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Evidence for Adenosine/Dopamine Receptor Interactions: Indications for Heteromerization

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Evidence has been obtained for adenosine/dopamine interactions in the central nervous system. There exists an anatomical basis for the existence of functional interactions between adenosine A_1R and dopamine D_1R and between adenosine A_{2A} and dopamine D_2 receptors in the same neurons. Selective A_1R agonists affect negatively the high affinity binding of D_1 receptors. Activation of A_{2A} receptors leads to a decrease in receptor affinity for dopamine agonists acting on D_2 receptors, specially of the high-affinity state. These interactions have been reproduced in cell lines and found to be of functional significance. Adenosine/dopamine interactions at the behavioral level probably reflect those found at the level of dopamine receptor binding and transduction. All these findings suggest receptor subtype-specific interactions between adenosine and dopamine receptors that may be achieved by molecular interactions (e.g., receptor heterodimerization). At the molecular level adenosine receptors can serve as a model for homomeric and heteromeric protein–protein interactions. A1R forms homodimers in membranes and also form high-order molecular structures containing also heterotrimeric G-proteins and adenosine deaminase. The occurrence of clustering also clearly suggests that G-protein- coupled receptors form high-order molecular structures, in which multimers of the receptors and probably other interacting proteins form functional complexes. In view of the occurrence of homodimers of adenosine and of dopamine receptors it is speculated that heterodimers between these receptors belonging to two different families of G-protein-coupled receceptors can be formed. Evidence that A1/D1 can form heterodimers in cotransfected cells and in primary cultures of neurons has in fact been obtained. In the central nervous system direct and indirect receptor–receptor interactions via adaptor proteins participate in neurotransmission and neuromodulation and, for example, in the establishment of high neural functions such as learning and memory.
[Neuropsychopharmacology 23:S50–S59, 2000]
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KEY WORDS: *G-protein-coupled receptors; Adenosine receptors; Dopamine receptors; Heterodimerization; Receptor–receptor interactions*

The hypothesis that G-protein-coupled receptors (GPCR) could interact in cell membranes was initially put forward in the early 1980s by Agnati and Fuxe (Agnati et al. 1982; Fuxe et al. 1983). It was found that in membrane preparations from many brain areas neuropeptide receptors can modulate the binding characteristics of monoamine receptors. This phenomenon, named "receptor–receptor" interaction, was the subject of an International Wennergren Center Symposium or-

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Received April 6, 2000; revised May 10, 2000; accepted May 10, 2000.

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655 Avenue of the Americas, New York, NY 10010

0893-133X/00/$–see front matter
PII S0893-133X(00)00144-5

ganized in Stockholm by Agnati and Fuxe (Fuxe and Agnati 1987). G-protein-coupled receptors are often regarded as transmembrane allosteric proteins of a monomeric character (see Changeux and Edelstein 1998). But already in 1982 the ordered formation and stabilization of GPCR clusters called receptor mosaics were postulated, as well as their participation in learning and memory (Agnati et al. 1982).

Dimerization of membrane receptors was first demonstrated for the tyrosine kinase receptor superfamily, a phenomenon which appeared to be essential for signal transduction including autophosphorylation and enhancement of affinity for the agonist (Schlessinger 1988; Schlessinger and Ullrich 1992). Heterodimerization was also clearly demonstrated for the tyrosine kinase receptors (Ullrich and Schlessinger 1990). In the 1980s there also appeared indications that functional G-protein-linked receptors exist in a dimeric form (Venter and Fraser 1983; Conn et al. 1982). Evidence for direct molecular crosstalk between GPCR was indeed obtained by Maggio and colleagues using chimeric muscarinic/adrenergic receptors (Maggio et al. 1993). Therefore, we postulated in 1993 that several cases of G-protein-coupled receptor–receptor interactions occurring in crude membrane preparations may be based on a process of heterodimerization, because the molecular mechanisms for dimerization may be conserved among different subclasses of GPCR (Zoli et al. 1993). The density of two or more interacting receptors and the number of receptors activated by the agonist in each population would then determine the proportion of monomers and homodimers and heterodimers and thus the overall action on target cell function (Zoli et al. 1993).

