337–2341.

ASSIE, M.B., COSI, C. & KOEK, W. (1997). 5-HT$_{1A}$ receptor agonist properties of the antipsychotic, nemonapride: comparison with bromerguride and colzapine. *Eur. J. Pharmacol.,* **334,** 141–147.

AUNE, T.M., GOLDEN, H.W. & MCGRATH, K.M. (1994). Inhibitors of serotonin synthesis and antagonists of serotonin 1A receptors inhibit T lymphocyte function in vitro and cell-mediated immunity in vivo. *J. Immunol.,* **153,** 489–498.

AUNE, T.M., MCGRATH, K.M., SARR, T., BOMBARA, M.P. & KELLEY, K.A. (1993). Expression of 5HT1a receptors on activated human T cells. Regulation of cyclic AMP levels and T cell proliferation by 5-hydroxytrayptamine. *J. Immunol.,* **151,** 1175–1183.

BALCELLS-OLIVERO, M., COUSINS, M.S. & SEIDEN, L.S. (1998). Holtzman and Sprague-Dawley rats: differences in DRL-72-sec performance and 8-hydroxy-di-propylamino teralin-induced hypothermia. *J. Pharmacol. Expt. Ther.,* **286,** 742–752.

BANERJEE, P., BERRY-KRAVIS, E., BONAFEDE-CHHABRA, D. & DAWSON, G. (1993). Heterologous expression of the serotonin 5-HT$_{1A}$ receptor in neural and non-neural cell lines. *Biochem. Biophys. Res. Commun.,* **192,** 104–110.

BARR, A.J., BRASS, L.F. & MANNING, D.R. (1997). Reconstitution of receptors and GTP-binding regulatory proteins (G proteins) in Sf9 cells. A direct evaluation of selectivity in receptor : G protein coupling. *J. Biol. Chem.,* **272,** 2223–2229.

BARR, A.J. & MANNING, D.R. (1997). Agonist-independent activation of G_z by the 5-hydroxytryptamine$_{1A}$ receptor co-expressed in *Spodoptera frugiperda* cells. Distinguishing inverse agonists from neutral antagonists. *J. Biol. Chem.,* **272,** 32979–32987.

BERG, A.A. & BALDWIN, A.S. (1993). The I$\kappa\beta$ proteins: multifunctional regulators of rel/NFκ-B transcription. *Genes Dev.,* **7,** 2064–2070.

BERRIDGE, M.J., BOOTMAN, M.D. & LIPP, P. (1998). Calcium – A life and death signal. *Nature,* **395,** 645–658.

BERTIN, B., FREISSMUTH, M., BREYER, R.M., SCHUTZ, W., STROSBERG, A.D. & MARULLO, S. (1992). Functional expression of the human serotonin 5-HT$_{1A}$ receptor in *Escherichia coli*. Ligand binding properties and interaction with recombinant G protein α-subunits. *J. Biol. Chem.,* **267,** 8200–8206.

BLACK, J.W., LEFF, P. & SHANKLEY, N.P. (1985). Pharmacological analysis of beta-adrenoceptor-mediated agonism in the guinea-pig, isolated, right atrium. *Br. J. Pharmacol.,* **84,** 561–571.

BLIER, P., BERGERON, R. & DE MONTIGNY, C. (1997). Selective activation of postsynaptic 5-HT$_{1A}$ receptors induces rapid antidepressant response. *Neuropsychopharmacology,* **16,** 333–338.

BODDEKE, H.W.G.M., FARGIN, A., RAYMOND, J.R., SCHOEFFTER, P. & HOYER, D. (1992). Agonist/antagonist interactions with cloned human 5-HT$_{1A}$ receptors: variations in intrinsic activity studied in transfected HeLa cells. *Naunyn-Schmiedebergs Arch. Pharmacol.,* **345,** 257–263.

BOGER, D.L., PATTERSON, J.E. & JIN, Q. (1998). Structural requirements for 5-HT$_{2A}$ and 5-HT$_{1A}$ serotonin receptor potentiation by the biologically active lipid oleamide. *Proc. Natl. Acad. Sci. U.S.A.,* **95,** 4102–4107.

BROWN, K., PARK, S. KANNO, T., FRANZOSA, G. & SEIBENLIST, U. (1993). Mutual regulation of the transcriptional activator NF-kB and its inhibitor. *Proc. Natl. Acad. Sci. U.S.A.,* **90,** 2532–2536.

BRÜSS, M., BÜHLEN, M., ERDMANN, J., GÖTHERT, M. & BÖNISCH, H. (1995). Binding properties of the naturally occurring human 5-HT1A receptor variant with the Ile28Val substitution in the extracellular domain. *Naunyn-Schmied. Arch. Pharmacol.,* **352,** 455–458.

BUSHFIELD, M., MURPHY, G.J., LAVAN, B.E., PARKER, P.J., HRUBY, V.J., MILLIGAN, G. & HOUSLAY, M.D. (1990). Hormonal regulation of G_{i2} α-subunits phosphorylation in intact hepatocytes. *Biochem. J.,* **268,** 449–457.

BUTKERAIT, P., ZHENG, Y., HALLAK, H., GRAHAM, T.E., MILLER, H.A., BURRIS, K.D., MOLINOFF, P.D. & MANNING, D.R. (1995). Expression of the human 5-hydroxytryptamine$_{1A}$ receptor in Sf9 cells. Reconstitution of a coupled phenotype by co-expression of mammalian G protein subunits. *J. Biol. Chem.,* **270,** 18691–18699.

