J Neural Transm (2007) 114: 49–75
DOI 10.1007/s00702-006-0589-0
Printed in The Netherlands

Journal of Neural Transmission

Intramembrane receptor–receptor interactions: a novel principle in molecular medicine*

K. Fuxe[1], M. Canals[1,3], M. Torvinen[1], D. Marcellino[3], A. Terasmaa[1], S. Genedani[2], G. Leo[2], D. Guidolin[2,9], Z. Diaz-Cabiale[4], A. Rivera[5], L. Lundstrom[6], U. Langel[6], J. Narvaez[4], S. Tanganelli[7], C. Lluis[3], S. Ferré[8], A. Woods[8], R. Franco[3], L. F. Agnati[2]

[1] Department of Neuroscience, Division of Cellular and Molecular Neurochemistry, Karolinska Institutet, Stockholm, Sweden
[2] Department of Biomedical Sciences, University of Modena and Reggio Emilia, Modena, and IRCCS of Venezia, Italy
[3] Department of Biochemistry and Molecular Biology, University of Barcelona, Barcelona, Spain
[4] Department of Physiology, Faculty of Medicine, University of Malaga, Malaga, Spain
[5] Department of Cell Biology, School of Science, University of Malaga, Malaga, Spain
[6] Department of Neurochemistry, University of Stockholm, Stockholm, Sweden
[7] Department of Clinical and Experimental Medicine, University of Ferrara, Ferrara, Italy
[8] National Institute on Drug Abuse, IRP, NIH, Department of Health and Human Services, Baltimore, MD, USA
[9] Department of Anatomy and Human Physiology, University of Padova, Padova, Italy

Received: January 1, 2006 / Accepted: October 4, 2006 / Published online: October 27, 2006

Summary In 1980/81 Agnati and Fuxe introduced the concept of intramembrane receptor–receptor interactions and presented the first experimental observations for their existence in crude membrane preparations. The second step was their introduction of the receptor mosaic hypothesis of the engram in 1982. The third step was their proposal that the existence of intramembrane receptor–receptor interactions made possible the integration of synaptic (WT) and extrasynaptic (VT) signals. With the discovery of the intramembrane receptor–receptor interactions with the likely formation of receptor aggregates of multiple receptors, so called receptor mosaics, the entire decoding process becomes a branched process already at the receptor level in the surface membrane. Recent developments indicate the relevance of cooperativity in intramembrane receptor–receptor interactions namely the presence of regulated cooperativity via receptor–receptor interactions in receptor mosaics (RM) built up of the same type of receptor (homo-oligomers) or of subtypes of the same receptor (RM type1). The receptor–receptor interactions will to a large extent determine the various conformational states of the receptors and their operation will be dependent on the receptor composition (stoichiometry), the spatial organization (topography) and order of receptor activation in the RM. The biochemical and functional integrative implications of the receptor–receptor interactions are outlined and long-lived heteromeric receptor complexes with frozen RM in various nerve cell systems may play an essential role in learning, memory and retrieval processes. Intramembrane receptor–receptor interactions in the brain have given rise to novel strategies for treatment of Parkinson's disease (A2A and mGluR5 receptor antagonists), schizophrenia (A2A and mGluR5 agonists) and depression (galanin receptor antagonists). The A2A/D2, A2A/D3 and A2A/mGluR5 heteromers and heteromeric complexes with their possible participation in different types of RM are described in detail, especially in the cortico-striatal glutamate synapse and its extrasynaptic components, together with a postulated existence of A2A/D4 heteromers. Finally, the impact of intramembrane receptor–receptor interactions in molecular medicine is discussed outside the brain with focus on the endocrine, the cardiovascular and the immune systems.

Keywords: A2A receptors, D2-like receptors, metabotropic glutamate receptor 5, neuropeptide receptors, receptor heteromers, receptor mosaics, basal ganglia, novel treatment strategies in neuropsychopharmacology, learning and memory

Introduction

The dawn of the concept

In 1980/81 Agnati and Fuxe introduced the concept of intramembrane receptor–receptor interactions and presented the first experimental observations for their existence in

* Dedicated to Prof. Rolf Luft, Department of Endocrinology, Karolinska Institutet, Stockholm, Sweden for His outstanding research and teaching that inspired an entire generation of scientists worldwide in the field of molecular medicine.

Correspondence: Kjell Fuxe, Department of Neuroscience, Division of Cellular and Molecular Neurochemistry, Karolinska Institutet, 17177 Stockholm, Sweden
e-mail: Kjell.Fuxe@ki.se

crude membrane preparations from various brain regions and from the spinal cord (Agnati et al., 1980; Fuxe et al., 1981) based on the ability of peptides via their receptors to modulate the binding characteristics of the heptaspanning monoamine receptors (see Lefkowitz, 2004). The main steps in the development of this concept are illustrated in Figs. 1–3. The first step (Fig. 1) was the indication that Substance P and cholecystokinins can modulate the affinity and density of high affinity 3H-5-HT binding sites and D2 antagonist binding sites, respectively in membrane preparations, indicating the possible existence of intramembrane

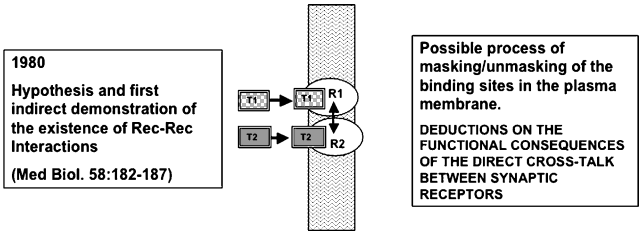


Fig. 1. Schematic illustration of the first indications of intramembrane receptor–receptor interactions based on neuropeptide induced changes in the binding characteristics of monoamine receptor subtypes as studied in membrane preparations from different brain regions (Agnati et al., 1980, 1983d; Fuxe et al., 1981, 1983a)

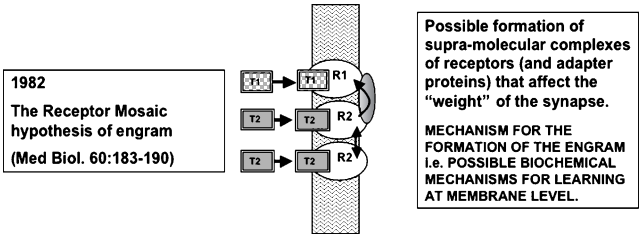


Fig. 2. Schematic illustration of the concept of the formation of supramolecular complexes of receptors called receptor mosaics built up of different types of receptors (tesserae) postulated to affect the synaptic weight (Agnati et al., 1982; Agnati and Fuxe, 1984; Fuxe et al., 1983c; Fuxe and Agnati, 1985)

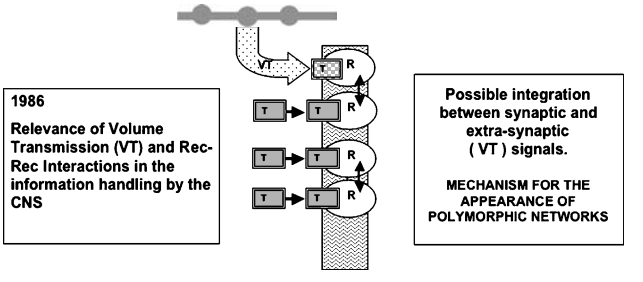


Fig. 3. Schematic illustration of the integration of wiring and volume transmission signals via receptor–receptor interactions, which inter alia contribute to the appearance of polymorphic networks (Agnati et al., 1986, 1990; Agnati and Fuxe, 2000; Fuxe et al., 1986a, b)

interactions between substance P and 5-HT receptors and between CCK and D2 receptors (Agnati et al., 1980, 1983d; Fuxe et al., 1981, 1983a). The molecular mechanisms for these intramembrane events were, e.g., suggested to involve activation of masking/unmasking processes of the binding sites of the monoamine receptors and/or changes in the interconversion between the high and low affinity state of the monoamine receptors.

The second step was the introduction of the receptor mosaic hypothesis of the engram (Fig. 2) (Agnati et al., 1982; Fuxe and Agnati, 1985). It was postulated that islands (clusters) of receptors could be formed via receptor–receptor interactions in the postsynaptic membrane under the influence of the synaptic activity to be learnt. These receptor islands were called receptor mosaics to underline their capability of working as a unique integrated input unit (Agnati et al., 1982, 2002) and it was postulated that their activation could favour ordered electrotonic sequences in the local circuits which could play an important role in learning and memory (Agnati et al., 1981, 2001, 2002). Thus, they represent at least part of the engram, which when activated can induce unique electrotonic sequences mimicking those of a previous (teaching) sequence and changes in synaptic weight leading to learning and memory can in this way take place.

The third step was the proposal that the existence of intramembrane receptor–receptor interactions made possible the circuit miniaturization with molecular networks formed in the surface membrane (Agnati and Fuxe, 1984). Finally, it was suggested that receptor–receptor interactions could allow the integration of synaptic (WT) and extrasynaptic (VT) signals (Fig. 3) (Agnati et al., 1986, 1990; Fuxe et al., 1986a, b), representing one of the mechanisms for the appearance of polymorphic networks (see Agnati and Fuxe, 2000).