At that time the first evidence of the existence of GPCR dimers was obtained by Ng et al. (1993, 1994a, 1994b, 1996) using antibodies specific for GPCR. It was shown in 1993 that the 5HT-1B receptor exists as monomers and dimers (Ng et al. 1993). This was followed by demonstration of dimers and oligomers of D_1 and D_2 receptors in infected Sf cells (Ng et al. 1994a, 1994b, 1996) and of A_1 receptors in a natural cell line and in mammalian brain (Ciruela et al. 1995). More recently, direct evidence for GPCR heterodimerization and higher order oligomerization (Figure 1) was obtained for GABA B (see Jones et al. 1999) and of kappa and delta opioid receptors (Jordan and Devi 1999). This opens a new field of drug development where molecular models of homo- and heterodimers will play an important role. At the same time the possible existence of additional mechanisms for receptor–receptor interaction must be considered, involving so-called anchoring (adaptor) proteins (see, e.g., Homers in the case of the metabotropic glutamate receptor 1 and 5), which can play a role in formation of heteromeric (and may be homomeric) complexes (Brakeman et al. 1997) (Figure 1). The G-protein network involving the receptor crosstalk through G-protein beta-gamma release and exchange should also be considered (see Zoli et al. 1993).

DOPAMINE-ADENOSINE INTERACTIONS IN THE CENTRAL NERVOUS SYSTEM

Adenosine is an endogenous nucleoside acting as a neuromodulator in the central nervous system. Its actions are mediated by adenosine receptors, four of which have been cloned and pharmacologically characterized: A_1, A_{2A}, A_{2B} and A_3 (Fredholm et al. 1994). Among those four subtypes A_1 and A_{2A} are the main targets of the behavioral effects occurring in animals treated with adenosine analogs (Ferré et al. 1992, 1993, 1997; Fredholm 1995). Caffeine, as an example of an antagonist acting at adenosine receptors, is today the most consumed psychostimulant drug in the world.

A_1 and A_{2A} receptors localized in the basal ganglia, and more precisely in the striatum, are responsible for the motor depressant effects of adenosine agonists and of the motor stimulatory effects of adenosine antagonists (Ferré et al. 1992, 1997). A vast majority of striatal adenosine receptors are located in the medium-sized spiny GABAergic neurons, efferent neurons that constitute more than 90% of the neuronal population in the striatum (Schiffmann et al. 1991; Rivkees et al. 1995). One subtype of GABAergic efferent neurons, the striopallidal ones, are those mainly containing D_2 dopaminergic receptors. A second subtype, the strionigro-strioentopeduncular neurons, contains D_1 dopaminergic receptors (see Ferré et al. 1997). The two subtypes of neurons both contain A_1 adenosine receptors, but only the striopallidal neurons contain A_{2A} receptors. These anatomical localizations indicate that in the basal ganglia A_{2A} receptors only exist in the D_2 receptor-containing neurons, whereas A_1 receptors exist in both D_1 and D_2 receptor-containing nerve cells. The anatomical localization of dopamine and adenosine receptor subtypes have provided the anatomical basis for the existence of functional interaction between A_1 and D_1 or between A_{2A} and D_2 receptors in the same neurons. These functional interactions have been investigated using a variety of techniques, from ligand binding and second messenger determinations to behavioral studies.

In terms of ligand binding the presence of adenosine analogues modify the affinity of dopamine analogues for the binding to D_1 receptors. Selective A_1R agonists affect negatively the high-affinity binding of dopamine to D_1 receptors (Ferré et al. 1998). These results have been obtained working with membranes from tissues or from cells cotransfected with the cDNA for the human versions of A_1R and D_1R. The adenosine A_1 agonists shift the high-affinity binding to the low-affinity states of the D_1 receptor. On the other hand, there is also an interaction at the adenylate cyclase level. A_1R are nega-