CAI, H., ERHARDT, P., SZEBERENYI, J., DIAZ-MECO, M.T., JOHANSEN, T., MOSCAT, J. & COOPER, G.M. (1992). Hydrolysis of phosphatidylcholine is stimulated by Ras proteins during mitogenic signal transduction. *Mol. Cell Biol.,* **12,** 5329–5335.

CAI, H., ERHARDT, P., TROPPMAIR, J., DIAZ-MECO, SITHANANDAM, G., RAPP, U.R., M.T., MOSCAT, J. & COOPER, G.M. (1993). Hydrolysis of phosphatidylcholine couples Ras to activation of Raf protein kinase during mitogenic signal transduction. *Mol. Cell Biol.,* **13,** 7645–7651.

CARMENA, M.J., CAMACHO, A., SOLANO, R.M., MONTALVO, L., GARCIA-LOPEZ, E., ARIAS, A. & PRIETO, J.C. (1998). 5-hydroxytryptamine$_{1A}$ receptor-mediated effects on adenylate cyclase and nitric oxide synthase activities in rat ventral prostate. *Cell. Signal.,* **10,** 583–587.

CHALMERS, D.T., LOPEZ, J.F., VAZQUEZ, D.M., AKIL, H. & WATSON, S.J. (1994). Regulation of hippocampal 5-HT$_{1A}$ receptor gene expression by dexamethasone. *Neuropsychopharmacology,* **10,** 215–222.

CHANDA, P.K., MINCHIN, M.C., DAVIS, A.R., GREENBERG, L., REILLY, Y., MCGREGOR, W.H., BHAT, R., LUBECK, M.D., MIZUTANI, S. & HUNG, P.P. (1993). Identification of residues important for ligand binding to the human 5-hydroxytryptamine1A serotonin receptor. *Mol. Pharmacol.,* **43,** 516–520.

CHAREST, A., WAINER, B.H. & ALBERT, P.R. (1993). Cloning and differentiation-induced expression of a murine serotonin 1A receptor in a septal cell line. *J. Neurosci.,* **13,** 914–923.

CLAUSTRE, Y., BENAVIDES, J. & SCATTON, B. (1988). 5-HT$_{1A}$ receptor agonists inhibit carbachol-induced stimulation of phosphoinositide turnover in rat hippocampus. *Eur. J. Pharmacol.,* **149,** 149–153.

CLAWGES, H.M., DEPREE, K.M., PARKER, E.M. & GRABER, S.G. (1997). Human 5-HT$_1$ receptor subtypes exhibit distinct G protein coupling behaviors in membranes from Sf9 cells. *Biochemistry,* **36,** 12930–12938.

COLINO, A. & HALLIWELL, J.V. (1987). Differential modulation of three separate K-conductances in hippocampal CA1 neurons by serotonin. *Nature,* **328,** 73–77.

COWEN, D.S., MOLINOFF, P.B. & MANNING, D.R. (1997). 5-hydroxytryptamine$_{1A}$ receptor-mediated increases in receptor expression and activation of NF-KB in transfected Chinese hamster ovary cells. *Mol. Pharmacol.,* **42,** 221–226.

COWEN, D.S., SOWERS, R.S. & MANNING, D.R. (1996). Activation of a mitogen-activated protein kinase (ERK2) by the 5-hydroxytryptamine$_{1A}$ receptor is sensitive not only to inhibitors of phosphatidylinositol 3-kinase, but to an inhibitor of phosphatidylcholine hydrolysis. *J. Biol. Chem.,* **271,** 22297–22300.

DANIEL-ISSAKANI, S., SPIEGEL, A.M. & STRULOVICI, B. (1989). Lipopolysaccharide response is linked to GTP binding protein, Gi_2, in the promonocytic cell line U937. *J. Biol. Chem.,* **264,** 20240–20247.

DASCAL, N., LIM, N.F., SCHREIBMAYER, W., WANG, W., DAVIDSON, N. & LESTER, H.A. (1993). Expression of an atrial G protein-activated potassium channel in *Xenopus* oocytes. *Proc. Natl. Acad. Sci. U.S.A.,* **90,** 6596–6600.

DELLA ROCCA, G.J., MUKHIN, Y.V., GARNOVSKAYA, M.N., DAAKA, Y., CLARK, G., LUTTRELL, L.M., LEFKOWITZ, R.J. & RAYMOND, J.R. (1999). Serotonin 5-HT$_{1A}$ receptor-mediated MAP kinase activation requires calcium/calmodulin-mediated receptor endocytosis. *J. Biol. Chem.,* **274,** 4749–4753.

DE VIVO, M. & MAAYANI, S. (1986). Characterization of 5-HT$_{1A}$ receptor-mediated inhibition of forskolin-stimulated adenylate cyclase activity in guinea pig and rat hippocampal membranes. *J. Pharmacol. Exp. Ther.,* **238,** 248–253.

DOHLMAN, H.G., CARON, M.G., DEBLASI, A., FRIELLE, T. & LEFKOWITZ, R.J. (1990). A role of extracellular disulfided bonded cysteines in the ligand binding function of the β_2 adrenergic receptor. *Biochemistry,* **29,** 2335–2342.

DOUPNIK, C.A., DAVIDSON, N., LESTER, H.A. & KOFUJI, P. (1997). RGS proteins reconstitute the rapid gating kinetics of G$\beta\gamma$-activated inwardly rectifying K^+ channels. *Proc. Natl. Acad. Sci. U.S.A.,* **94,** 10461–10466.

DOUPNIK, C.A., DESSAUER, C.W., SLEPAK, V.Z., GILMAN, A.G., DAVIDSON, N. & LESTER, H.A. (1996). Time resolved kinetics of direct G $\beta_1/\gamma 2$ interactions with the carboxyl terminus of Kir3.4 inward rectifier K^+ channel subunits. *Neuropharmacology,* **35,** 923–931.