The first observations indicating the existence of dimerization of GPCR were made in 1982 (Fraser and Venter, 1982; Paglin and Jamieson, 1982) and the first symposium on receptor–receptor interactions was held in Stockholm in 1986 (Fuxe and Agnati, 1987). In 1987 dimerization was demonstrated as the crucial event in the activation of the epidermal growth hormone receptor by EGF (Yarden and Schlessinger, 1987). A major breakthrough in the receptor–receptor interaction field came with the discovery of the GABA B receptor heterodimer in 1998/1999 (see Marshall et al., 1999; Marshall, 2005). The heteromerization as the molecular basis for the receptor–receptor interaction had been postulated by our group in 1993 (Zoli et al., 1993). For reviews, see (Franco et al., 2000; Angers et al., 2002; Agnati et al., 2003a; Milligan, 2004).

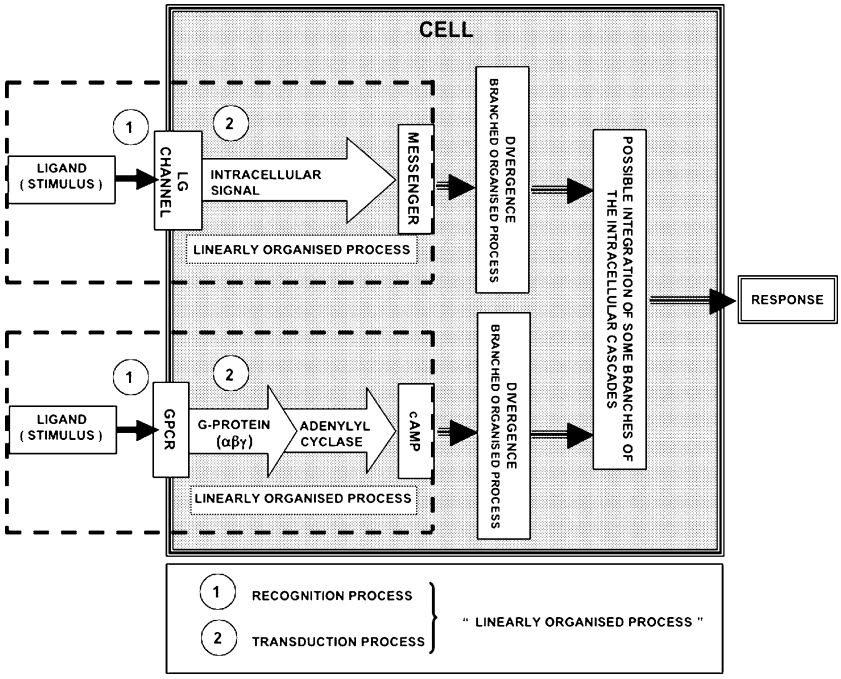


Fig. 4. Scheme on the development of our knowledge on the process of cell activation by GPCRs. Before the receptor–receptor interaction discovery the model of the process appears as a linearly organized process until it reaches the second messenger level where a branched process develops with integration in the phosphorylation cascades

A retrospective view of the development of the concept: the classical view vs the novel view of the decoding process

In the eighties the recognition-transduction process was looked upon as a linearly organized event with divergence only developing at the second messenger level in the cytoplasm (Fig. 4). With the discovery of the intramembrane receptor–receptor interactions with the likely formation of receptor aggregates of multiple receptors, so called receptor mosaics, the entire decoding process becomes a branched process already at the receptor level in the surface membrane (Fig. 5). The receptor mosaic works as an integrative functional unit causing an integrated activation of multiple transduction lines followed by an integrated regulation of multiple effectors with high divergence and the development of syndromic responses.

The classical and the novel views on crosstalk between receptors in the surface membrane are summarized

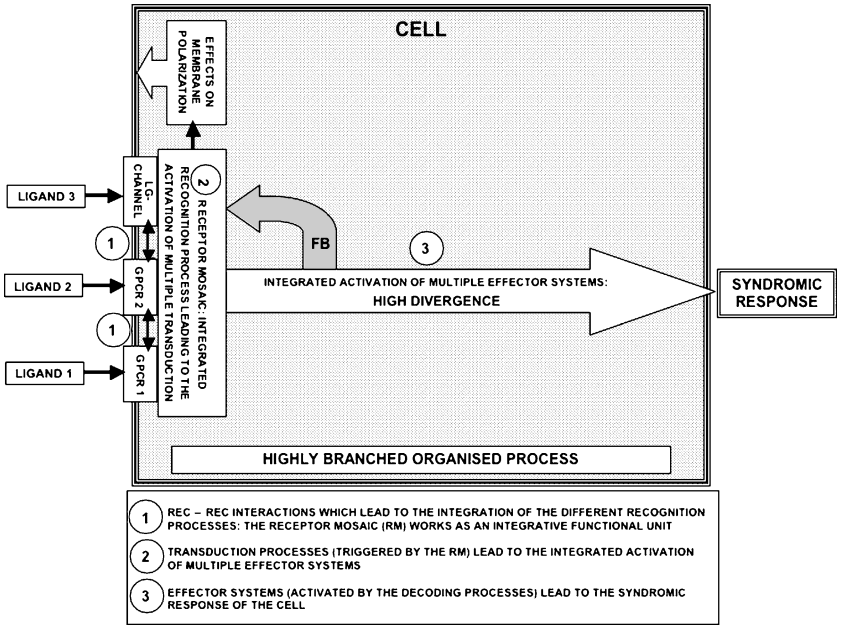


Fig. 5. Scheme on the development of our knowledge on the process of cell activation by GPCRs. With discovery of the receptor–receptor interactions including the receptor mosaic concept the model of the process develops as a branched decoding process already at the receptor level in the surface membrane

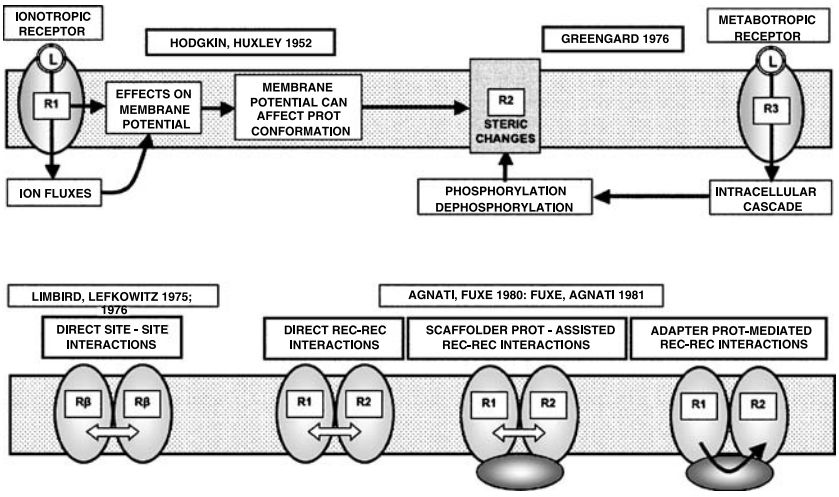


Fig. 6. Scheme of the classical and novel view of receptor–receptor interactions, the old view regarding them as a result of indirect interactions via changes in membrane polarization or changes in phosphorylation/dephosphorylation of receptors. The novel view focuses on the existence of direct receptor–receptor interactions, which are mainly based on heteromerization but sometimes can be mediated via an adapter protein and sometimes require the assistance of scaffolding proteins to allow the direct interaction to occur. For further details, see text

in Fig. 6. The old view maintained that crosstalk between receptors took place exclusively through indirect receptor–receptor interactions by an ionotropic receptor (R1) changing the membrane potential or a G protein coupled receptor (R3) changing the phosphorylation/dephosphorylation cascades. The change in membrane potential or the altered phosphorylated state would then cause a conformational change in the other receptor (R2) leading to altered recognition and signaling in R2. In 1975/76 Lefkowitz, Limbird and colleagues discovered negative cooperativity in beta adrenergic receptors (Limbird et al., 1975; Limbird and Lefkowitz, 1976) which could be explained on the basis of the existence of homodimers leading to site–site interactions. In 1980/81 the first indications were obtained for the existence of direct receptor–receptor interactions (type 1) in the membrane among different types of G protein coupled receptors (Agnati et al., 1980; Fuxe et al., 1981), which were proposed to be widened to take place also between different classes of macromolecules such as receptors, ion channels and ion pumps (Fuxe and Agnati, 1987). Such physical direct receptor–receptor interactions may sometimes require scaffolding proteins to link (tether) the GPCRs together to allow the receptors to interact and change the conformational state of each other via oligomerization (Fig. 6). Thus, other proteins are part of the macromolecular complex besides the receptors (direct receptor–receptor interactions of type 2). Finally, there is a third mechanism where adapter proteins linked to two receptors without direct contact to each other undergo conformational changes and in this way transfer the conformational change in one receptor to an adjacent receptor (adapter protein mediated receptor–receptor interactions) (Fig. 6). It is called receptor–receptor interactions of type 3.

Recent developments of the concept: relevance of cooperativity in intramembrane receptor–receptor interactions

Cooperative binding can take place when a multimeric receptor binds more than one molecule of the same transmitter. Cooperativity means that the binding of a ligand alters the affinity of the same ligand to bind to the other subunits of the multimeric protein (see Changeux and Edelstein, 2005) This becomes possible through allosteric changes developing in the contact zones of the protein subunits as the first ligand causes conformational changes in its subunit. In this way the conformational change can be intermolecularly transferred to the other subunits (see Changeux and Edelstein, 2005; Agnati et al., 2005a). Thus, cooperativity is the phenomenon where the first ligand causes a sequential change in subunit conformations and represents in multimeric proteins a self-regulation mechanism (Changeux and Edelstein, 2005). In the tetrameric hemoglobin where cooperativity has been extensively studied both a concerted (all-or-nothing) and a sequential (mixed conformational states) mechanism may exist (see Ackers et al., 1992).