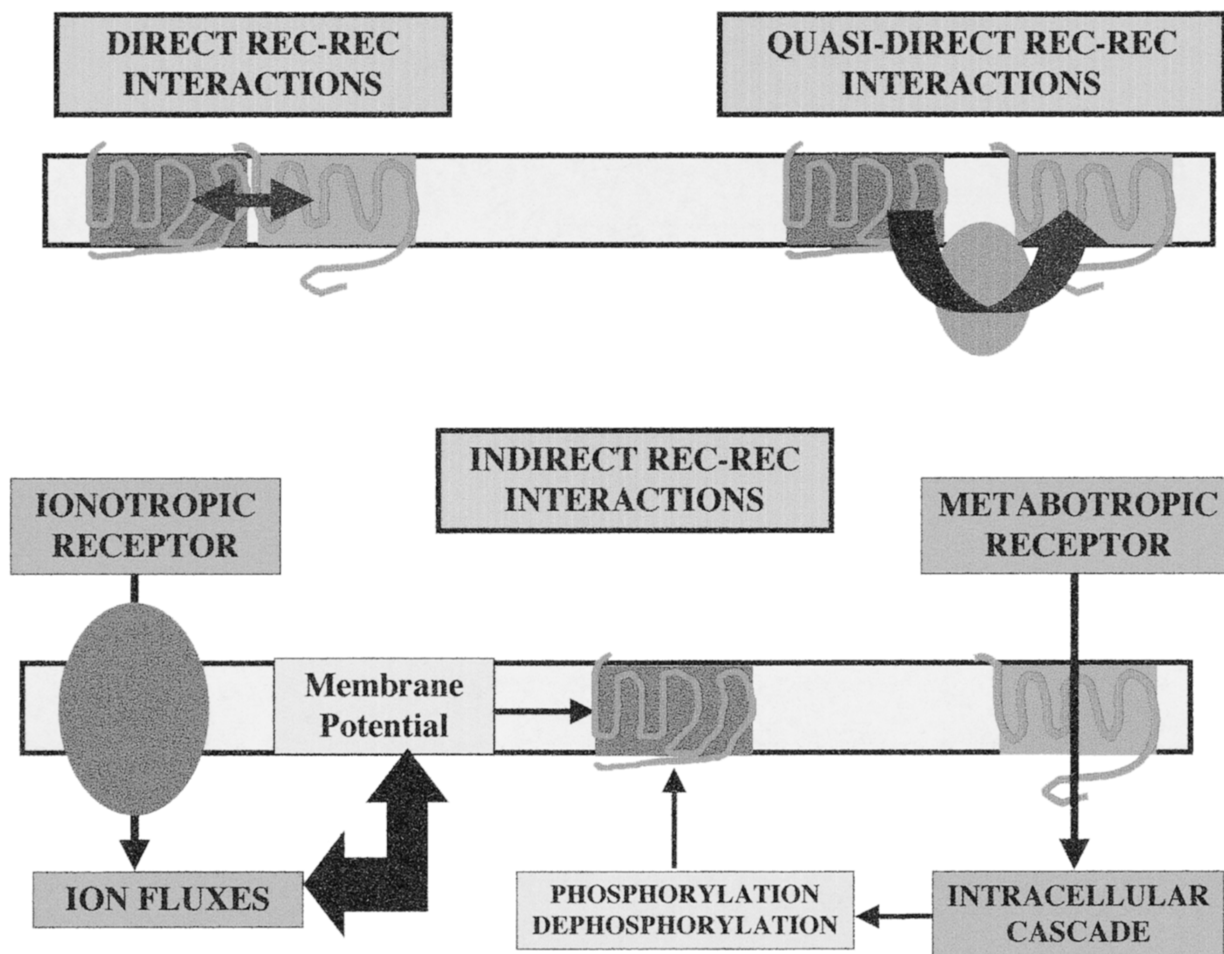

Figure 1. Different types of receptor–receptor interactions. The so-called "direct" receptor–receptor interactions would be exemplified by receptor heterodimerization. "Quasi direct" receptor–receptor interactions would involve anchoring proteins (such as Homers in the case of metabotropic glutamate receptors).

tively coupled to the adenylate cyclase whereas D_1 receptors are positively coupled to this enzyme. Thus, the presence of adenosine agonists leads to a decrease in the dopamine D_1-induced cAMP production whereas, in turn, the presence of antagonists acting on A_1R leads to the potentiation in the cAMP responses occurring by activation of D_1 receptors (Ferré et al. 1998).

Adenosine agonists acting upon A_{2A} receptors instead counteract the effect of dopamine acting upon D_2R. In fact, activation of A_{2A} receptors leads to a decrease in receptor affinity for dopamine agonists, especially of the high- affinity state (Ferré et al. 1991; Dasgupta et al. 1996; Kull et al. 1999). Even in cotransfected CHO cells treatment with the A_{2A} selective agonist CGS21680 leads to a marked reduction in the binding of dopamine to D_2 receptors (Figure 2). Recently, in a neuroblastoma cell line, which constitutively expresses $A_{2A}R$, it has been shown that transfection of D_2R leads to an antagonistic adenosine/dopamine crosstalk which is mediated by A_{2A} and D_2 receptor interactions. This functional interaction is demonstrated to occur in acute treatments in which the dopamine-induced counteraction of the increase in intracellular calcium concentration evoked by KCl is blocked via simultaneous activation of $A_{2A}R$ (Salim et al. 2000).