DRUMMOND, A.H. (1985). Bidirectional control of cytosolic free calcium by thyrotropin-releasing hormone in pituitary cells. *Nature,* **315,** 752–755.

DUNLOP, J., ZHANG, Y., SMITH, D.L. & SCHECHTER, L.E. (1998). Characterization of 5-HT1A receptor functional coupling in cells expressing the human 5-HT1A receptor as assessed with the cytosensor microphysiometer. *J. Pharmacol. Toxicol. Methods,* **40,** 47–55.

EDAGAWA, Y., SAITO, H. & ABE, K. (1998). 5-HT1A receptor-mediated inhibition of long-term potentiation in rat visual cortex. *Eur. J. Pharmacol.,* **349,** 221–224.

EMERIT, M.B., MIQUEL, M.C., GOZLAN, H. & HAMON, M. (1991). The GTP-insensitive component of high affinity [^{3}H]-8-hydroxy-2(di-*n*-propylamino)tetralin binding in the rat hippocampus corresponds to an oxidized state of the 5-HT$_{1A}$ receptor. *J. Neurochem.,* **56,** 1705–1716.

ERDMANN, J., SHIMRON, ABARBANELL, D., CICHON, S., ALBUS, M., MAIER, W., LICHTERMAN, D., MINGES, J., REUNER, U., FRANZEK, E., ERTL, M.A., HEBEBRAND, J., REMSCHMIDT, H., LEHMKUHL, G., POUSTKA, F., SCHMIDT, M., FIMMERS, R., KÖRNER, J., RIETSCHEL, M., PROPPING, P. & NÖTHEN, M.M. (1995). Systematic screening for mutations in the promoter and coding regions of the 5-HT$_{1A}$ gene. *Am. J. Med. Genetics,* **60,** 393–399.

FARGIN, A., RAYMOND, J.R., LOHSE, M., KOBILKA, B.K., CARON, M.G. & LEFKOWITZ, R.J. (1988). A genomic clone which resembles a β-adrenergic receptor sequence encodes the 5-HT$_{1A}$ receptor. *Nature,* **335,** 358–360.

FARGIN, A., RAYMOND, J.R., REGAN, J.W., COTECCHIA, S., LEFKOWITZ, R.J. & CARON, M.G. (1989). Effector coupling mechanisms of the cloned 5-HT$_{1A}$ receptor. *J. Biol. Chem.,* **264,** 14848–14852.

FARGIN, A., YAMAMOTO, K., COTECCHIA, S., GOLDSMITH, P.K., SPIEGEL, A.M., LAPETINA, E.G., CARON, M.G. & LEFKOWITZ, R.J. (1991). Dual coupling of the cloned 5-HT$_{1A}$ receptor to both adenylyl cyclase and phospholipase C is mediated via the same G_i protein. *Cell Signal.,* **3,** 547–557.

FENRICK, R., POU, C., BELIVEAU, M. & FARGIN, A. (1996). The human 5-hydroxytryptamine 1A receptor differentially modulates phospholipase C and adenylyl cyclase activities. *Gen. Pharmacol.,* **27,** 263–268.

FLETCHER, A., FORSTER, E.A., BILL, D.J., BROWN, G., CLIFFE, I.A., HARTLEY, J.E., JONES, D.E., MCLENACHAN, A., STANHOPE, K.J., CRITCHLEY, D.J., CHILDS, K.J., MIDDLEFELL, V.C., LANFUMEY, L., CORRADETTI, R., LAPORTE, A.M., GOZLAN, H., HAMON, M. & DOURISH, C.T. (1996). Electrophysiological, biochemical, neurohormonal, and behavioral studies with WAY-100635, a potent, selective, and silent 5-HT$_{1A}$ receptor antagonist. *Behav. Brain. Res.,* **73,** 337–353.

FRASER, C.M. (1989). Site-directed mutagenesis of β-adrenergic receptors. Identification of conserved cysteine residues that independently affect ligand binding and receptor activation. *J. Biol. Chem.,* **264,** 9266–9270.

FUJIWARA, Y., NELSON, D.L., KASHIHARA, K., VARGA, E., ROESKE, W.R. & YAMAMURA, H.I. (1990). The cloning and sequence analysis of the rat serotonin-1A receptor gene. *Life Sci.,* **47,** 949–958.

FUJIWARA, Y., TOMITA, H., HIKIJI, M., KASHIHARA, K., OTSUKI, S., OHNUKI, T., HAMAGISHI, Y., OKI, T., SORA, I., ROESKE, W.R. & YAMAMURA, H.I. (1993). Characterization of a cloned rat serotonin 5-HT$_{1A}$ receptor expressed in the HeLa cell line. *Life Sci.,* **52,** PL127–PL132.

GADDUM, H. & PICARELLI, Z.P. (1957). Two kinds of tryptamine receptor. *Br. J. Pharmacol. Chemother.,* **12,** 323–328.

GARNOVSKAYA, M.M., GETTYS, T.W., VAN BIESEN, T., CHUPRUN, J.K., PRPIC, V. & RAYMOND, J.R. (1997). G protein-coupled 5-HT$_{1A}$ receptor activates Na^+/H^+ exchange in CHO-K1 cells through $G_{i\alpha 2}$ and $G_{i\alpha 3}$. *J. Biol. Chem.,* **272,** 7770–7776.

GARNOVSKAYA, M.N., MUKHIN, Y.V. & RAYMOND, J.R. (1998). Rapid activation of sodium-proton exchange and extracellular signal-regulated protein kinase in fibroblasts by G protein-coupled 5-HT$_{1A}$ receptor involves distinct signalling cascades. *Biochem. J.,* **330,** 489–495.

