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Overview

The 5-HT_{1A} Receptor : An Overview of Recent Advances

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Progress in the field of neuronal receptor research has accelerated during the last few years due to developments in pharmacology and molecular biology. This is particularly true in the case of the serotonin 5-HT_{1A} receptor. In 1983 the very selective, high affinity 5-HT_{1A} agonist 8-OH-DPAT was developed which allowed the pharmacology and distribution of the 5-HT_{1A} receptor in the central nervous system of the rat and man to be extensively characterized. By 1987, the gene encoding this receptor protein was cloned and sequenced, allowing not only elucidation of its structure, but also better insight into the nature of its coupling to transmembrane signal transduction systems. Thus in a short period of time considerable knowledge has accumulated on how serotonin exerts its functions in the central nervous system via the 5-HT_{1A} receptor. In the present review we will briefly discuss some of the latest developments regarding the 5-HT_{1A} receptor.

KEY WORDS: Serotonin; 5-HT; 5-HT receptor subtype; 5-HT_1 receptor; 8-OH-DPAT; receptor purification; receptor cloning; antipeptide antibodies.

INTRODUCTION

When Gaddum and Picarelli first described serotonin (5-hydroxytryptamine, 5-HT) receptors more than 30 years ago, they initially characterized two subtypes (29). Currently, four types and no less than ten subtypes of 5-HT receptors have been described (for review see ref. 7, 18). Type 1 receptors (5-HT_1R) have a high affinity for 5-HT, whereas types 2, 3 and 4 have a moderate to low affinity for the monoamine. The situation is even more complex within the first group, where five subtypes have been defined (5-HT_{1A-E}, for review, see ref. 38 and 46).

The 5-HT_{1A} receptor has been the subject of intense interest over the last decade for three reasons: i) a very specific, high affinity radioligand (^{3}H-8-OH-DPAT) has been available since 1983 to label these binding sites, ii) several new clinically relevant anxiolytics (buspirone, ipsapirone and gepirone) have been developed which have high affinity ligands for the 5-HT_{1A} receptor, and iii) the gene encoding the 5-HT_{1A} receptor has been cloned and sequenced (31,42,69).

Recently, many new ligands for serotonin binding sites have been developed and in particular three which exhibit high affinity for the 5-HT_{1A} receptor. Gepirone, ipsapirone and buspirone appear to represent a promising new family of non-benzodiazepine, antidepressant/anxiolytic drugs, as evidenced by the recent marketing of Buspar© (i.e., Buspirone). However, 8-OH-DPAT remains the most important and useful 5-HT_{1A} receptor ligand. To date, this tetraline derivative is the most highly selective ligand for 5-HT_{1A} receptors. Because of this selectivity, considerable knowledge has accumulated on the clinical and behavioral effects of 5-HT_{1A} receptor stimulation (8).

The 5-HT_{1A} receptor subtype was first described in 1981 (56), and its gene was cloned and sequenced in

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1987 (42). The 5-HT_{1A} receptor was the first G protein-coupled receptor to be cloned using low-stringency cross-hybridization techniques. Seeking to clone the human β_1-adrenergic receptor (β_1-AR), Kobilka and colleagues used the full length human β_2-AR cDNA to isolate a gene clone, termed G21, from a human genomic library (15, 42). The intronless G21 clone was found to encode a putative membrane-bound protein which shared a high degree of amino acid sequence homology with the β_2-AR and other G protein-coupled receptors. However, this gene was not found to encode an adrenergic receptor. The β_1-AR was subsequently isolated in a fortuitous fashion by using the G21 DNA to probe a human placental cDNA library (27). The identity of the protein encoded by G21 remained unknown until Fargin and colleagues demonstrated specific binding of the β-AR selective antagonist [^{125}|]iodocyanopindolol to membrane from COS-7 cells transfected with G21 DNA. These cells were screened for radioligand binding with adrenergic ligands. The moderate affinity (≈10 nM) of [^{125}I]iodocyanopindolol for the G21-encoded binding site was more consistent with a 5-HT_1 receptor than with an adrenergic receptor. Subsequently, transfected COS-7 membranes were labeled with the specific 5-HT_{1A} agonist, [^{3}H]-8-OH-DPAT, thus confirming that G21 encoded the human 5-HT_{1A} receptor (25).