Agnati and Fuxe have, therefore, suggested that there exist three main types of receptor mosaics: Receptor mosaic (RM), namely RM-type 1, RM-type 2 and RM of a mixed type (Agnati et al., 2005a).

– RM1 is built up of the same type of receptor (homo-oligomers) or of subtypes of the same receptor (special type of hetero-oligomer) and cooperativity can develop. In Fig. 7 we list and illustrate DA receptor mosaics of type 1. The RM1 is shown as a crucial branch point in the membrane in Fig. 8, where it interacts not only with membrane associated proteins to form the horizontal molecular networks but also with proteins in the extra-

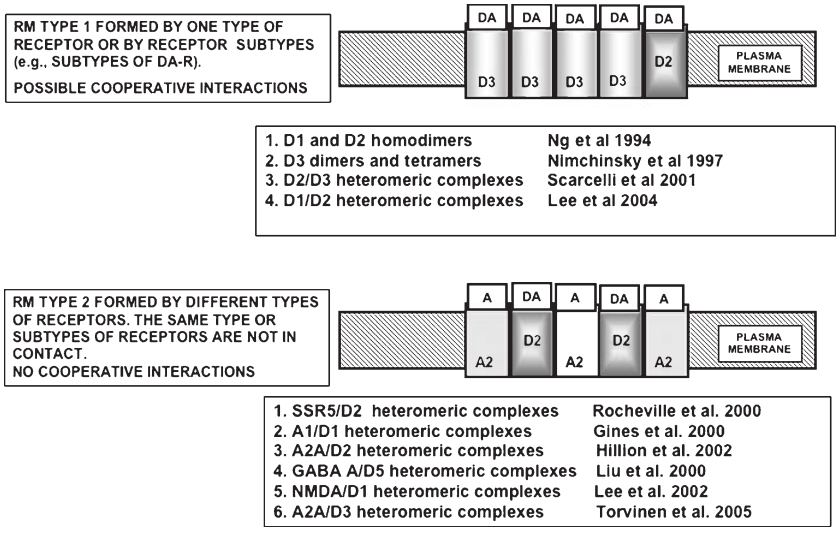


Fig. 7. Illustration of examples (references are given) of types 1 and 2 DA receptor mosaics with type 1 able to show cooperativity by having direct contacts among identical DA receptors or DA receptor subtypes. In contrast, in receptor mosaic 2 the DA receptors are not in direct contact and cooperative interactions cannot develop

cellular matrix and in the cytoplasm forming the so called vertical molecular networks (Agnati et al., 2003). Such branch points may show regulated cooperativity being modulated by allosteric interactions with other membrane associated proteins and by ions (Agnati et al., 2005c, d; Armstrong and Strange, 2001).

– RM2 is built up of different types of receptors (hetero-oligomers) and may include several non-contacting receptors of the same receptor or receptor subtype. Cooperativity can therefore not develop. In Fig. 7 DA receptor mosaics of type 2 are also listed and illustrated.
– RM of the mixed type are built up of RM2 which include receptor mosaics of type 1 (islands of RM1). Thus, in these islands cooperativity may exist and they may represent allosteric cooperative units (van Holde et al., 2000; Agnati et al., 2005a, d).

In view of above it seem likely that in RM1 and in RM of the mixed type cooperativity can be an important mechanism involved in the intramembrane receptor–receptor interactions. It is also clear from above that the GPCR and probably all types of receptors are part of the RM outlined here and therefore the major molecular mechanism underlying the conformational cafeteria theory of receptors by Kenakin (2003) is probably the multiple intramembrane receptor–receptor interactions ongoing in these RM.

These receptor–receptor interactions will to a large extent determine the various conformational states of the receptors and their operation will be determined by the composition, the spatial organization (topography) and order of receptor activation in the RM (see Agnati et al., 2005a, d).

Positive cooperativity is a mechanism to sharpen the responsiveness of a receptor system to a change of its ligand in a narrow range of concentrations. Negative cooperativity is a mechanism to dampen the responsiveness of a receptor system to a change of its ligand in a broad range of concentrations to avoid overactivation of the receptor system. It seems likely that negative cooperativity plays a major role in synaptic transmission with high concentrations of the ligand reaching the receptor mosaics of type 1 or of the mixed type. In contrast, positive cooperativity may play a major role in such receptor mosaics operating in volume transmission with low nanomolar concentrations reaching the extrasynaptic receptor mosaics (Agnati et al., 2005a).

As an example may be mentioned the possible role of possible positive and negative cooperativity in the DA filtering action on glutamate inputs to dendritic spines of medium-sized striatal neurons, where DA acts as a high pass filter. With low glutamate transmission the extrasynaptic inhibitory DA D2 like receptor mosaic of type 1 or of the mixed type located on the corticostriatal terminals may operate via positive cooperativity to reduce glutamate release and glutamate transmission. With high glutamate transmission this positive cooperativity in the D2 like RM may be abolished due, e.g., to conformational changes induced in the D2 like RM via the frequent depolarization of the membrane potential and/or their altered phosphorylation state. In this way this DA RM may fail to effectively inhibit the high glutamate release process contributing to the high pass filter function of DA. As to the postulated negative cooperativity development in the synaptic DA D2 RM of type 1 or of the mixed type it may instead become enhanced in the state of high glutamate transmission via similar mechanisms as described above further dampening the inhibitory activity of the D2 RM. Thus, opposite alterations in the positive (reduction) and

negative (increase) cooperativity of the extrasynaptic and synaptic D2 RM, respectively upon high glutamate transmission may contribute to a further increase in the upstate of the striato-pallidal GABA neurons. In the case of the direct pathway rich in D1 receptors it is of interest that in the upstate they become coupled to L-type Ca channels enhancing their function causing a further upregulation of the high glutamate transmission in the direct GABA pathway to the entopeduncular nucleus and the substantia nigra, zona reticulata (Nicola et al., 2000).

Recently Franco and Canela have introduced a novel dimer-based model for heptaspanning membrane receptors (Franco et al., 2005). The model predicts cooperativity in binding and thus the existence of non-linear Scatchard plots as well as the various responses of full, partial, and inverse agonists and of neutral antagonists.

The important parameters in the model are *alpha*, representing the intrinsic efficacy of the first ligand A entering the dimer, *teta*, representing the intrinsic efficacy of the second A molecule entering the dimer, and *mu*, representing the binding cooperativity between the first and second A molecule.

In the assembly of protein mosaics in general a hub protein may exist which can interact with several protein monomers (tesserae) to form the mosaic, the assembly being modulated by the chemico-physical influences of the environment. In this process also the morpheein model should be considered (Jaffe, 2005) which gives a new structural paradigm for allosteric regulation and can have several important physiological and pathological implications as discussed below.

Thus, the monomers can exist in more than one conformation, each favouring quaternary structures with different multiplicities and likely different functions. Novel protein mosaics can therefore be formed with a novel spectrum of emergent functions (Jaffe, 2005). This may also be true for receptor mosaics and gives an increased understanding of their dynamics in terms of, e.g., development of cooperativity. The receptor monomer in terms of its conformational state will then determine the oligomerization state of the receptors e.g., a dimeric, trimeric or tetrameric state. Thus, the morpheein concept (Jaffe, 2005) gives a new structural aspect also to the allosteric regulation of receptors.

On the physiological and pathological relevance of intramembrane receptor–receptor interactions among heptaspanning membrane receptors

Let us discuss two main implications of receptor–receptor interactions, namely the basic biochemical one and the

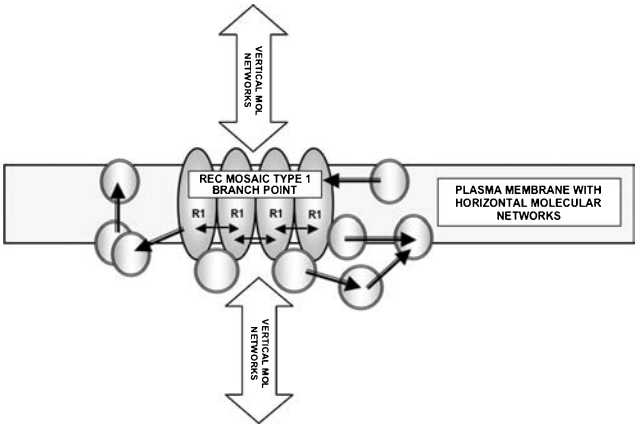


Fig. 8. Schematic illustration of a receptor mosaic 1 as a branch point with cooperativity showing links to horizontal molecular networks in the membrane and with the vertical molecular networks in the cytoplasm and in the extracellular matrix

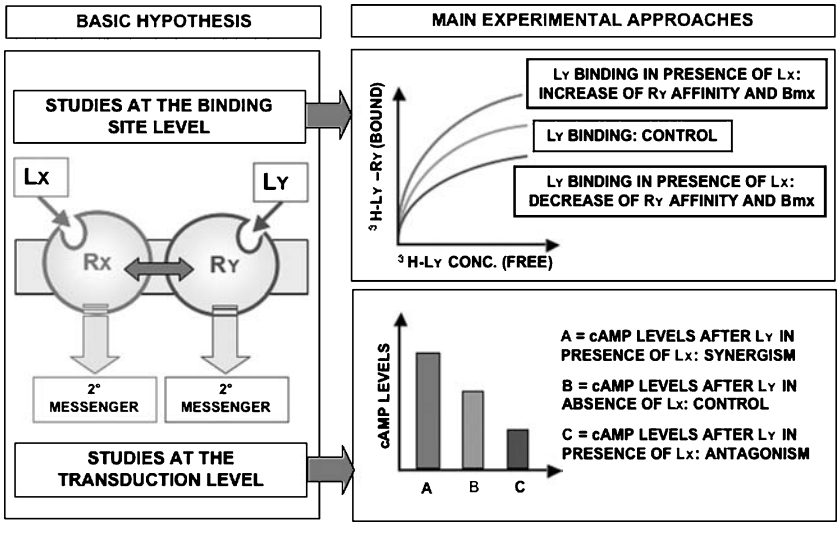


Fig. 9. Illustration of some experimental approaches to obtain indications of receptor–receptor interactions at the level of receptor recognition (radioligand binding) (Agnati et al., 1980; Fuxe et al., 1981) and at the level of transduction (cAMP measurements)

functional integrative one and for both these ample topics the physiological and pathological aspects.