GARNOVSKAYA, M.N., VAN BIESEN, T., HAWES, B., RAMOS, S.C., LEFKOWITZ, R.J. & RAYMOND, J.R. (1996). Ras-dependent activation of fibroblast mitogen-activated protein kinase by 5-HT$_{1A}$ receptor *via* a G protein $\beta\gamma$-subunit-initiated pathway. *Biochemistry,* **35,** 13716–13722.

GETTYS, T.W., FIELDS, T.A. & RAYMOND, J.R. (1994). Selective activation of inhibitory G-protein α-subunits by partial agonists of the human 5-HT$_{1A}$ receptor. *Biochemistry,* **33,** 4283–4290.

GILBERT, F., DOURISH, C.T., BRAZELL, C., MCCLUE, S. & STAHL, S.M. (1988). Relationship of increased food intake and plasma ACTH levels to 5-HT$_{1A}$ receptor activation in rats. *Psychoneuroendocrinology,* **13,** 471–478.

GOZLAN, H., EMERIT, M.B., EL MESTIKAWY, S., COSSERY, J.M., MARQUET, A., BESSELEVIÈVRE, A. & HAMON, M. (1988). Photoaffinity labeling and solubilization of the central 5-HT$_{1A}$ receptor binding site. *J. Receptor Res.,* **7,** 195–221.

GUAN, X.M., PEROUTKA, S.J. & KOBILKA, B.K. (1992). Identification of a single amino acid residue responsible for the binding of a class of β-adrenergic receptor antagonists to 5-hydroxytryptamine$_{1A}$ receptors. *Mol. Pharmacol.,* **41,** 695–698.

HARADA, S., OKUBO, T., TSUTUMI, M., TAKASE, S. & MURAMATSU, T. (1996). Investigation of genetic risk factors associated with alcoholism. *Alcohol Clin. Exp. Res.,* **20** (suppl. 9), 293A–296A.

HARRINGTON, M.A., SHAW, K., ZHONG, P. & CIARENAELLO, R.D. (1994). Agonist-induced desensitization and loss of high affinity binding sites of stably expressed human 5-HT$_{1A}$ receptors. *J. Pharmacol. Expt. Ther.,* **268,** 1098–1106.

HENSLER, J.G., CERBERA, L.S., MILLER, H.A. & CORBITT, J. (1996). Expression and modulation of 5-hydroxytryptamine$_{1A}$ receptors in P11 cells. *J. Pharmacol. Expt. Ther.,* **278,** 1138–1145.

HO, B.Y., KARSCHIN, A., BRANCHEK, T., DAVIDSON, N. & LESTER, H.A. (1992b). The role of conserved aspartate and serine residues in ligand binding and in function of the 5-HT$_{1A}$ receptor: a site-directed mutagenesis study. *FEBS Lett.,* **312,**

HO, B.Y., KARSCHIN, A., RAYMOND, J.R., BRANCHEK, T., LESTER, H.A. & DAVIDSON, N. (1992a). Expression in animal cells of the 5-HT$_{1A}$ receptor by a vaccinia virus vector system. *FEBS Lett.,* **301,** 303–306.

HURLEY, J.H., (1999). Structure, mechanism, and regulation of mammalian adenylyl cyclase. *J. Biol. Chem.,* **274,** 7599–7602.

IKEN, K., CHENG, S., FARGIN, A., GOULET, A.C. & KOUASSI, E. (1995). Serotonin upregulates mitogen-stimulated B lymphocyte proliferation through 5-HT$_{1A}$ receptors. *Cell. Immunol.,* **163,** 1–9.

ISHIZUKA, J., BEAUCHAMP, R.D., TOWNSEND, C.M., GREELEY, G.H. & THOMPSON, J.C. (1992). Receptor-mediated autocrine growth-stimulatory effect of 5-hydroxytryptamine on cultured human pancreatic carcinoid cells. *J. Cell. Physiol.,* **150,** 1–7.

KARSCHIN, A., HO, B.Y., LABARCA, C., ELROY-STEIN, O., MOSS, B., DAVIDSON, N. & LESTER, H.A. (1991). Heterologously expressed serotonin 1A receptors couple to muscarinic K^+ channels in heart. *Proc. Natl. Acad. Sci. U.S.A.,* **88,** 5694–5698.

KAWANISHI, Y., HARADA, S., TACHIKAWA, H., OKUBO, T. & SHIRAISHI, H. (1998). Novel mutations in the promoter and coding region of the human 5-HT1A receptor gene and association analysis in schizophrenia. *Am. J. Med. Genetics.,* **81,** 434–439.

KENAKIN, T.P. & MORGAN, P.H. (1988). Theoretical effects of single and multiple transducer receptor coupling proteins on estimates of the relative potency of agonists. *Mol. Pharmacol.,* **35,** 214–222.

KOBILKA, B.K., FREILLE, T., COLLINS, S., YANG-FENG, T., KOBILKA, T.S., FRANCKE, U., LEFKOWITZ, R.J. & CARON, M.G. (1987). An intronless gene encoding a potential member of the family of receptors coupled to guanine nucleotide regulatory proteins. *Nature,* **329,** 75–79.

KONINGS, P.N.M., SHAHID, M., VAN ALEBECK, C., MAKKINK, W.K., STAM, N.J., RUIGT, G.S.F. & VANDERHEYDEN, P.M.L. (1995). Combined is situ hybridization, Northern blot analysis, and receptor binding studies in clones expressing different levels of the human 5-HT1A receptor. *J. Receptor Signal. Transduction Res.,* **15,** 443–455.