The 5-HT_{1A} receptor, although only very recently described, already has a rich history. This review will try to describe the most striking advances made in this field, from clinical, pharmacological, anatomical, biochemical, and molecular biological studies.

Clinical Correlates

The hypothesized role of the serotoninergic system and various 5-HT receptors in stress-induced behaviour, depression, pain, and anxiety has stimulated considerable interest. For example, brain serotonin has been implicated in the modulation of benzodiazepine action on the GABA/Cl-channel, and benzodiazepines decrease the activity of central 5-HT neurones (9). The specific involvement of the 5-HT_{1A} receptor in anxiety has been advanced by the recent introduction of a new class of non-benzodiazepine anxiolytics which act directly on serotoninergic neurones through this receptor (8, 69). These piperazine derivatives (buspirone, gepirone and ipsapirone) inhibit the firing of serotoninergic neurones from the nucleus raphe dorsalis (68) and all three compounds have nanomolar affinity for rat brain 5-HT_{1A} receptors (69). Although some of these compounds are active on other systems (i.e. dopaminergic, ref. 35), the evidence strongly suggests that 5-HT_{1A} receptors (among others) play a major role in the control of anxiety.

An important clinical issue is the extent of serotoninergic modulation of ascending nociception. Serotoninergic neurones that originate in the nucleus raphe magnus project to the superficial layer of the dorsal horn of the spinal cord and are involved in the descending control of nociception (52). Autoradiographic studies in rhizotomized or capsaicin-treated animals have demonstrated the presence of 5-HT_{1A} receptors on primary afferent fibres in the dorsal horn of the spinal cord (13) suggesting a potential role for 5-HT_{1A} receptors in pain perception. Moreover, in animal models, 5-HT_{1A} agonists such as 8-OH-DPAT and buspirone have been shown to have an analgesic effect (19). Therefore, the 5-HT_{1A} receptor provides a novel potential target for the control of nociception.

Finally it should be empahsized that 5-HT_{1A} receptors may be important in disease states or normal physiological functions, such as i) migraine, ii) modulation of the immune function, iii) regulation of sleep and iv) control of vascular tone (see ref. 8).

Pharmacology

Many radioligands have been used to label the 5-HT_{1A} receptor. These include [^{3}H]5-HT (57), [^{3}H]8-OH-DPAT (32), [^{3}H]ipsapirone (TVX Q-7821, ref. 30), [^{3}H]-WB4101 (53), [^{3}H]spiroxatrine (37), [^{3}H]1-2-(4-aminophenyl)ethyl)-4-(3-trifluoromethyl-phenyl) piperazine ([^{3}H]-PAPP) (59), [^{3}H]5-MeO-DPAC (12) and [^{125}I]-BH-8-MeO-N-PAT (33). Among these 5-HT_{1A} agonists the 8-hydroxy-aminotetraline derivatives (i.e., 8-OH-DPAT and I-BH-8-MeO-N-PAT) have proven to be the most useful due to their high affinity and selectivity for 5-HT_{1A} receptors. Other radioligands are much less specific. For example, ipsapirone and WB-4101 bind to α_1-AR, buspirone binds to D_2-dopamine receptors (D_2-DAR) and spiroxatrine binds to D_2-DAR and μ-opiate receptors (for review see ref. 47). Although 5-HT_{1A} agonist pharmacology is well characterized, selective antagonists are still lacking. This difficulty has often been circumvented by using agents such as β-adrenergic receptor blockers or the neuroleptic spiperone in lieu of a specific 5-HT_{1A} receptor antagonist (56, 67). Thus, the development of a "pure" 5-HT_{1A} antagonist remains an important future goal.