Biochemical implications

It is possible to consider four major aspects:

1. Via the intramembrane regulation of receptor signalling the biochemical pathways in the cytoplasm towards especially the nucleus can work on ''conditioned'' signals (Agnati et al., 1986; Fuxe et al., 1986b) The regulation by receptor–receptor interactions involves modulation of receptor recognition (Kd and B_{max} values) and of G protein coupling leading to modulatory actions in the activation or inhibition of multiple effector systems associated with the plasma membrane such as ion channels and enzymes e.g., adenylate cyclase. The basic hypothesis and the main experimental approaches are shown in Fig. 9. Already in the early eighties it was underlined that by means of these molecular events it becomes possible for the receptor–receptor interactions to filter incoming signals to one receptor based on the state of the target cell and the activity of other incoming signals (Agnati et al., 1983a, c, 1986; Fuxe et al., 1983a, b, 1986a, b).

   From a pathological point of view it should be considered that any alteration in one of these processes can cause abnormalities in the sensitivities of several receptors and in the proper activation and balance of the multiple effector systems often triggered by the ligand binding to one and the same receptor.
2. The receptor–receptor interaction may make possible the appearance of novel receptor subtypes like the GABA B receptor (see Marshall, 2005). Also the A1R/P2Y1R heteromerization leads to the appearance of an A1 receptor with a binding site showing P2Y agonist like pharmacology (Nakata et al., 2005). Thus, the pharmacology of the binding pockets in the receptors participating in the formation of the heteromer may become markedly modulated. In pathological conditions ''abnormal'' receptors may be formed via the interactions of monomers that should not interact. Such pathological interactions can also be thought of to occur through a ''morpheein-like'' phenomenon with the formation of an aberrant receptor assembly due to a pathologic conformational state in the monomer (Agnati and Fuxe, in preparation).
3. The conformational changes in the receptors caused by the receptor–receptor interactions may lead to the formation of novel interactions with other membrane proteins especially other receptors including ligand gated ion channels and different types of G proteins. Thus, novel RM may be formed and others may disappear and with the RM having novel interactions with other membrane associated proteins like scaffolding and adapter proteins. Together they form the horizontal molecular networks (HMN) in the lipid rafts which are specialized liquid-ordered platforms in the surface membrane for HMN involved in signal integration and transduction and where the RM forms a crucial node (see Fig. 8) (see Agnati et al., 2005d). In pathological conditions ''abnormal'' HMN may be formed and either inactive or pathological protein mosaics may appear (Agnati and Fuxe, in preparation).
4. The receptor–receptor interactions also have a major impact on receptor cotrafficking like receptor maturation, cell surface expression and internalization (Bouvier, 2001). Such events are of high relevance for sensitization and desensitization of receptors and especially for their crossmodulation. Experimental studies for analysis of receptor colocation and cotrafficking including coclustering and cointernalization with focus on computer assisted image analysis are indicated in Fig. 10 (see Hillion et al., 2002; Agnati et al., 2005b; Genedani et al., 2005).

An important methodology for analysis of colocation and cotrafficking of receptors will be atomic force microscopy (AFM). In Fig. 11 the A2A receptors labelled with 15 nm immunogold particles are visualized with this technique (Agnati and Fuxe, unpublished data).

Studies have been carried out in CHO cells, which have been cotransfected with human HA tagged-A2A and human D2 long cDNAs (Torvinen et al., 2004, 2005b). These cells are known to contain A2A/D2 heteromers and with the AFM technique immunogold clusters of A2A receptors and their area can be determined in a sampled area. In the controls a large number of small clusters of A2A receptors are detected.

After incubation with the D2 agonist quinpirole (50 μM) for 3 h a reduction in the number of clusters of A2A receptors has taken place associated with an increase in their size (Fig. 11). These results can be explained by a coclustering of A2A/D2 heteromers upon the D2 activation (see Hillion et al., 2002). After 8 h with quinpirole there is a further reduction in the number of clusters of A2A receptors associated with a reduction in the size of the clusters. Such results indicate a preferential internalization of the large size clusters of A2A receptors and can be explained by a preferential cointernalization of large size clusters of

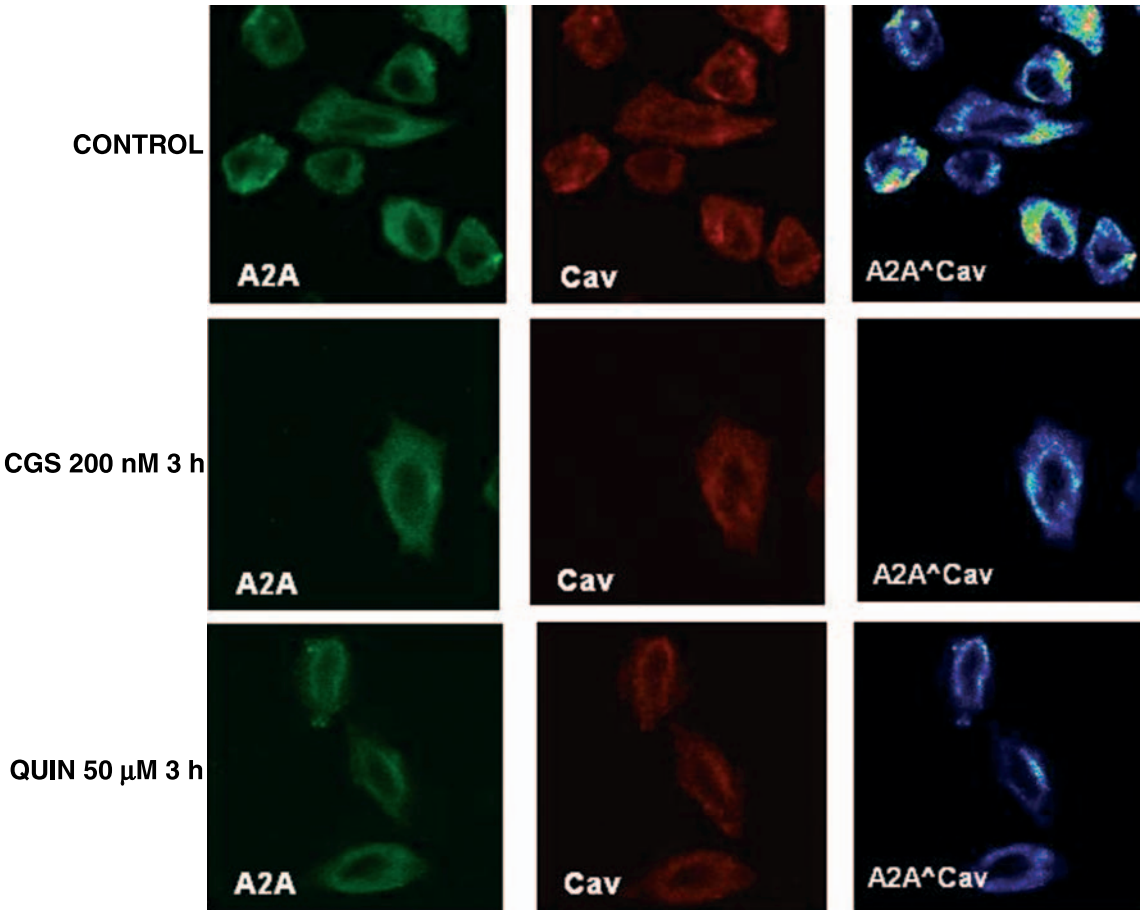
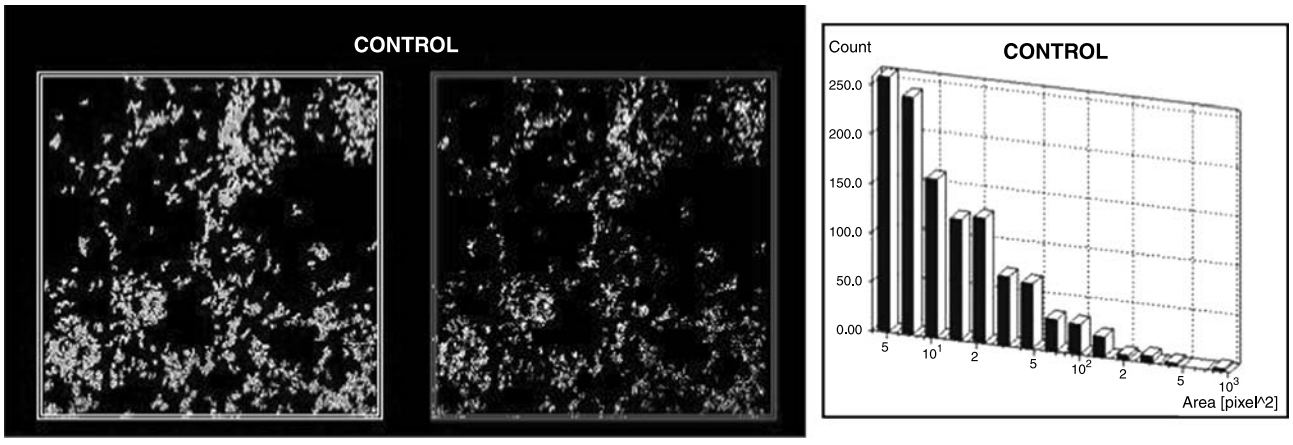