KUIPERS, W., LINK, R., STANDAAR, P.J., STOIT, A.R., VAN WIJNGAARDEN, I., LEURS, R. & IJZERMAN, A.P. (1997). Study of the interaction between aryloxypropanolamines and Asn386 in helix VII of the human 5-hydroxytryptamine$_{1A}$ receptor. *Mol. Pharmacol.,* **51,** 889–896.

KUIPERS, W., VAN WIJNGAARDEN, I. & IJZERMAN, A.P. (1994). A model of the serotonin 5-HT$_{1A}$ receptor: agonist and antagonist binding sites. *Drug. Design Discovery,* **11,** 231–249.

LAM, S., SHEN, Y., NGUYEN, T., MESSIER, T., BRANN, M., COMINGS, D., GEORGE, S.B. & O'DOWD, B. (1996). A serotonin receptor gene (5-HT$_{1A}$) variant found in a Tourette's syndrome patient. *Biochem. Biophys. Res. Commun.,* **219,** 853–858.

LANGLOIS, X., EL MESTIKAWY, S., ARPIN, M., TRILLER, A., HAMON, M & DARMON, M. (1996). Differential addressing of 5-HT$_{1A}$ and 5-HT$_{1B}$ receptors in transfected LLC-PK1 epithelial cells: a model of receptor targeting in neurons. *Neuroscience,* **74,** 297–302.

LAUDER, J.M. (1993). Neurotransmitters as growth regulatory signals: roles of receptors and second messengers. *Trends Pharmacol. Sci.,* **16,** 233–239.

LEMBO, P.M. & ALBERT, P.R. (1995). Multiple phosphorylation sites are required for pathway-selective uncoupling of the 5-hydroxytrypatamine$_{1A}$ receptor by protein kinase C. *Mol. Pharmacol.,* **48,** 1024–1029.

LEMBO, P.M., GHAHREMANI, M.H., MORRIS, S.J. & ALBERT, P.R. (1997). A conserved threonine residue in the second intracellular loop of the 5-hydroxytryptamine$_{1A}$ receptor directs signaling specificity. *Mol. Pharmacol.,* **52,** 164–171.

LEONE, M., ATTANASIO, A., CROCI, D., FERRARIS, A., D'AMICO, D., GRAZZI, L., NESPOLO, A. & BUSSONE, G. (1998). 5-HT1A receptor hypersensitivity in migraine is suggested by the m-chlorphenylpiperazine test. *Neuroreport,* **9,** 2605–2608.

LIU, Y.F. & ALBERT, P.R. (1991). Cell-specific signaling of the 5-HT$_{1A}$ receptor. Modulation by protein kinases C and A. *J. Biol. Chem.,* **266,** 23689–23697.

LIU, Y.F., JAKOBS, K.H., RASENICK, M.M. & ALBERT, P.R. (1994). G protein specificity in receptor-effector coupling. Analysis of the roles of G_o and G_{i2} in GH_4C_1 pituitary cells. *J. Biol. Chem.,* **269,** 13880–13886.

LIMBIRD, L.E. (1988). Receptors linked to inhibition of adenylate cyclase: additional signaling mechanisms. *FASEB J.,* **2,** 2686–2695.

LUTTRELL, L.M., VAN BIESEN, T., HAWES, B.E., KOCH, W.J., KRUEGER, K.M., TOUHARA, K. & LEFKOWITZ, R.J. (1997). G-protein-coupled receptors and their regulation: activation of the MAP kinase signaling pathway by G-protein-coupled receptors. *Adv. Second Messenger Phosphoprotein Res.,* **31,** 263–277.

MANGEL, A.W., RAYMOND, J.R. & FITZ, J.G. (1993). Regulation of high-conductance anion channels by G proteins and 5-HT$_{1A}$ receptors in CHO cells. *Am. J. Physiol.,* **264,** F490–F495.

MARRACCI, S., CINI, D. & NARDI, I. (1997). Cloning and developmental expression of 5-HT1A receptor gene in *Xenopus laevis*. *Brain Mol. Brain. Res.,* **47,** 67–77.

MARSHALL, C.J. (1995). Specificity of receptor tyrosine kinase signaling: transient versus sustained extracellular signal-regulated kinase activation. *Cell,* **80,** 179–185.

MASWOOD, N., CALDAROLA-PASTUSZKA, M. & UPHOUSE, L. (1998). Functional integration among 5-hydroxytryptamine receptor families in the control of female rat sexual behavior. *Brain Res.,* **802,** 98–103.

MARKSTEIN, R., HOYER, D. & ENGEL, G. (1986). 5-HT$_{1A}$ receptors mediate stimulation of adenylate cyclase in rat hippocampus. *Naunyn-Schmiedeberg's Arch. Pharmacol.,* **333,** 335–341.

MESSIER, T.L., DORMAN, C.M., BRAUNER-OSBORNE, H., EUBANKS, D. & BRANN, M.R. (1995). High throughput assays of cloned adrenergic, muscarinic, neurokinin, and neurotrophin receptors in living mammalian cells. *Pharmacol. Toxicol.,* **76,** 308–311.

MICZEK, K.A., HUSSAIN, S. & FACCIDOMO, S. (1998). Alcohol-heightened aggression in mice: attenuation by 5-HT1A receptor agonists. *Psychopharmacology,* **139,** 160–168.

MIDDLETON, J.P., RAYMOND, J.R., WHORTON, A.R. & DENNIS, V.W. (1990). Short-term regulation of Na^+/K^+ adenosine triphosphatase by recombinant human serotonin 5-HT$_{1A}$ receptor expressed in HeLa cells. *J. Clin. Invest.,* **86,** 1799–1805.