Adenosine/dopamine interactions at the behavioral level probably reflect those found at the level of receptor binding and signaling. Thus, adenosine receptor antagonist-induced motor activation is counteracted by treatments that cause an acute dopamine depletion or blockade of D_1 or D_2 receptors (Ferré et al. 1992, 1997). Furthermore, adenosine receptor agonists inhibit and adenosine receptor antagonists potentiate the motor activating effects of dopamine agonists (Ferré et al. 1992, 1997). Specifically, low doses of A_1 and A_{2A} receptor agonists selectively counteract the motor activating effects induced by D_1 and D_2 receptor agonists, respectively. On the other hand, selective A_1R antagonists selectively potentiate D_1R receptor agonist-induced motor activation whereas A_{2A} receptor antagonists potentiate D_2 receptor agonist-mediated motor effects (see Ferré et al. 1997 for review).

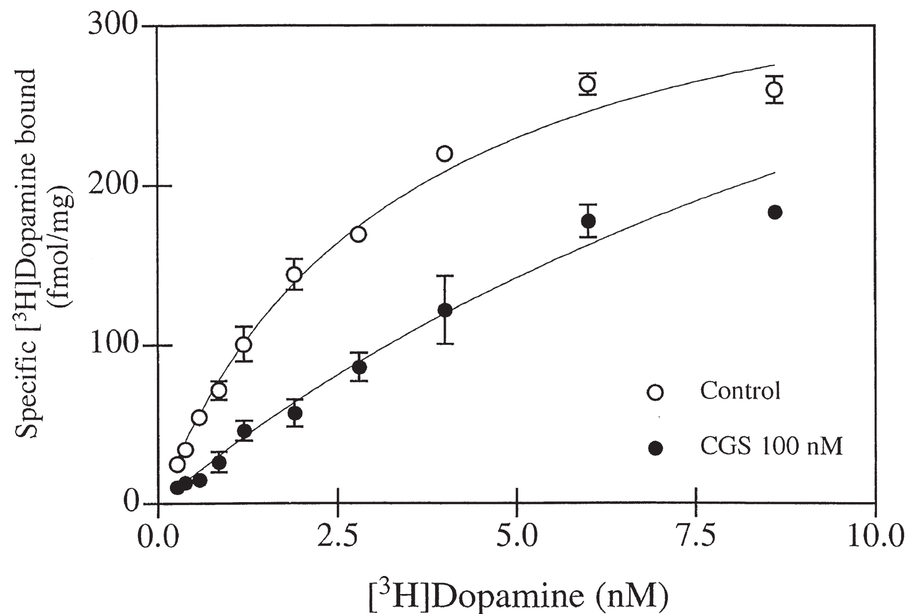

Figure 2. Representative saturation curves of specific binding of [^{3}H]dopamine ([7,8-^{3}H]dopamine; 40 Ci/mmol; Amersham Pharmacia) in membrane preparations from CHO cells stably cotransfected with human adenosine A_{2A} and rat dopamine D_{2S} receptor cDNAs (for details about the transfection and maintenance of the cells, as well as the membrane preparation, see Kull et al. 1999) in the presence and absence of the adenosine A_{2A} agonist CGS 21680 (100 nM). Apomorphine (0.2 mM) was used for nonspecific binding. Results represent means ± standard deviation of triplicate data of one single experiment. B_{max} and K_D values were 382 fmol/mg prot. and 3.3 nM, respectively, for the control curve and 578 fmol/mg prot. and 15.3 nM, respectively, in the presence of CGS 21680. The results show a predominant modulatory effect of adenosine A_{2A} receptors on the affinity of dopamine D_{2S} receptors (a five-fold decrease), since [^{3}H]dopamine labels the high-affinity component of the transfected dopamine D_{2S} receptors, at the concentrations used in the present experiment (original data).

Altogether, the correlation among the data obtained at the cellular level, at the behavioral level and even at the level of neuronal function (see Ferré et al. 1997) strongly suggest that the receptor subtype-specific interaction between adenosine and dopamine receptors (A_{2A}/D_2 and A_1/D_1) play an essential role in the modulation of basal ganglia function. To some extent, these interactions are a consequence of specific molecular interactions achieved by means of heteromerization (see below).

ADENOSINE RECEPTORS AS A MODEL FOR HOMOMERIC AND HETEROMERIC PROTEIN–PROTEIN INTERACTIONS

The work of Franco and colleagues on A_1 adenosine receptors has provided a better understanding of how ligand binding and signal transduction is affected by homotypic and heterotypic protein–protein interactions (Franco et al. 1996, 1997; Ginés et al. 2000; Sarrió et al. 2000).