Fig. 10. Illustration of the quinpirole (D2 agonist, 50 μM; 3 h) and CGS 21680 (A2A agonist, 100 nM, 3 h) induced internalization (disappearance from cell membrane) of A2A/caveolin-1 IR in A2A/D2 cotransfected CHO cells using the multiply method. The degree of colocation is shown in pseudocolors in the A2A/CAV-1 picture. The high intensity product pixels are shown in red to white where you have the high physical association of the two signals and thus of the caveolin-1 and A2A IR. The low intensity product pixels are shown in blue to green and represent pixels with low association of the two signals and thus of Cav-1 and A2A IR. The D2 and A2A agonist induced internalization of the A2A/Cav-1 IR is seen as a disappearance of the colocated hot spots and is explained by the existence of A2A/D2 heteromers leading to the cointernalization of a A2A/D2/Cav-1 macrocomplex. For details, see Genedani et al. (2005)

A2A/D2 heteromers upon prolonged D2 activation. In pathological conditions ''abnormal'' receptor (and/or protein) mosaics are formed and hence membrane associated proteins may show altered cotrafficking.

Functional integrative implications

We will only deal with the intramembrane receptor–receptor interaction and its possible role in learning and memory. Learning in neuronal networks takes place by changing their synaptic weights leading to changes in their synaptic efficacies (Hawkins et al., 1993) The receptor mosaic hypothesis states that this may be brought about by reorganization of the available RM structurally and/or by resetting the multiple receptor–receptor interactions in these RM as well as by the formation of novel RM via alterations in WT and VT signals (see Fig. 12) (see Agnati et al., 1982, 2003b, 2004b; Fuxe et al., 1983c). Already at the Congress in Sigtuna ''On the role and control of random events in biological systems'' in 1995, Agnati and Fuxe proposed that, in some instances, RM can behave as random Boolean networks and this possible model has been further developed (see Zoli et al., 1996; Agnati et al., 2003b, 2004b) until the most recent submitted paper (see Agnati, Guidolin, Fuxe: this special issue). The basic tenet of this model is the possibility that circulation of information within a RM moves towards spontaneous order. In general terms, the behaviour of the information handling in the RM depends on the Boolean switching rules and/or the number of inputs involved (see Kauffman, 1993; see Agnati, Guidolin and Fuxe, this special issue). The RM may therefore rapidly reach a transient frozen state which may be the molec-



CHO CELLS: DETECTION OF CLUSTER OF IMMUNOGOLD PARTICLES

Immunolabelling: A2A (15 nm); AFM tapping mode

		AREA	
	COUNTS	**MEDIAN (nm^2)**	**MEAN (nm^2)**
CONTROL	**951**	**138**	**930**
QUINPIROLE (50 μM; 3 h)	**147**	**608**	**9601**
QUINPIROLE (50 μM; 8 h)	**144**	**590**	**2217**

Fig. 11. CHO cell were cultured as described in previous papers (see, e.g., Torvinen et al., 2005). CHO cells were stably transfected with a double hemagglutinin-tagged (HA-tagged) dog adenosine A2A receptor cDNA (a kind gift from Dr. M. Olah, 1230 kb cDNA fragment cloned into the pcDNA/Hygro +, conferring resistance to Hygromycin), with lipefect AMINE plus reagent (Life Technologies, Inc). For coexpression of HA-A2A and D2 receptors, the human dopamine D2L (long form) receptor cDNA (2600 kb cDNA fragment cloned into the Plxsn-vector, which confers resistance to geneticin), was similarly transfected into the CHO cell line expressing stable A2A receptors (HA-A2A/D2 cell line), and the clones resistant to geneticin and hygromycin were selected (for further details, see Torvinen et al., 2005). As far as the immunogold staining cells were grown on glass slides (Chamber Slide Culture, Labtek/Nunc, VWR International srl, Milano, Italy) coated with poly-L-lysine (Sigma, Milano, Italy). Cells were then rinsed in PBS, fixed in 4% paraformaldehyde and gluteraldehyde 2% for 20 min and washed with PBS containing 20 mM glycine and subsequently treated with PBS/20 mM glycine/1% BSA for 30 min at room temperature. Immunostaining was performed with the affinity purified mouse anti-HA antibody (Roche SpA, Milano, Italy) in PBS, pH 7.4, supplemented with 1% normal serum at 4°C overnight. The cells were then rinsed three times for 10 min in Tris pH 7.4, three times for 5 min in tris pH 7.4 + BSA 0.2%, one time for 15 min in Tris pH 8.2 + BSA 1% and incubated with gold particle (15 nm) coniugated anti-mouse antibody (1:25) in Tris pH 8.2 + BSA 1% for 1 h at room temperature. Cells were then rinsed twice for 10 min in Tris pH 7.4. Atomic force microscopy (AFM, PARK Autoprobe CP instrument) was carried out on the A2A/D2 cotransfected CHO cells after immunogold labelling of A2A receptors with 15 nm immunogold particles. An area of 2×2 μm was scanned by the AFM tip (in tapping mode) to image regions with different visco-elastic properties. By means of this approach the effects of the D2 agonist quinpirole (50 μM 3 h and 8 h) on the clusters of immunogold particles have been analized. The results are shown in the table of the figure with an increase of the mean cluster area and a reduction in their number at 3 h after quinpirole (Agnati, Fuxe et al., in preparation)

ular basis for a transient engram and thus for short term memory, leading to a change in the synaptic weight.

The engram consolidation and thus long term memory may be brought about by the transcriptional panorama (involving partially internalized RM) induced by the repeated activation of the novel or altered transient RM and its associated HMN and VMN. This will lead to the activation of immediate early genes and the postulated formation of unique adapter and scaffolding proteins which stabilize the RM by binding to them. In this way long-lived heteromeric receptor complexes are formed where the frozen RM represents the memory trace. It is possible that phosphorylation events can participate in this memory process via enabling stronger electrostatic epitope–epitope interactions in the heteromeric complex (Woods et al., 2005). It may also be considered that reconsolidation of these frozen RM may take place by the ability of the unique adapter proteins to cause a certain constitutive activity of one of the recep-

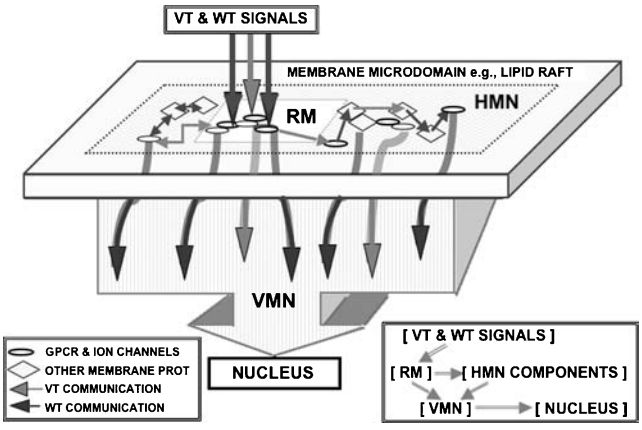


Fig. 12. Illustration of the receptor mosaics in the lipid rafts of the surface membrane reached by WT and VT signals and being part of horizontal molecular networks of GPCR, ion channels and other types of membrane proteins. Their activation will change the WT and VT communication in the cytoplasm to change the transcriptional panorama and change gene expression