Localization

The anatomic distribution of 5-HT_{1A} receptors has been defined through a combination of autoradiography

and radioligand binding studies in control or lesioned animals. High levels of 5-HT_{1A} receptors have been consistently observed in human, mouse, rat, guinea-pig and porcine brain limbic areas (i.e., hippocampus, lateral septum, and frontal cortex), in the nucleus raphe dorsalis and in the dorsal horn of the spinal cord. No labeling has been observed in extrapyramidal structures such as the substantia nigra, the globus pallidus or the caudate nucleus, or in the cerebellum (13, 39, 40, 49, 54, 55, 70). The preferential distribution in limbic areas is consistent with involvement of 5-HT_{1A} receptors in the control of mood and anxiety.

Following injection of 5,7-dihydroxytryptamine (5,7-DHT), the destruction of serotoninergic neurones originating in the nucleus raphe dorsalis is associated with a severe (i.e., $\approx$50%) loss of 5-HT_{1A} receptors in this structure, but with no change in the hippocampus. These data suggest that 5-HT_{1A} receptors are (partly) located presynaptically on serotoninergic cell bodies and dendrites in the nucleus raphe dorsalis. In contrast, 5-HT_{1A} receptors appear to be entirely postsynaptic in the hippocampus (70, 72). In this structure, it should be noted that 5-HT_{1A} receptors hyperpolarize pyramidal cells of the CA1 field by opening K^+ channels (3, 4). Kainic acid injection into the hippocampus causes only a 50% reduction of 5-HT_{1A} binding site. Thus, half of the hippocampal 5-HT_{1A} receptors appear to be located on "non-serotoninergic" terminals, whereas the other half are apparently located on "non-serotoninergic" neuronal cell bodies (32, 34).

Although autoradiography at the light microscopic level has proved very powerful in delineating brain regions with high to moderate levels of 5-HT_{1A} receptors, more sensitive techniques are required for cellular and subcellular localization. The availability of the 5-HT_{1A} receptor amino acid sequence has allowed, i) the design of antipeptide antibodies able to specifically imunoprecipitate the receptor and immunolabel brain slices (Figure 1) (22,61), and ii) the synthesis of oligonucleotide probes for in situ hybridization visualization. In the near future, the conjunction of both techniques will ultimately permit the ultrastructural localization of the receptor.

Signal Transduction

The nature of the intracellular second messenger linked to agonist occupancy of the 5-HT_{1A} receptor has been a point of considerable controversy [for review see 36 and 38]. Some investigators have reported the 5-HT_{1A} receptor to be coupled to stimulation of adenylyl cyclase (50, 66), whereas others have demonstrated an inhibitory link with adenylyl cyclase (6, 14, 17, 35, 36, 48, 64, 71). Moreover, electrophysiological studies have demonstrated a 5-HT_{1A} receptor-mediated stimulation of K^+ conductance (2, 3, 4, 11). Finally, a potent inhibition of phospholipase C activity (IC_{50} = 11nM) has recently been attributed to 5-HT_{1A} receptors (10). Since these data were mainly obtained with hippocampal preparations, which contain heterogeneous cell types, they suggest either that different receptor subtypes have been examined or that a single receptor subtype modulates the activity of multiple signal transduction pathways.

Recently, transfection of a "pure" 5-HT_{1A} gene into mammalian cells has allowed partial clarification of this point (26). Using whole cells and membranes derived from transiently transfected COS-7 cells, and HeLa and CHO cells stably expressing the human 5-HT_{1A} receptor, potent 5-HT induced inhibition of the cAMP production was demonstrated (26). 5-HT_{1A} mediated inhibition of adenylyl cyclase was sensitive to Pertussis toxin treatment, thus implicating a direct coupling of 5-HT_{1A} receptors to a Gi protein. A 5-HT_{1A} receptor coupling to Gs was not detected in any of these three host cell lines, since stimulation with 5-HT and 8-OH-DPAT did not elevate whole cell cAMP levels or membrane adenylyl cyclase activity, even in cell lines expressing very high levels of the 5-HT_{1A} receptor (A. Fargin personal communication). This result is not due to an absence of Gs in these cells, since incubation of non-transfected COS-7 cells with the β-agonist, isoproterenol (2 μM), and HeLa cells with vasoactive intestinal peptide, mediates a strong stimulation of cAMP production (26, 62).