The binding of [^{3}H]-2-chloroadenosine to A_1 receptors present in rat brain membranes was first studied in two laboratories. Basically, depending upon the absence or presence of exogenous adenosine deaminase (ADA), one single (low-affinity; Wu et al. 1980; Wu and Phillis 1982) or two binding sites (low- and high-affinity; Williams and Risley 1980a, 1980b) were found. The appearance of a high-affinity binding site in the presence of ADA was explained by the disappearance of endogenous adenosine, which acts as a competitor of A_1R agonists, or by assuming that ADA had an extracatalytic high-affinity binding site for 2-chloroadenosine (Phillis and Wu 1981), which is not the case according to the X-ray structure of the enzyme (Wilson et al. 1991). Subsequent studies have demonstrated that A_1R present two different affinities for agonists that depend on the coupling to heterotrimeric G-proteins (Lohse et al. 1984); coupled receptor G-protein complexes display high affinity for agonists (K_d = 0.1–0.2 nM), whereas uncoupled receptors display low affinity (1–2 nM) (Lohse et al. 1984; Casadó et al. 1990).

Although for many years it has been considered that exogenous ADA acts by removing endogenous adenosine, this may not have been the true explanation for the revelation of a high-affinity binding site. Franco's laboratory has accumulated sufficient evidence to be sure that ADA and A_1R interact and that both proteins are functionally coupled. First, in pig brain cortex membranes where the adenosine concentration was undetectable, it was found that ADA was necessary for the identification

of the high-affinity component of the binding to A_1R. In fact, in the absence of ADA, a single low-affinity binding was found (Table 1). If the high-affinity site corresponds to the receptor G-protein complex, ADA would be necessary for the coupling of A_1R to G-proteins. However, the cluster-arranged cooperative model, which accounts for the kinetics of ligand binding to A_1R (Franco et al. 1996), shows that high- and low-affinity sites are a consequence of the negative cooperativity of agonist binding and may not be related to the content of free and GPCR. Therefore, ADA would affect cooperativity without affecting the A_1R-G protein coupling. Further investigations of the molecular interaction between ADA and A_1R was performed in a smooth muscle cell line (DDT_1MF-2), which is currently used as a model of A_1R-expressing cells. Furthermore, the expressed A_1R display similar binding kinetics as A_1R present in cerebral cortex. In these cells, apart from the confirmation of an increase in the affinity for agonists only in the presence of ADA, a more molecular approach was used (Ciruela et al. 1996). Thus, the proof of an ADA/A_1R interaction was investigated by means of confocal microscopy, affinity chromatography and coimmunoprecipitation. Some of these experiments were made possible by the development of antibodies against A_1R which worked well in immunocytochemical, immunoprecipitation and immunoblotting assays. All the approaches tried were positive since ADA and A_1R coprecipitated, A_1R was specifically retained in a matrix of ADA-Sepharose and both ADA and A_1R colocalized on the surface of DDT_1MF-2 cells. In these cells, the degree of colocalization between ADA and CD26, an ADA-anchoring protein in lymphocytes, was lower than the colocalization between ADA and A_1R. Interestingly, when cells were preincubated with commercial ADA (from calf intestine), the degree of colocalization ADA/A_1R approached 100%.

These data constituted the first evidence demonstrating an interaction between a degradative ectoenzyme and the receptor whose ligand is the enzyme substrate. Due to the fact that the interaction of ADA with CD26 on the surface of lymphocytes leads to signal transduction it was suspected that the interaction ADA/A_1R might have a role in the modulation of the receptor-mediated responses. Using a compound able to inhibit the enzymatic activity it was demonstrated that the ADA/A_1R interaction was needed for an efficient signaling via the adenosine receptor. Therefore, ADA is acting catalytically but also in an extraenzymatic way as a co-stimulatory molecule. The actual scenario is that at low adenosine concentrations ADA is mainly facilitating signaling whereas at high adenosine concentrations, by which the ADA/A_1R interaction is disrupted, the low affinity state of the receptor predominates and an autologous mechanism of desensitization is devised (see Franco et al. 1997 for review).

Apart from this heterotypic interaction A_1R do form homodimers in membranes from a variety of tissues or cell lines. The first evidence was obtained by immunoblotting using antibodies that recognized, in samples from pig brain cortical membranes, a specific band of 39 kDa and, in addition, a second band of 74 kDa (Figure 3). This high molecular weight band did not dissociate in a reducing environment or by the treatment at 100° with detergent and did not contain G-proteins that could be forming a stable complex with the receptor (Figure 3). Therefore the band probably reflected the existence of dimers in membranes from pig brain. The bands corresponding to the monomer and to the dimer were present in extracts from different pig and rat tissues, the dimer being specially abundant in samples from cortex and striatum.