MOISEWITSCH, J.R., RAYMOND, J.R., TAMIR, H. & LAUDER, J.M. (1998). Regulation by serotonin of tooth-germ morphogenesis and gene expression in mouse mandibular explant cultures. *Arch. Oral Biol.,* **43,** 789–800.

MONS, N., DECORTE, L., JAFFARD, R. & COOPER, D.M. (1998). Ca^{2+}-sensitive adenylyl cyclases, key regulators of cellular signalling. *Life Sci.,* **62,** 1647–1652.

MULHERON, J.G., CASAÑAS, S.J., ARTHUR, J.M., GARNOVSKAYA, M.N., GETTYS, T.W. & RAYMOND, J.R. (1994). Human 5-HT$_{1A}$ receptor expressed in Sf9 insect cells couples to endogenous G_o-like G proteins. *J. Biol. Chem.,* **269,** 12954–12962.

NAKHAI, B., NIELSEN, D.A., LINNOILA, M. & GOLDMAN, D. (1995). Two naturally occurring amino acid substitutions in the human 5-HT1A receptor: glycine 22 to serine 22 and isoleucine 28 to valine 28. *Biochem. Biophys. Res. Commun.,* **210,** 530–536.

NEBIGIL, C.G., GARNOVSKAYA, M.N., CASAÑAS, S.J., MULHERON, J.G., PARKER, E.M., GETTYS, T.W., RAYMOND, J.R. (1995). Agonist-induced phosphorylation and desensitization of 5-HT$_{1A}$ receptor expressed in Sf9 insect cells. *Biochemistry,* **34,** 11954–11962.

NEWMAN-TANCREDI, A., CHAPUT, C., VERRIELE, L. & MILLAN, M.J. (1996). S 15535 and WAY 100,635 antagonise 5-HT-stimulated [^{35}S]-GTPγS binding at cloned 5-HT$_{1A}$. *Eur. J. Pharmacol.,* **307,** 107–111.

NEWMAN-TANCREDI, A., CONTE, C., CHAPUT, C., VERRIELE, L. & MILLAN, M.J. (1997). Agonist and inverse agonist efficacy at human recombinant serotonin 5-HT$_{1A}$ receptors as a function of receptor:G-protein stoichiometry. *Neuropharmacology,* **36,** 451–459.

NEWMAN-TANCREDI, A., GAVAUDAN, S., CONTE, C., CHAPUT, C., TOUZARD, M., VERRIELE, L., AUDINOT, V. & MILLAN, M.J. (1998). Agonist and antagonist actions of antipsychotic agents at 5-HT$_{1A}$ receptors: a [^{35}S]GTPγS binding study. *Eur. J. Pharmacol.,* **355,** 245–256.

NEWMAN-TANCREDI, A., WOOTTON, R. & STRANGE, P.G. (1992). High-level stable expression of recombinant 5-HT$_{1A}$; 5-hydroxytryptaminereceptors in Chinese hamster ovary cells. *Biochem. J.,* **285,** 933–938.

NI, Y.G., PANICKER, M.M. & MILEDI, R. (1997). Efficient coupling of 5-HT$_{1A}$ receptors to phospholipase C pathway in *Xenopus* oocytes. *Mol. Brain. Res.,* **51,** 115–122.

PARKS, C.L., ROBINSON, P.S., SIBILLE, E., SHENK, T. & TOTH, M. (1998). Increased anxiety of mice lacking the serotonin$_{1A}$ receptor. *Proc. Natl. Acad. Sci. U.S.A.,* **95,** 10734–10739.

PAUWELLS, P.J., TARDIF, S., WURCH, T. & COLPAERT, F.C. (1997). Stimulated [^{35}S]GTPγS binding by 5-HT$_{1A}$ receptor agonists in recombinant cell lines. Modulation of apparent efficacy by G protein activation state. *Naunyn-Schmiedeberg's Arch. Pharmacol.,* **356,** 551–561.

PAUWELS, P.J., VAN GOMPEL, P. & LEYSEN, J.E. (1993). Activity of serotonin (5-HT) receptor agonists, partial agonists and antagonists at cloned 5-HT$_{1A}$ receptors that are negatively coupled to adenylate cyclase in permanently transfected HeLa cells. *Biochem. Pharmacol.,* **45,** 375–382.

PEDIGO, N.W., YAMAMURA, H.I. & NELSON, D. (1981). Discrimination of multiple [^{3}H]5-hydroxytryptamine binding sites by the neuroleptic in spiperone in rat brain. *J. Neurochem.,* **36,** 220–226.

PENNINGTON, N.J. & KELLY, J.S. (1990). Serotonin receptor activation reduces calcium current in an acutely dissociated adult central neuron. *Neuron,* **4,** 751–758.

POU, C., NENONENE, E.K., READER, T.A. & FARGIN, A. (1997). The human 5-HT$_{1A}$ receptor: comparison of its binding properties in transfected cells and cortical tissue. *Gen. Pharmacol.,* **29,** 737–747.

RAMBOZ, S., OOSTING, R., AMARA, D.A., KUNG, H.F., BLIER, P., MENDELSOHN, M., MANN, J.J., BRUNNER, D. & HEN, R. (1998). Serotonin receptor 1A knockout: An animal model of anxiety-related disorder. *Proc. Natl. Acad. Sci. U.S.A.,* **95,** 14476–14481.

RAPPORT, M.M., GREEN, A.A. & PAGE, I.H. (1948). Crystalline serotonin. *Science,* **108,** 329–330.

RAYMOND, J.R. (1991). Protein kinase C induces phosphorylation and desensitization of the human 5-HT$_{1A}$ receptor. *J. Biol. Chem.,* **266,** 14747 – 14753.