In permanently transfected Hela cells, Fargin and colleagues also found a 5-HT_{1A}-dependent stimulation of phosphatidylinositol (PI)-turnover (26, 63). In this case, the magnitude of the half-maximal stimulating dose for 5-HT (EC_{50} $\approx$ 3μM) made the notion of direct coupling of the 5-HT_{1A} receptor to this transduction pathway very questionable. However, this coupling has subsequently been shown to occur at the level of the plasma membrane, providing compelling evidence for a direct coupling of this receptor to the phospholipase C effector system (A. Fargin, unpublished data). Additionally, this receptor couples to phospholipase C activation in CHO cells (J. R. Raymond and J.P. Middleton, unpublished observations). Moreover, the activation of phospholipase C in HeLa cells and CHO cells is sufficient to activate protein kinase C-induced phosphorylation of the MARCKS protein (63) and to mobilize intracellular calcium (51). These signal transduction pathways also modulate sodium-dependent phosphate transport (63) and (Na^+/K^+)-ATPase (51) activation in HeLa cells, sug-

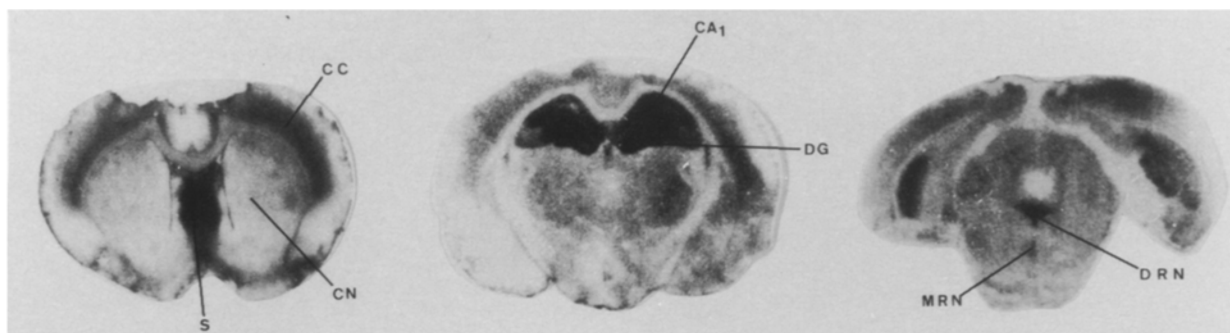

Fig. 1. Immunoradiographic distribution of 5-HT_{1A} receptors in rat brain. Coronal sections (20μm) were incubated in the presence of an antipeptide antibody (see figure 3) recognizing the third cytoplasmic loop of the 5-HT_{1A} receptor. Immunolabeled structures were revealed with ^{35}S-protein A. Abbreviations : CA1, pyramidal cells of Ammon's horn; CC, cerebral cortex; CN, caudate nucleus; DG, dentate gyrus; DRN dorsal raphe nucleus; MRN, median raphe nucleus; S, septum.

gesting that these accessory pathways may modulate physiological functions of cells.

The possibility of direct inhibition of PI turnover and stimulation of K^+ conductance mediated by 5-HT_{1A} receptors in these and other expression systems is not yet resolved. Currently, it has been clearly established that the 5-HT_{1A} receptor is capable of direct coupling to adenylyl cyclase inhibition and stimulated K^+ conductance in various parts of the brain. Thus, for the present, the 5-HT_{1A} receptor seems to be a "multifunctional" receptor. However, the physiological relevance of coupling of the 5-HT_{1A} receptor to various transduction pathways (i.e., andenylyl cyclase stimulation, PI turnover, Ca^{2+} conductance, etc.) within the brain, remains to be established.