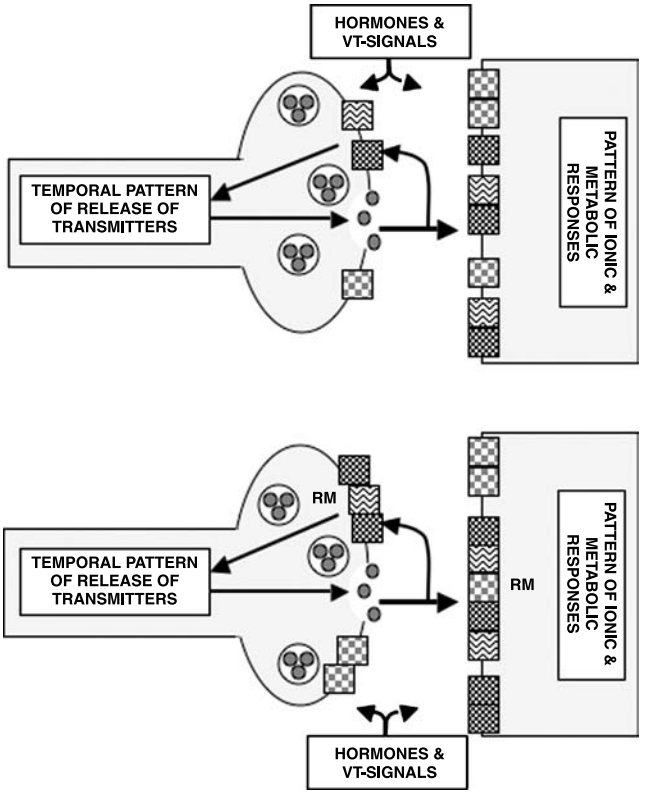


Fig. 13. Schematic representation of the molecular basis of the Hebb's synapse. Basal state is on top and the trained state below. In training the chemical transmitter code is learnt by producing a unique ionic metabolic state of the postsynaptic cell caused by a reorganization of the postsynaptic and extrasynaptic receptor mosaics on the postsynaptic side leading to a unique firing pattern of the cell linked to the presynaptic firing pattern to be learned. The reorganization in the presynaptic and extrasynaptic receptor mosaics on the presynaptic side will help maintain the pattern of transmitter release and intrasynaptic and extrasynaptic transmitter levels which is the code to be learned by the postsynaptic receptor mosaics (see Agnati et al., 2003a)

tors leading to an ordered activation of the RM, rehearsal of the electrotonic events and reappearance of the transcriptional panorama with continued formation of the unique adapter proteins and maintenance of the frozen RM and thus of the engram.

This hypothesis agrees with the Hebbian rule that memory is associated with simultaneous firing of the pre and postsynaptic nerve cells causing permanent changes in the functional properties of the postsynaptic nerve cell (Hebb, 1949). We can now give a molecular basis to this rule by postulating that the repeated temporal pattern of a transmitter and modulator code in the synaptic cleft becomes linked to a special firing pattern and metabolic activity thanks to the formation and resetting of RM in the postsynaptic membrane (Fig. 13) (Agnati et al., 2003b). Rapid and transient changes in the RM involving also formation of novel RM may also take place in the presynaptic membrane in order to favour the pattern of neurotransmitter release to be learnt. Activation of prejunctional receptors via pre and postsynaptic VT and WT signals as well as retrograde signals may importantly contribute to the plasticity changes in the presynaptic RM (Fig. 13) (Agnati et al., 2003b). Subsequently, with time novel adapter and scaffolding proteins may be formed and reach the terminals to form long-lived heteromeric receptor complexes containing the frozen RM as postulated for the postsynaptic membrane.

The engram retrieval may take place via scanning of the target networks by the arousal systems until the correct tuning of the synaptic weights has been obtained leading to the reappearance of the engram (Agnati et al., 2004a).

In the striosomal GABA system of the basal ganglia the consolidated RM may have a special role in motivational learning of motor skills (Agnati et al., 2003b). In conclusion, according to our hypothesis long-lived heteromeric receptor complexes with frozen RM in various nerve cell systems play an essential role in learning, memory and retrieval processes where the molecular engrams can be integrated by extensive reciprocal feedback loops giving rise to coherent synchronized neuronal activity in the participating nerve cell populations.

It seems possible that ''pathological'' RM could be at the basis of neuropsychiatric disorders. For example, tardive dyskinesia could be caused by the formation of special RM in the basal ganglia. Thus, it has been postulated that upon activation of such RM and the molecular circuits they are part of in neuronal networks of the striatum especially the islandic networks, abnormal activities may develop in the indirect and direct pathways of the basal ganglia leading to the dyskinesias

(Agnati et al., 2003b). Similarly, fobic and compulsive behaviours could be favoured by the formation and the continuous rehearsal of the ordered activation of pathological RM in the basal ganglia and underline the learning related functions of the basal ganglia (Graybiel, 2005).

Pathological implications and new drug developments: intramembrane receptor–receptor interactions and novel treatments of Parkinson's disease, schizophrenia and depression

Studies in animal models of human diseases can uncover pathological mechanisms underlying neuropsychiatric diseases and hence device new treatment strategies. We are focusing our attention on the CNS but receptor–receptor interactions have certainly an important role also in the peripheral apparatuses (see below). Thus, alterations in receptor–receptor interactions may play a role for systemic diseases and hence also for these pathologies new drugs will be developed on the basis of this biochemical mechanism (see Quitterer et al., 2004). Some relevant brain pathologies will now be analyzed.

Development of A2A receptor antagonists in treatment of Parkinson's disease (PD) based on the A2A/D2 receptor interaction in the dorsal striatum

This approach began with the behavioural observations that caffeine and theophyllamine can enhance the effects of L-dopa and DA receptor agonists at supersensitive DA receptors in an unilateral lesion model of Parkinson's disease based on an analysis of contralateral turning behaviour (Fuxe and Ungerstedt, 1974, 1976). Subsequently, it became clear that methylxanthines may act as adenosine receptor antagonists (Fredholm et al., 1976) to produce such effects. Furthermore, intramembrane antagonistic A2A/D2 receptor–receptor interactions may be involved in these behavioral actions, since A2A activation reduced the affinity of the agonist binding site of the D2 receptors, especially the high affinity component in striatal membrane preparations (Ferré et al., 1991) and also the D2/Gi protein coupling (see Ferré et al., 1997; Fuxe et al., 1998). Later on the A2A receptor antagonists were also demonstrated to show antiparkinsonian actions in rat and nonhuman primate models of PD including reserpinized mice and haloperidol exposed cataleptic mice (Pinna et al., 1996; Pollack and Fink, 1996; Fenu et al., 1997; Le Moine et al., 1997; Kanda et al., 1998; Shiozaki et al., 1999; Strömberg et al., 2000; Ferré et al., 2001; Fuxe et al., 2001; Morelli and Wardas, 2001). A2A antagonists can also dose-dependently increase the locomotor activity of subthreshold doses of L-dopa and D2 like agonists in reserpinized mice, which can be explained by the blockade of the A2A receptor in the A2A/D2 heteromer located in the striato-pallidal GABA neurons (see below), leading to enhancement of D2 signalling (Tanganelli et al., 2004). This molecular mechanism may also explain the ability of A2A antagonists to counteract parkinsonian symptoms in presence of low doses of L-dopa (Hauser et al., 2003; Chase et al., 2003; Xu et al., 2005) sometimes without the appearance of increased amount of dyskinesias (Bara-Jimenez et al., 2003). It should be considered that lowering of the L-dopa dose will reduce the intermittent activation of the transcriptional panorama by L-dopa in the direct D1 rich GABA pathway and in the D2 rich striato-pallidal GABA neurons, which may lead to decreased development of dyskinesias. Thus, the fine tuning via A2A receptor antagonists may represent a more physiological way of enhancing D2 signalling (see Fuxe et al., 2003). An additional mechanism of action by A2A antagonists in models of PD may also be blockade of the increased A2A signalling that develops with deficits in D2 signalling due to the removal of the D2 mediated inhibition of A2A activated adenylate cyclase and of other mechanisms (see Fuxe et al., 2001; Morelli and Wardas, 2001; Antonelli et al., 2006).

In conclusion, A2A antagonists may represent novel antiparkinsonian drugs targeting the A2A/D2 heteromer, where the antagonistic A2A/D2 receptor interaction takes place leading to reduced D2 mediated inhibition of the striato-pallidal GABA pathway which causes motor inhibition.

Development of mGluR5 antagonists for treatment of Parkinson's disease based on multiple mGluR5/A2A/D2 receptor interactions in the dorsal striato-pallidal GABA pathway

In 1984 Fuxe, Agnati and Celani found that glutamate reduced the affinity of the high affinity D2 agonist binding sites in striatal membrane preparations (Fuxe et al., 1984). In 1999 evidence was found that group I mGluR subtypes may mediate this receptor–receptor interaction (Ferré et al., 1999). A2A and group I mGluR synergistically increased the Kd value of the high affinity D2 agonist binding sites, which was associated with an ability of these two receptors when activated to synergistically counteract D2 agonist induced contralateral turning behaviour in a rat model of PD (Ferré et al., 1999). In 2001 similar results were obtained with the mGluR5 agonist CHPG giving evidence for intramembrane antagonistic mGluR5/D2 interactions involving interactions with the A2A receptors to strongly re-

duce D2 signaling (Popoli et al., 2001). These results were further strengthened by the discovery of a mGluR5/A2A heteromeric receptor complex (Ferré et al., 2002), which could be the basis for the synergistic A2A/mGluR5 mediated antagonism of phencylidine-induced motor activity (D2 dependent; Ferré et al., 2002) and by results from microdialysis experiments showing synergism of A2A agonists and mGluR5 agonists in increasing GABA release in the ventral striato-pallidal GABA pathway (Diaz-Cabiale et al., 2002).

This series of papers indicated the usefulness of employing not only A2A antagonists but also mGluR5 antagonists and their combinations in the treatment of PD by increasing D2 signalling in A2A/mGluR5/D2 receptor mosaics located mainly in perisynaptic regions of glutamate and DA synapses on dendritic spines of striato-pallidal GABA neurons (see Agnati et al., 2003a; Ferré et al., 2002, 2004; Fuxe et al., 2003) and by reducing glutamate release from corticostriatal glutamate terminals, where A2A and mGluR5 can interact synergistically in A2A/mGluR5/D4 receptor mosaics to increase glutamate release (Pintor et al., 2001; Tanganelli et al., 2004; Rodrigues et al., 2005).