RAYMOND, J.R., ALBERS, F.J. & MIDDLETON, J.P. (1992). Functional expression of human 5-HT$_{1A}$ receptors and differential coupling in CHO cells. *Naunyn-Schmied. Arch. Pharmacol.,* **346,** 127 – 137.

RAYMOND, J.R., ALBERS, F.J., MIDDLETON, J.P., LEFKOWITZ, R.J., CARON, M.G. & DENNIS, V.W. (1990). 5-HT$_{1A}$ and histamine H$_1$ receptors in HeLa cells stimulate phosphoinositide hydrolysis and phosphate uptake *via* distinct G protein pools. *J. Biol. Chem.,* **266,** 372 – 379.

RAYMOND, J.R., FARGIN, A., MIDDLETON, J.P., GRAFF, J.M., HAUPT, D.M., CARON, M.G., LEFKOWITZ, R.J. & DENNIS, V.W. (1989). The human 5-HT$_{1A}$ receptor expressed in HeLa cells stimulates sodium-dependent phosphate uptake *via* protein kinase C. *J. Biol. Chem.,* **264,** 21943 – 21950.

RAYMOND, J.R., KIM, J.L., BEACH, R.E. & TISHER, C.C. (1993a). Immunohistochemical mapping of cellular and subcellular distribution of 5-HT$_{1A}$ receptors in rat and human kidney. *Am. J. Physiol.,* **264,** F9 – F19.

RAYMOND, J.R. & OLSEN, C.L. (1994). Protein kinase A induces phosphorylation of the human 5-HT$_{1A}$ receptor and augments its desensitization by protein kinase C in CHO cells. *Biochemistry,* **33,** 11264 – 11269.

RAYMOND, J.R., OLSEN, C.L. & GETTYS, T.W. (1993b). Cell-specific physical and functional coupling of human 5-HT$_{1A}$ receptors to inhibitory G protein α-subunits and lack of coupling to G$_{sz}$. *Biochemistry,* **32,** 11064 – 11073.

RAZANI, H., HEDLUND, P.B., HANSSON, A.C., FUXE, K. & OGREN, S.O. (1997). 5-HT$_{1A}$ receptor activation: short-term effects on the mRNA expression of the 5-HT$_{1A}$ receptor and galanin in the raphe nuclei. *Neuroreport,* **8,** 3565 – 3570.

ROUSSELLE, J.C., PLANTEFOL, M., FILLION, M.P., MASSOT, O., PAUWELS, P.J. & FILLION, G. (1998). Specific interaction of 5-HT-moduline with human 5-HT1b as well as 5-HT1d receptors expressed in transfected cultured cells. **358,** 279 – 286.

ROUTLEDGE, C. (1996). Development of 5-HT$_{1A}$ receptor antagonists. *Behav. Brain Res.,* **73,** 153 – 156.

RYU, S.H., KIM, U., WAHL, M.I., BROWN, A.B., CARPENTER, G., HUANG, K. & RHEE, S.G. (1990). Feedback regulation of PLC-b by protein kinase C. *J. Biol. Chem.,* **265,** 17941 – 17945.

SCHOEFFTER, P., BOBIRNAC, I., BODDEKE, E. & HOYER, D. (1997). Inhibition of cAMP accumulation *via* recombinant human serotonin 5-HT$_{1A}$ receptors: considerations on receptor coupling across systems. *Neuropharmacology,* **36,** 429 – 437.

SELETTI, B., BENKELFAT, C., BLIER, P., ANNABLE, L., GILBERT, F. & DE MONTIGNY, C. (1995). Serotonin$_{1A}$ receptor activation by flesinoxan in humans. Body temperature and neuroendocrine responses. *Neuropsychopharmacology,* **13,** 93 – 104.

SEUWEN, K., MAGNALDO, I. & POUYSSÉGUR, J. (1988). Serotonin stimulates DNA synthesis in fibroblasts acting through 5-HT$_{1B}$ receptors coupled to a G$_i$ protein. *Nature,* **335,** 254 – 256.

SHENKER, A., MAAYANI, S., WEINSTEIN, H. & GREEN, J.P. (1985). Two 5-HT receptors linked to adenylate cyclase in guinea pig hippocampus are discriminated by 5-carboxamidotryptamine and spiperone. *Eur. J. Pharmacol.,* **109,** 427 – 429.

SHIAH, I.S., YATHAM, L.N., LAM, R.W., TAM, E.M. & ZIS, A.P. (1998). Cortisol, hypothermic and behavioral responses to ipsapirone in patients with bipolar depression and normal controls. *Neuropsychobiology,* **38,** 6 – 12.

SINGH, J.K., CHROMY, B.A., BOYERS, M.J., DAWSON, G. & BANERJEE, P. (1996b). Induction of the serotonin$_{1A}$ receptor in neuronal cells during prolonged stress and degeneration. *J. Neurochem.,* **66,** 2361 – 2372.

SINGH, J.K., YAN, Q., DAWSON, G. & BANERJEE, P. (1996a). Cell-specific regulation of the stably expressed serotonin 5-HT$_{1A}$ receptor and altered ganglioside synthesis. *Biochim. Biophys. Acta.,* **1310,** 201 – 211.

STAM, N.J., VAN HUIZEN, F., VAN ALEBEEK, C., BRAND, S.J., DIJKEMA, R., TONNAER, J.A. & OLIJVE, W. (1992). Genomic organization coding sequence and functional expression of human 5-HT$_2$ and 5-HT$_{1A}$ receptor genes. *Eur. J. Pharmacol.,* **227,** 153 – 162.

STRADER, C.D., CANDELORE, M.R., HILL, W.S., SIGAL, I.S. & DIXON, R.A.F. (1989). Identification of two serine residues involved in agonist activation of the β-adrenergic receptor. *J. Biol. Chem.,* **264,** 13572 – 13578.