A_1R, like other GPCR are multifunctional proteins which are able to form high- order molecular structures containing at least two receptor molecules, heterotrimeric G-proteins and adenosine deaminase. The search of other proteins interacting with A_1R are underway and at least one more protein able to interact with an intracellular loop of those receptors has been discovered (Sarrió et al. 2000). This protein, hsc73, affects the binding of adenosine deaminase to A_1R and the preliminary re-

Table 1. Equilibrium Parameters of [^{3}H]R-PIA Binding to DDT1MF-2 Cells and to Cell Membranes in the Absence or Presence of ADA

Presence of ADA	Affinity State	Kd (nM)	B_{max} (pmol/mg prot)
None	High-affinity	–	–
	Low-affinity	–	–
	Very low-affinity	50 ± 10	0.4 ± 0.1
0.2 U/ml	High-affinity	0.79 ± 0.09	0.28 ± 0.03
	Low-affinity	9 ± 2	0.15 ± 0.05
	Very low-affinity	–	–
0-2 U/ml plus Hg2+	High-affinity	1.5 ± 0.5	0.25 ± 0.05
	Low-affinity	9 ± 2	0.13 ± 0.07
	Very low-affinity	–	–

Hg^{2+} was used to abolish the enzymatic activity of ADA without disrupting the interaction with A_1 adenosine receptor (modified from Ciruela et al. 1996).

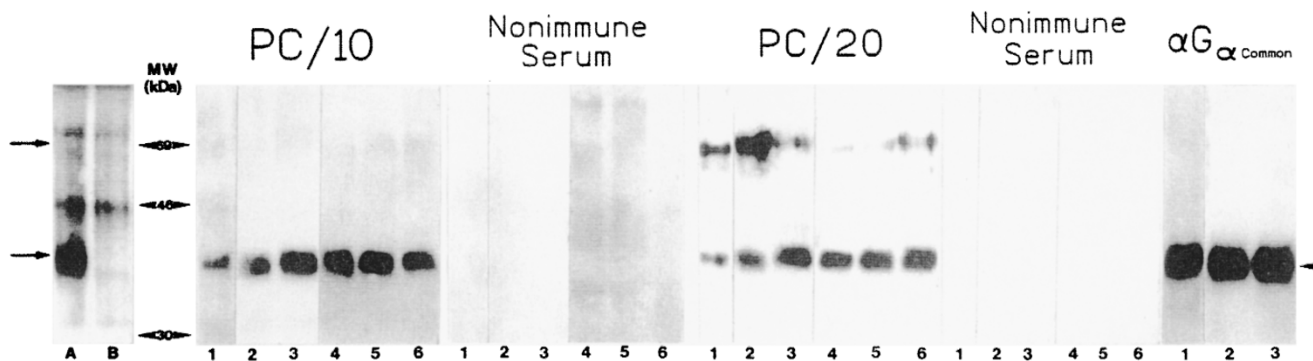

Figure 3. Immunoblotting analysis of the pig brain adenosine A_1R. Pig cortical brain membranes were photoaffinity labelled with [^{125}I]R-AHPIA in the absence **(A)** or in the presence **(B)** of an excess of unlabelled R-AHPIA. Seventy micrograms of protein from cortical pig brain crude membranes (lane 1) or detergent extracts (lanes 2–6) were used. Detergent extracts were prepared from untreated membranes (lane 2) or from membranes treated with either R-AHPIA (lane 4), DPCPX (lane 5) or R-PIA plus Gpp(NH)p (lane 6). In lane 3 the acetone precipitated of detergent extracts was applied. After transferring and blotting, the PVDF membrane was incubated with two anti-A_1R antibodies: PC10 (against an intracellular loop) and PC20 (against an extracellular loop), with antibodies anti G-alpha, or with nonimmune serum. Arrows: bands corresponding to monomers and dimers of A_1R. Arrowhead: band corresponding to G-alpha subunits (from Ciruela et al. 1995, with permission).

sults indicate that A_1R cannot bind to both proteins at the same time. It should be noted that all these interactions play a role in ligand binding and in signaling but also in traffic and down-regulation of the receptors.

DIMERS AND CLUSTERS OF GPCRs

Since the description of the existence of homodimers for 5HT1B, D1, D2 (Ng et al. 1993, 1994a, 1994b, 1995) and A1 receptors (Ciruela et al. 1995) a number of reports have described the occurrence of homodimers for a variety of GPCRs. In fact it now seems that any member of the GPCR superfamily can be present in form of dimers in the plasma membrane. To our knowledge reports on the existence of dimers have been disclosed for dopamine (Ng et al. 1993, 1994a, 1994b, 1995; Zawarynski et al. 1998), adrenergic (Hebert et al. 1996), metabotropic glutamate (Romano et al. 1996), serotonin (Pauwels et al. 1998; Xie et al. 1999), muscarinic acetylcholine (Maggio et al. 1996; Zeng and Wess 1999), opioid (Cvejic and Devi 1997) and chemokine (Rodriguez-Frade et al. 1999) receptors.