Biochemistry

The biochemistry of the 5-HT_{1A} receptor had not been thoroughly investigated until recently. The minimal size of the 5-HT_{1A} binding subunit from various sources has been determined by radiation inactivation and photoaffinity labeling techniques (23, 32, 60, 61) and a ≈ 60-70 kilodalton (kD) molecular weight binding site is generally agreed upon. Using the zwitterionic detergent, CHAPS (21,24), it was demonstrated that the 5-HT_{1A} receptor complex from the rat hippocampus is a glycoprotein with an apparent mass of 150 kD that can be dissociated under the influence of GTP into two components of 60 and 90 kD respectively. The small subunit is the receptor itself, while strong evidence suggests that the 90 kD element comprises a Pertussis toxin-sensitive G-protein.

Treatment of CHAPS-solubilized hippocampal membranes with the crosslinking reagent, disuccinimidyl suberate, resulted in a change of the 5-HT_{1A} molecular weight from 150 kD to 300 kD (24). Among many other possibilities, it can be hypothesized from this result that the 5-HT_{1A} binding subunit from hippocampal membranes is strongly associated with one G-protein subunit and loosely attached to a 150 kD unknown element. Interestingly, adenylyl cyclase has a molecular weight around 150kD (44). However upon complete purification of native 5-HT_{1A} receptor from rat hippocampus, both non-receptor elements are lost, as can be seen from the autoradiogram of an iodinated purified preparation in Fig. 2 (El Mestikawy, unpublished observation).

Although most of the G protein-coupled receptors are substrates for various kinases, so far no data has been obtained on 5-HT_{1A} receptor phosphorylation by various protein kinases. Thanks to the existence of, i) a rapid and efficient purification protocol (21), ii) specific antibodies (22, 26, 61), and iii) cell lines permanently expressing high levels of the 5-HT_{1A} receptor (26), this important pathway for receptor regulation will soon be explored.

Molecular Biology

The 5-HT_{1A} receptor and the two other serotonin receptors (5-HT_{1C} and 5-HT_2) that have been cloned and sequenced belong to the G protein-coupled receptor superfamily (25, 41, 58). As such, the three 5-HT receptors cloned so far share structural homology with rhodopsin and β-AR.

5-HT_{1A} receptors from human and rat brain are ≈ 420 amino acids in length (1, 43) and can be organized into hydrophobic and hydrophilic regions. Seven segments of ≈ 25 hydrophobic amino acids putatively form seven membrane-spanning domains. Four hydrophilic