STRASSHEIM, D. & MALBON, C.C. (1994). Phosphorylation of G$_{i2}$ attenuates inhibitory adenylyl cyclase in neuroblastoma/glioma (NG108-15) cells. *J. Biol. Chem.,* **269,** 14307 – 14313.

SUNDARAM, H., NEWMAN-TANCREDI, A. & STRANGE, P.G. (1992). Pharmacological characterisation of the 5-HT$_{1A}$ receptor using the agonist [^{3}H]8-OH-DPAT, and the antagonist [^{3}H]spiperone. *Biochem. Soc. Trans.,* **20,** 145S.

SUNDARAM, H., TURNER, J.D. & STRANGE, P.G. (1995). Characterisation of recombinant 5-HT$_{1A}$ receptors expressed in Chinese hamster ovary cells: the agonist [^{3}H]lisuride labels free receptor and receptor coupled to G protein. *J. Neurochem.,* **65,** 1909 – 1906.

TAUSSIG, R. & ZIMMERMAN, G. (1998). Type-specific regulation of mammalian adenylyl cyclases by G protein pathways. *Adv. Second Messenger Phosphorortien Res.,* **32,** 81 – 98.

UEZONO, Y., BRADLEY, J., MIN, C., MCCARTY, N.A., QUICK, M., RIORDAN, J.R., CHAVKIN, C., ZINN, K., LESTER, H.A. & DAVIDSON, N. (1993). Receptors that couple to 2 classes of G proteins increase cAMP and activate CFTR expressed in *Xenopus oocytes. Receptors Channels,* **1,** 233 – 241.

VAN HUIZEN, F., BRANSEE, M. & STAM, N.J. (1993). Agonist-induced down-regulation of human 5-HT$_{1A}$ and 5-HT$_2$ receptors in Swiss 3T3 cells. *Neuroreport,* **4,** 1327 – 1330.

VARRAULT, A. & BOCKAERT, J. (1992). Differential coupling of 5-HT$_{1A}$ receptors occupied by 5-HT or 8-OH-DPAT to adenylyl cyclase. *Naunyn Schmiedebergs Arch. Pharmacol.,* **346,** 367 – 374.

VARRAULT, A., BOCKAERT, J. & WAEBER, C. (1992a). Activation of 5-HT$_{1A}$ receptors expressed in NIH-3T3 cells induces focus formation and potentiates EGF effect on DNA synthesis. *Mol. Biol. Cell,* **9,** 961 – 969.

VARRAULT, A., JOURNOT, L., AUDIGIER, Y. & BOCKAERT, J. (1992b). Transfection of human 5-hydroxytryptamine$_{1A}$ receptors in NIH-3T3 fibroblasts: effects of increasing receptor density on the coupling of 5-hydroxytryptamine$_{1A}$ receptors to adenylyl cyclase. *Mol. Pharmacol.,* **41,** 999 – 1007.

VARRAULT, A., NGUYEN, D.L., MCCLUE, S., HARRIS, B., JOUIN, P. & BOCKAERT, J. (1994). 5-HT$_{1A}$ receptor synthetic peptides. Mechanisms of adenylyl cyclase inhibition. *J. Biol. Chem.,* **269,** 367 – 374.

WANG, S.J., CHENG, L.L. & GEAN, P.W. (1999). Cross-modulation of synaptic plasticity by β-adrenergic and 5-HT1A receptors in the rat basolateral amygdala. *J. Neurosci.,* **19,** 570 – 577.

WEISS, S., SEBBEN, M., KEMP, D.E. & BOCKAERT, J. (1986). Serotonin 5-HT$_1$ receptors mediate stimulation of cyclic AMP production in neurons. *Eur. J. Pharmacol.,* **120,** 227 – 230.

WHITAKER-AZMITIA, P.M. (1991). Role of serotonin and other neurotransmitter receptors in brain development. Basis for developmental pharmacology. *Pharmacol. Rev.,* **43,** 347 – 352.

WONG, Y.H., CONKLIN, B.R. & BOURNE, H.R. (1992). G$_2$-mediated hormonal inhibition of cyclic AMP accumulation. *Science,* **255,** 339 – 342.

YAMADA, J., SUGIMOTO, Y. & YOSHIKAWA, T. (1998). Effects of adrenalectomy on hyperphagia-induced by the 5-HT1A receptor agonists 8-OH-DPAT and 2-deoxy-D-glucose in rats. *Neuroreport,* **9,** 1831 – 1833.

YAMAGUCHI, F. & BRENNER, S. (1997). Molecular cloning of 5-hydroxytryptamine (5-HT) type 1 receptor genes from the Japanese puffer fish, *Fugu rubripes. Gene,* **191,** 219 – 223.

YATOMI, Y., ARATA, Y., TADA, S., KUME, S. & UI, M. (1992). Phosphorylation of the inhibitory-guanine-nucleotide-binding protein as a possible mechanism of inhibition by protein kinase C of agonist-induced Ca^{2+}-mobilization in human platelet. *Eur. J. Biochem.,* **205,** 1003 – 1009.

ZGOMBICK, J.M., BECK, S.G., MAHLE, C.D., CRADDOCK-ROYAL, B. & MAYAANI, S. (1989). Pertussis toxin-sensitive guanine nucleotide-binding protein(s) couple adenosine A$_1$ and 5-hydroxytryptamine$_{1A}$ receptors to the same effector systems in rat hippocampus. Biochemical and physiological studies. *Mol. Pharmacol.,* **35,** 484 – 494.

(Received October 5, 1998
Revised April 23, 1999
Accepted May 17, 1999)