In view of the recent discovery of dimers for GPCR there are efforts to know the molecular mechanism of the interaction. Although an indirect interaction through a bridge protein cannot be discarded, the evidence so far indicates that some transmembrane domains participate in the interaction and that the interaction involves mainly nonpolar residues. The use of theoretical biology approaches by Gouldson and colleagues (Gouldson et al. 1997, 1998) have predicted that protein–protein interaction for the homodimer formation (and eventually of heterodimers) is mediated by domain swapping involving transmembrane regions 5 and 6, and probably other helical transmembrane domains as well. The only exception to this general trend are metabotropic glutamate 5 receptors for which a disulfide-linked structure has been demonstrated for the dimer (Romano et al. 1996). This may be rather unique to these receptors since they show little homology with other members of the GPCR family.

Assuming that there is an equilibrium between monomeric and heteromeric forms of GPCR in membranes, the agonists displace the equilibrium toward the formation of the dimer. It seems, at least for some members of the GPCR family, that this displacement occurs because dimers are more functional that monomers. But interestingly, when observed under a microscope in immunocytochemical assays, adenosine receptors cluster in the presence of agonists (Figure 4). It is highly probable that clustering occurs for several GPCRs when they are activated by their ligands. Occurrence of clustering clearly reflects that GPCR form high molecular order structures in which multimers of the receptors and, probably, other interacting proteins form functional complexes whose precise role in the biochemistry and physiology of the receptor will require more experimental effort in the future.

DOPAMINE-ADENOSINE RECEPTOR–RECEPTOR INTERACTIONS

Some of the functional interactions between dopamine and adenosine can be explained by downstream interactions. Thus the A_1/D_1 receptor antagonism at the level of the cAMP formation can be easily explained by the fact that G-proteins for A_1R and D_1R are differently

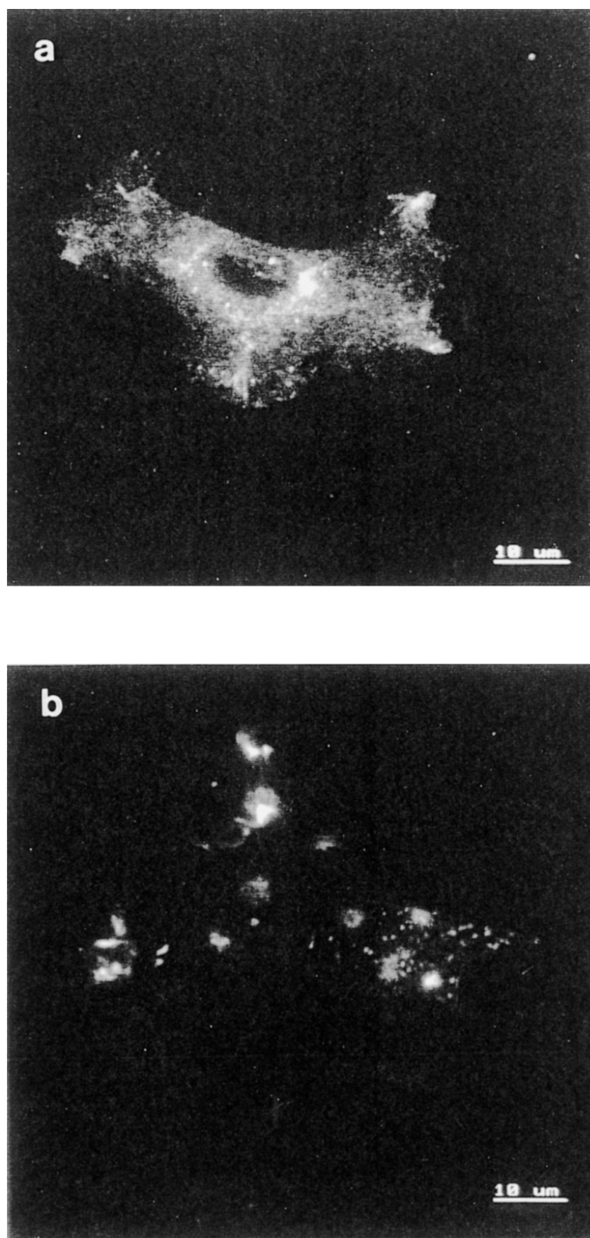

Figure 4. Ligand-induced clustering of A_1R in DDT1-MF2 cells. Anti-A_1R antibodies was used to stain control cells **(A)** or cells treated with 50 nM R-PIA for 5 min **(B)**. Scale bar: 10 μm.