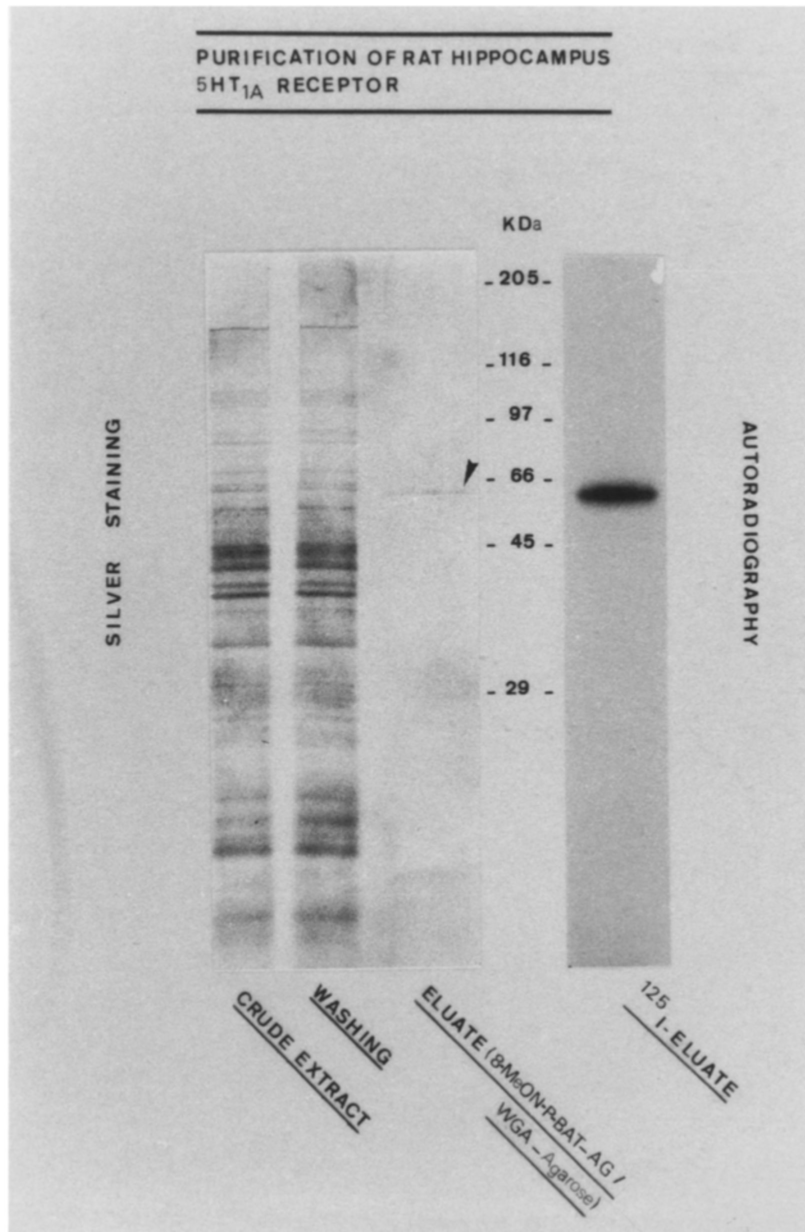

Fig. 2. Solubilized rat hippocampal 5-HT$_{1A}$ receptors were purified with an 8-OH-DPAT derivative affinity column and a wheat germ-agglutinin-agarose column (21). Purified proteins were subsequently iodinated with ^{125}I-Bolton-Hunter reagent, concentrated, and separated by polyacrylamide gel electrophoresis. Silver staining and autoradiography revealed only one band with an apparent molecular weight of 64 kilodaltons.

segments of various lengths are thought to face outside the cell membrane, whereas four others face the intracellular space. The glycosylated N-terminus is apparently oriented extracellularly and the short C-terminus (19 amino acids) is thought to reside inside the cell.

Putative transmembrane segments (TMS) are generally well conserved among the G protein-coupled receptors (as summarized in Table I). Based on evidence derived from rhodopsin and β_2-AR deletion mutants and β_1/β_2-AR chimeric receptors, the amine binding site is apparently located within the TMS (15, 28). TMS-3, -6 and -7 are highly conserved between α_2-, β_1- and β_2-AR and the 5-HT$_{1A}$ receptor (respectively, 76, 68 and 56% amino acid identity). The amino acid identity between D_2-DAR and 5-HT$_{1A}$R in TMS-3 and TMS-7 is also very high (65 and 48% respectively). This high degree of amino acid identity

Table I. Homology of Various Segments of G Protein-Coupled Receptors with the Human 5-HT_{1a} Receptor

	TMS1	TMS2	TMS3	TMS4	TMS5	TMS6	TMS7	IL1	IL2	IL3
Cyclase -										
Hum. α2 C10 AR	32	38	76	32	40	32	60	30	53	20
Rat D2 DAR	38	43	61	32	24	44	48	40	29	7
Hum. M2 AChR	24	33	44	24	32	36	44	40	47	7
Hum. M3 AChR	27	27	39	21	32	37	36	50	41	23
Cyclase +										
Hum. β1 AR	40	46	64	40	40	76	52	27	47	19
Hum. β2 AR	52	38	56	28	40	76	70	45	41	5
Hum. M1 AChR	24	25	44	24	32	48	44	36	41	14
Pi turnover										
Rat 5-HT1C	23	36	65	22	36	41	36	30	35	12
Rat 5-HT2	23	32	65	26	36	41	36	50	35	5

Abbreviation : TMS, transmembrane segment; IL, intracellular loop; Cyclase − or +, the receptor is coupled in an inhibitory or stimulatory fashion respectively, to adenylyl cyclase; Pi turnover, receptors which couple to the breakdown of inositol phosphates.