In the same time period chronic treatment with the mGluR5 antagonist MPEP was shown to counteract motor deficits in Parkinsonian rats (see Coccurello et al., 2004) and acutely MPEP could reduce haloperidol induced muscle rigidity and catalepsy (Ossowska et al., 2001), indicating in fact that mGluR5 antagonists can improve motor function also beyond the D2 receptors by actions, e.g., on the striatal glutamate release (see above) and on the mGluR5 in the subthalamic glutamate system reducing its activity. Functional interactions between A2A and mGluR5 receptors in the striatum were early on demonstrated by Kearney and Albin (1995). Recently, Schwarzschild, Chen, Young and colleagues (Kachroo et al., 2005) have obtained convincing evidence of interactions between mGluR5 and A2A receptors in normal and Parkinsonian mice, involving the use of single and double A2A and mGluR5 knockout mice including a forebrain specific conditional knockout of the A2A receptor. This work strongly supports the combined use of A2A antagonists and mGluR5 antagonists as a novel strategy for the treatment of PD. In addition, both types of drugs also show neuroprotective potential besides their antiparkinsonian effects mediated via transmission changes in the basal ganglia (Marino et al., 2003; Xu et al., 2005; Battaglia et al., 2004; Aguirre et al., 2005).

As to the mechanism of action of mGluR5 antagonists it should also be considered that mGluR5 enhances the NMDA currents in the medium sized spiny neurons (see Conn et al., 2005) probably via formation of a heteromeric complex with the NMDA receptor involving scaffolding-anchoring-adapter proteins like PSD-95, Shank and Homer in the postsynaptic membrane of the striatal glutamate terminals. Thus, blockade of the mGluR5 in this synaptic mGluR5/NMDA heteromeric complex found in many glutamate synapses all over the brain may contribute to the antiparkinsonian action of mGluR5 antagonists although the major targets may be the extrasynaptic postjunctional A2A/mGluR5/D2 RM and the extrasynaptic prejunctional A2A/mGluR5/D4 RM in view of the strong interactions between mGluR5 receptors and the A2A receptors (Fig. 14). The present evidence would strongly favour the development of antiparkinsonian drugs with combined mGluR5 and A2A antagonist properties to increase D2 signalling via a non-dopaminergic therapy.

Development of A2A agonists for treatment of schizophrenia based on the intramembrane A2A/D2 receptor interaction in the ventral striato-pallidal GABA pathway

Blockade of D2 receptors still plays a key role in mediating the antipsychotic actions of neuroleptic drugs (Kapur

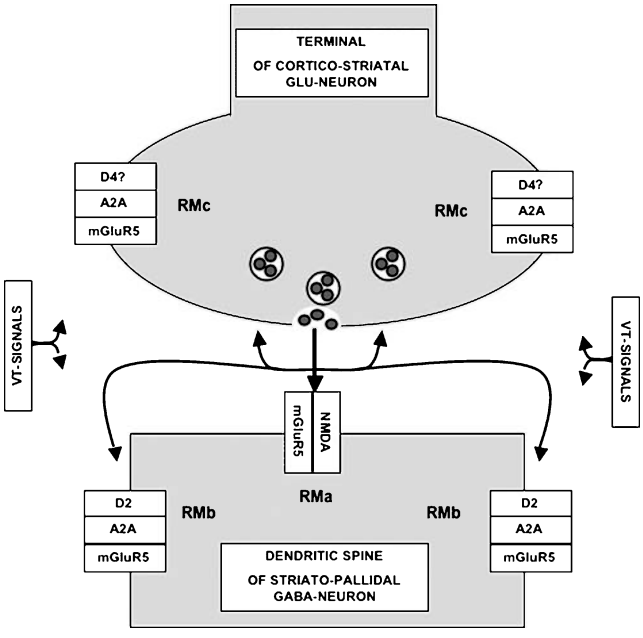


Fig. 14. Schematic representation of possible receptor mosaics (RM) in the corticostriatal glutamate synapse on the dendritic spine of the striatopallidal GABA nerve cell. RMa show the synaptic NMDA/mGluR5 RM in the postsynaptic membrane; RMb shows the postjunctional extrasynaptic mGluR5/A2A/D2 RM on the dendritic spine outside the glutamate and dopamine synapses. RMc shows the prejunctional extrasynaptic mGluR5/A2A/D4? RM. Their existence and the multiple receptor–receptor interactions within them can explain a large number of observations. For details, see text

and Mamo, 2003). The first evidence that antipsychotic drugs blocks DA receptors was obtained by Carlsson and Lindquist (1963), supported subsequently by further evidence obtained by Anden et al. (1966, 1970) in a combined neurochemical and functional analysis. The present DA hypothesis of schizophrenia, however, has become more complex and now includes also the glutamate hypofunction hypothesis. It proposes that there exists a hypofunction of the mesocortical DA systems in response to hypofrontality in the prefrontal cortex with reduced activity in prefrontal glutamate afferents to the ventral tegmental area DA cell bodies projecting back to the neocortex (Carr and Sesack, 2000). In this process reduced NMDA mediated glutamate transmission seems to play a major role and the resulting reduction of neocortical D1 mediated transmission may contribute to the deficits in cognition and to the negative symptoms of schizophrenia (see Goldman-Rakic et al., 2004). Results of this type have been obtained in the phencyclidine (NMDA channel antagonist) model of schizophrenia.

However, in contrast a hyperactive mesolimbic DA system develops in the PCP model of schizophrenia (see Jentch and Roth, 1999; Svensson, 2000). In agreement, hypofrontality predicts enhanced striatal DA activity in schizophrenia (Meyer-Lindenberg et al., 2002). The results may be explained by neuroanatomical findings indicating that prefrontal glutamate afferents modulating the activity in the mesoaccumbens DA neurons operate via inhibitory GABA interneurons (Carr and Sesack, 2000). A reduced glutamate drive will therefore result in reduced GABA inhibition and increased activity in the meso-limbic DA neurons (Murase et al., 1993). The increase in D2 mediated limbic DA transmission especially in the nucleus accumbens will via the ventral pallidum lead to a reduced glutamate drive from the mediodorsal thalamic nucleus to the prefrontal cortex and further reduce the hypoglutamatergia in the cortex (see Fuxe et al., 1998), and thus worsen the impairment of the cortical NMDA mediated glutamate transmission. This part of the revised DA hypothesis of schizophrenia can thus explain the antipsychotic effects of D2 receptor antagonists, especially with regard to the improvement of positive symptoms seen as highly intense emotional manners and behaviours in response to delusions and hallucinations in view of the involvement of the mesolimbic DA neurons in emotional behaviours like fear and motivation.

In a series of papers (Ferré et al., 1994, 1997; Rimondini et al., 1997) we have advanced the proposal based on the DA hypothesis of schizophrenia outlined above that A2A agonists may be novel antipsychotic drugs by antagonizing the D2 receptor signalling via an A2A/D2 intramembrane receptor–receptor interaction in the ventral striato-pallidal GABA system. The A2A agonist CGS21680 was shown to have an atypical antipsychotic profile by reducing the amphetamine and PCP induced locomotor activity in doses failing to cause catalepsy. Furthermore, the injection of the A2A agonist into the nucleus accumbens reversed the inhibition of prepulse inhibition by apomorphine (Hauber and Koch, 1997) and combined treatment with subthreshold doses of a D2 antagonist and an A2A agonist led to an activation of the ventral striato-pallidal GABA pathway (Ferré et al., 1994). In line with these results the increase in fos-like immunoreactivity in the nucleus accumbens after treatment with antipsychotic drugs like clozapine and hapoperidol was counteracted by treatment with an A2A antagonist (Pinna et al., 1999). CGS 21680 also potently reduces the affinity of DA receptors in the nucleus accumbens (Diaz-Cabiale et al., 2001). In higher doses CGS 21680 but not an A1 receptor agonist could antagonize the DA receptor agonist induced stereotyped behaviours, which are elicited from DA receptors in the dorsal striatum (Rimondini et al., 1998). It is of substantial interest that the A2A agonist demonstrates antipsychotic like activity in Cebus apella monkeys without production of extrapyramidal side effects (Andersen et al., 2002) underlining the development of novel A2A agonists as a strategy for treatment of schizophrenia based on their atypical antipsychotic profile.

It is true that A2A antagonists have not been found to cause psychotic episodes in man. However, this may be related inter alia to low endogenous A2A receptor activity in the subcortical limbic regions due to low extracellular levels of adenosine. This would also make the accumbens A2A receptors more sensitive to A2A agonists vs those in the dorsal striatum, where A2A antagonists cause motor activation.

A2A/D3 receptor heteromers with antagonistic A2A/D3 receptor interactions have also been demonstrated in cotransfected CHO cell lines (Torvinen et al., 2005a). However, their possible existence in the ventral striato-pallidal GABA neurons remains to be clarified as well as their functional interactions in the nucleus accumbens.