coupled to adenylate cyclase. However, some of the functional interactions occur even in acute treatments (see above) which suggested that a direct interaction may occur. Due to the occurrence of homodimers of adenosine and of dopamine receptors it is tempting to speculate that heterodimers between receptors belonging to two different families of GPCR can be formed. Until recently it had not been possible to study this possibility due to the lack of suitable tools in the form of specific antibodies working for immunoprecipitation, immunoblotting and immunofluorescence studies. We have now enough evidence to be sure that A_1 and D_1 form heterodimers in both cotransfected cells and in primary cultures of neurons. In fact it has been possible to see a high colocalization between A_1R and D_1R even in primary cultures and also antibodies against the A_1R are able to coimmunoprecipitate the D_1R (Ginés et al. 2000). A further proof of the specificity of the interaction has been obtained by studying the effect of ligands on the distribution of the receptors in the membrane. Thus, whereas agonists for A_1R cluster the two receptors in cotransfected cells, the ligand for D_1R cluster D_1R but not A_1R and, therefore, the interaction is lost (Ginés et al. 2000). It is rewarding to have been able to prove our hypothesis emitted in the 1980s and note that ligands can modulate the distribution of the receptors in the membrane in what we can call the dancing of receptors.

Similar experiments are currently underway to try to demonstrate the existence of such interaction between A_{2A} and D_2 receptors. While we are raising antibodies useful for coimmunoprecipitation experiments we have obtained excellent results in terms of colocalization between A_{2A} and D_2 receptors in cell lines and in primary cultures from striatum. Hopefully we will be able to demonstrate soon that the two receptors coimmunoprecipitate.

HOMO- AND HETEROMERIZATION OF GPCR: UNDERSTANDING THE NERVOUS SYSTEM

In 1999 the first reports on heteromerization involving GPCR started to appear in the literature. They corresponded to heterodimerization among receptors for the same ligand. Heterodimers consisting of two subtypes of opioid receptors (κ and δ) have a pharmacological profile that differs from that corresponding to each of the receptor subtypes when expressed alone (Jordan and Devi 1999). The case of heterodimerization of GABABR1 and GABABR2 receptors is paradigmatic since cells only express these receptors when they are assembled together in the endoplasmic reticulum (Jones et al. 1999; Kaupmann et al. 1999; White et al. 1999). An interaction between a GPCR for dopamine (D5 receptors) and a ligand gated receptor for GABA (GABAA receptors) has just been reported (Liu et al. 2000). Also our report on heteromerization of two GPCR receptors for two structurally different ligands (A_1R and D_1R) has just appeared (Ginés et al. 2000). Taken together the existence of homodimers and heterodimers of various types indicates that the operation of those receptors may involve homotypic and heterotypic interactions which are crucial for GPCR function and for ligand-gated channels. It is quite reasonable that the interacting proteins might assemble and disassemble depending upon the composition of the cell membrane (i.e., the types of receptors present there), and upon the presence of the different neuromodula-

tors in the extracellular medium. As an example, there is evidence that A_1R interact with metabotropic glutamate receptors in cerebellar neurons (Ciruela et al., data in preparation). Therefore, the heterotypic interactions established with A_1R in a given cell would depend upon the presence of D_1R, of metabotropic glutamate receptors or both. Furthermore, the geometry of the interactions in the clusters formed after activation of the receptors may depend upon the combination of neuromodulators present in the extracellular medium, and more precisely in the synapses.

In the central nervous system this scenario is very attractive because receptor–receptor interactions are likely involved in neurotransmission and neuromodulation as well as in development or in the establishment of higher neural functions such learning and memory (Agnati et al. 1982). At this point you are reminded of the model of the fluid mosaic of receptors that we devised many years ago. We think that at a given plasma membrane a multiple interaction between receptors and adaptor proteins, such as ADA itself, exists (Figure 1). This leads to a mosaic which is specific for a given neuron and is responsible for a given action of, say, a given neuromodulator. On the other hand, this mosaic is dynamic because ligands might affect the composition and the geometry of the mosaic. This may constitute the basis for a better understanding of how the central nervous communication network is supported by the multifunctional role of neuronal GPCR.

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