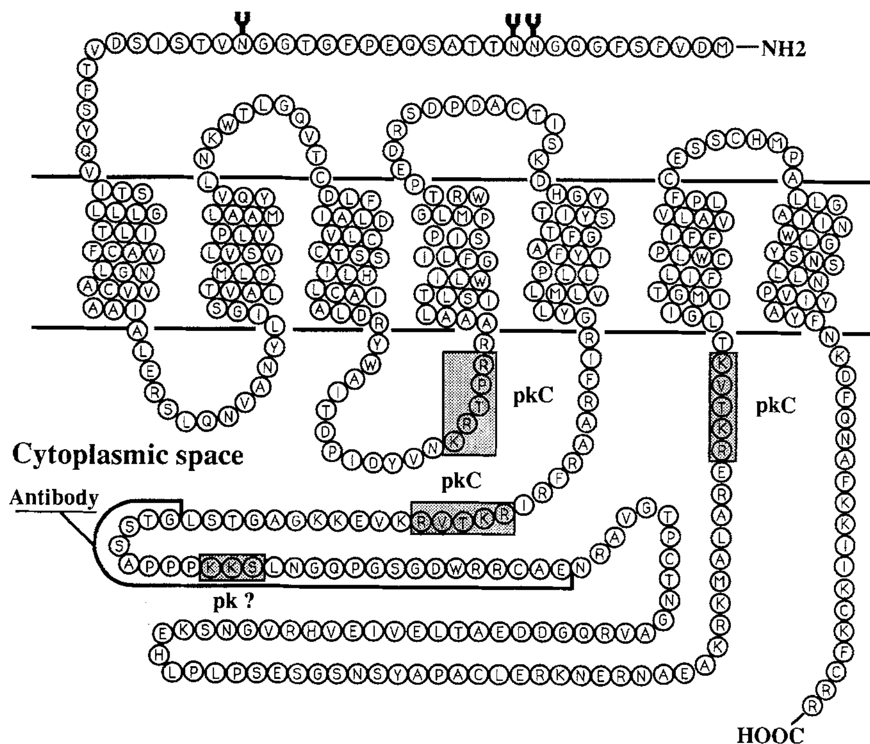

Fig. 3. Schematic representation of the rat 5-HT_{1A} receptor primary structure (according to ref. 1). Four potential phosphorylation sites are indicated in grey boxes, three of them might correspond to protein kinase C phosphorylation sites. The sequence of the 26-amino acid peptide in the third cytoplasmic loop (underlined) was utilized to produce an antipeptide antiserum specifically recognizing the 5-HT_{1A} receptor (22).

might explain why β-blockers like iodo-cyanopindolol or propranolol, and the neuroleptic spiperone can be used as $5\text{-HT}_{1A}R$ antagonists.

Apparently, intracellular peptide sequences are involved in signal transduction, whereas extracellular domains might be involved in axonal transport of the

Table II. Sequences of Portions of Intracellular Loops from Various Receptors Negatively (Cyclase −) or Positively (Cyclase +) Coupled to Adenylyl Cyclase Were Aligned

	IL1	IL2	IL3
Cyclase −:			
Hum. α2C10	TSRALKAPQN	RYWSI...KRTP	RIY...KRRTRVPPS......REKRFT
Hum. M2	VNRHLQTVNN	RYFCV...KRTT	HIS...KSRIKKDKK......REKKVT
Hum. M3	VNRQLQTVNN	RYFCV...RRTT	HIS...RSRRVHKHR......RERKVT
Hum. 5HT1A	LERSLQNVAN	RYWAI...KRTP	RIF...RFRIRKTVK.......RERKTV
Rat 5HT1A	LERSLQNVAN	RYWAI...KRTP	RIF...RFRIRKTVR.......RERKTV
Rat D2	REKALQTTTN	RYTAV...RYSS	KIY..RKRRKRVNT......KEKKAT
Cyclase +:			
Hum. β1	KTPRLQTLTN	RYLAI...LLT−	RVF...QKQVKKIDSC.....REQKAL
Hum. β2	KFERLQTVTN	RYFAI...LLT−	RVF...KRQLQKIDKS.....KEHKAL

Potential PKC-phosphorylated threonine residues are underlined.

receptor. Like all receptors negatively coupled to adenylyl cyclase, the 5-HT_{1A} receptor has a long hydrophilic third intracellular loop (IL3 ≈125 aa). This region is distinctive among G-protein-coupled receptors. As shown in Table I, the homology between the IL3 of the 5-HT_{1A} receptor and other receptors is very limited (i.e., ≈5% with 5-HT_2R and β_2-AR, ≈7% with M_2 muscarinic receptors and D_2-DAR, and ≈12% with 5-HT_{1C}R). Therefore, this region was selected to raise specific antibodies to the 5-HT_{1A}R (Figure 1, and 61).

"Border" regions of the second and the third intracellular loop (IL2 and IL3) are well conserved among receptors coupled to G proteins, while the ten amino acids of IL1 are more divergent (as can be seen in Table II). Thus, it is tempting to hypothesize that an interaction between receptors and G proteins takes place in the border regions of IL2 and IL3.

In the human and the rat 5-HT_{1A} receptor, threonines (T) surrounded by the putative consensus sequence for protein kinase C (PKC) are found twice in IL3 (RK[T]VK - position 226 −230 and RK[T]VR - position 340-344) and once in IL2 (KR[T]PR - position 147-151) (Table II, underlined residues). If the interaction between the receptor and the G protein takes place in these regions, then phosphorylation of threonine residues may play a regulatory role in signal transduction. Interestingly, PKC has been demonstrated to uncouple α-AR from their transduction pathways (45).

Surprisingly, over the 422 and 421 amino acids of the rat and human 5-HT_{1A} receptors, no serine or threonine surrounded by putative consensus sequence for cyclic-AMP dependent protein kinase (PKA = R/K-R/K-X-T/S-) is found, although this kinase is known to be involved in β-AR regulation (5). Along the same line, although calcium/calmodulin-dependent protein kinase type II is very abundant in hippocampal neurones, where it represents 2% of total proteins and 10-50% of post-synaptic density proteins (65), no putative consensus phosphorylation sites for PKII are found on the 5-HT_{1A} receptor.

The presence of putative PKC consensus sequences within the intracellular regions of the 5-HT_{1A} receptor suggest that this kinase may modulate receptor function. Such a regulatory process could occur by homoregulation, or by heteroregulation by other transmitter receptors which activate protein kinase C. The classical regulatory phenomena such as homologous and heterologous densensitization, as defined by Benovic et al. (5), remain to be investigated for the 5-HT_{1A} receptor. However, the availability of specific antisera, cell lines expressing high levels of the receptor, and the cDNA of the receptor for site-directed mutagenesis, should help to shed light on this issue.

CONCLUSION

Thanks to very specific tools which have recently become available (specific antisera, cell lines expressing high levels of receptor, and the receptor cDNA/gene), our understanding of the structure and function of the 5-HT_{1A} receptor has increased dramatically over the last 5 years. It is conceivable that much more progress will be made in the coming years. However, there is still a large gap between clinical and molecular knowledge regarding the physiological functions of the 5-HT_{1A} receptor. We hope that the acquisition of the new tools described above will help to bridge this gap.

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