Development of agonists with combined A2A agonist/mGluR5 agonist properties for the treatment of schizophrenia based on the multiple mGluR5/A2A/D2 receptor interactions in the ventral striato-pallidal GABA pathway

The experimental evidence suggests that synergistic interactions between A2A and mGluR5 receptors based on the

existence of A2A/mGluR5 heteromeric complexes (Ferré et al., 2002) in postulated extrasynaptic mGluR5/A2A/D2 receptor mosaics of the ventral striato-pallidal GABA neurons play a major role in increasing activity of this pathway and removing it from D2 mediated inhibition. This has been demonstrated in dual probe microdialysis studies on this GABA system using coperfusion with A2A agonists and mGluR5 agonists (Diaz-Cabiale et al., 2002). Also in a behavioural analysis central coadministration of CGS 21680 and the mGluR5 agonist CHPG counteracted PCP induced motor activation known to be mediated via D2 receptor activity (Ferré et al., 2002). This behavioural inhibition by A2A and mGluR5 coactivation was correlated with a synergistic activation of c-FOS IR in the nucleus accumbens, which may be caused by a synergistic increase of the ERK1/2 phosphorylation as observed in HEK 293 cells (Ferré et al., 2002).

Based on the above observations it seems reasonable to suggest that drugs with combined A2A agonist and mGluR5 agonist properties may have antipsychotic properties by restoring the drive in the ventral striato-pallidal GABA pathway through counteraction of D2 signalling in the RM discussed above based on the multiple receptor–receptor interactions.

Such drugs will thus via the circuitry controlled by this pathway increase the activity in the cortical glutamate afferents from the medio-dorsal thalamic nucleus to the prefrontal cortex, increase glutamate transmission in this region and counteract the hypofrontality. Also D2 mediated emotional responses in the limbic regions will be reduced. These combined agonists may also increase glutamate transmission by synergistically increasing glutamate release in the subcortical limbic regions via coactivation of A2A and mGluR5 receptors in prejunctional RM on the cortico-limbic glutamate terminals (Pintor et al., 2001). It may be that partial rather than full mGluR5 agonist properties in these postulated novel antipsychotic drugs may be preferred in view of possible excitotoxic actions caused by full mGluR5 agonists (see Jeffrey Conn et al., 2005).

It should be considered that as recently shown by the Agnati and Fuxe groups the antipsychotic D2 receptor antagonists can stabilize the D2 receptor on the cell membrane and reduce the cointernalization of the postulated mGluR5/A2A/D2 receptor mosaic (Torvinen et al., 2005). Such an action may contribute to D2 receptor supersensitivity development under antipsychotic therapy resulting in resistance development. Such a molecular mechanism may, however, be counteracted by giving drugs with A2A and MGluR5 agonist properties which would increase the internalization of this RM (Hillion et al., 2002; Torvinen et al., 2005b) and allowing a reduction of the dose of the D2 antagonist, leading to reduced side-effects.

Development of galanin receptor antagonists for treatment of depressive illness based on the galR/5-HT1A receptor interactions

In 1988 galanin was shown to reduce the affinity of 5-HT1A receptors in the ventral limbic cortex (Fuxe et al., 1988a), giving the first evidence for the existence of antagonistic intramembrane GalR/5-HT 1A receptor interactions. In 1991 the reciprocal interaction was demonstrated with evidence that 5-HT1A receptor activation leads to an increase in the affinity of galanin receptors in various regions of the tel- and diencephalons (Hedlund and Fuxe, 1991). This increase of galanin recognition may be part of an intramembrane inhibitory feedback mechanism to reduce overactivation of 5-HT 1A signalling taking place via the interface in a postulated GalR/5-HT 1A heteromeric complex. The same year the relevance of this receptor–receptor interaction for depression was discussed in the frame of the 5-HT hypothesis of depression (Fuxe et al., 1991) and the proposal was made that galanin receptor antagonists by enhancing postjunctional 5-HT1A mediated transmission in the forebrain may represent novel antidepressant drugs. In line with this proposal it was also found that chronic treatment with imipramine could increase the affinity of the galanine receptor binding sites in the tel and diencephalon (Hedlund and Fuxe, 1991) probably as a result of increased activation of 5-HT1A receptors due to increased extracellular levels of 5-HT caused by the blockade of the 5-HT transporter by imipramine. Thus, a galanin receptor antagonist should increase the therapeutic actions of known antidepressant drugs targeting and blocking the serotonin transporter.

Galanin receptor antagonists may also produce antidepressant effects by blocking galanin receptors in the dorsal raphe, which inhibit the 5-HT releasing activity and firing of the ascending 5-HT pathways to the tel- and diencephalon (Fuxe et al., 1988b; Kehr et al., 2002; Xu et al., 1998). The galanin receptor on the HT cell bodies interacts with the 5-HT1A autoreceptor (Razani et al., 2000) but the functional outcome of this interaction remains to be clarified in terms of 5-HT1A autoreceptor signalling. It is of substantial interest that an increased density of galanin receptor agonist binding sites has been found in the dorsal raphe in a genetic model of depression (Bellido et al., 2002), leading to a feed-back inhibition of galanin synthesis with reductions of galanin immunoreactivity in the dorsal raphe (Bellido et al., 2002) and indications of reduced extracel-

lular release of 5-HT. The antidepressant-like behaviour found in mice lacking the 5-HT 1A receptor may be related to the disappearance of the inhibitory 5-HT 1A autoreceptor with increased activity in the ascending 5-HT pathways (Heisler et al., 1998; Parks et al., 1998).

In view inter alia of the indications that classical antidepressants may block 5-HT2 receptors (Fuxe et al., 1977; Ögren et al., 1979; Peroutka and Snyder, 1979) and that inhibition of 5-HT7 receptors and their inactivation produces antidepressant-like behaviour (Hedlund et al., 2005) the 5-HT hypothesis of depression should be modified to state that depression may be induced when a unbalance in the activation of the various 5-HT receptor subtypes in the brain takes place. The role of the galanin receptor antagonist would be to enhance the postjunctional 5-HT1A signalling and to increase the activity of the ascending 5-HT pathways. However, it still remains to test if galanin receptors can modulate the function also of other 5-HT receptor subtypes.

It is still unclear which galanin receptor subtype is involved in forming the postulated heteromeric complex with 5-HT1A receptors. Based on the existence of electrostatic epitope–epitope interactions in the interface of heteromers (Woods et al., 2005) it seems likely that Galanin R3 is importantly involved. Thus, the strongest electrostatic interaction can be demonstrated between the GalR3 and the 5-HT1A receptor. In agreement it has recently been shown that galR3 antagonists possess antidepressant like behavioural activity (Swanson et al., 2005).

It is of substantial interest that only specific N terminal gal fragment binding sites have been found in the dorsal hippocampus (Hedlund et al., 1992) and in this region only galanin (1–15) but not galanin (1–29) modulates the 5-HT1A receptors (Hedlund et al., 1994). Thus, galanin receptor subtypes still not cloned and preferentially binding N terminal galanin fragments may be forming heteromers with the 5-HT1A receptors. Alternatively, galanin receptors acquire different binding characteristics according to the RM in which they are involved or finally other unknown neuropeptide receptors in the dorsal hippocampus binding N terminal galanin fragments with high affinity may be interacting with the hippocampal 5-HT1A receptors.

The A2A heteromerization example

Vast information has been collected on the adenosine receptor interactions with other receptors for classical transmitters. It could be an important field to investigate whether adenosine receptors can interact also with non-classical transmitter receptors. As a matter of fact it seems that adenosine, in agreement with its functional role as modulator of neuronal activity (Fredholm and Svenningsson, 2003), can have as target several RM formed by different heteromers. Some of these have been extensively studied and have been shown to be of highest interest for the development of new therapeutical approaches (Fuxe et al., 2003).

The A2A/D2 heteromer

The first evidence for the existence of an A2A/D2 heteromeric receptor complex was obtained in coimmunoprecipitation experiments in neuronal cell lines and fibroblast cell lines, showing also a lack of coimmunoprecipitation of A2A and D1 receptors (Hillion et al., 2002). Subsequently, coimmunoprecipitation of A2A and D2 receptors was also observed in rat striatal tissue (Patkar et al., in prep.).

Evidence for a direct and specific interaction between A2A and D2 receptors was obtained with a quantitative BRET analysis and sensitized emission FRET as well as acceptor photo-bleaching FRET analysis (Kamiya et al., 2003; Canals et al., 2003). However, the stoichiometry in the A2A/D2 heteromer is unknown. Nevertheless, even if D2 receptors exist in proportion 4:1 vs the A2A receptors, according to the Agnati and Fuxe model (Fuxe et al., 2006) A2A may still exert an efficient antagonistic control of D2 function by regulating cooperativity in the D2 tetramer, which depends on the topography of the participating A2A and D2 receptors (Fig. 15) (see Fuxe et al., 2006). Recently, indications have been obtained in Dr. A. S. Woods' laboratory using mass spectrometry that two A2A epitopes may bind to one D2 epitope (Fig. 16) opening up the possibility that two A2A receptors can bind to one D2 receptor.

A2A homodimers exist and have been detected on the cell surface with time resolved FRET (Kamiya et al., 2003; Canals et al., 2004) and the A2A/D2 heteromers are in balance with the A2A homodimers and the D2 homodimers (Lee et al., 2000) at the membrane and the cytoplasmatic level (Fig. 17). This balance will have a major impact on the electrical and metabolic activity of the striato-pallidal GABA pathway and thus on striatal function.

Based on the use of D1/D2 chimeras the third cytoplasmatic loop and the 5th transmembrane domain of the D2 receptor appears to be part of the A2A/D2 interface (Torvinen et al., 2004). This is in agreement with the results obtained with mass-spectrometry and biochemical pull-down assays by Woods, Franco, Ciruela and colleagues (Ciruela et al., 2004) showing epitope–epitope electrostatic

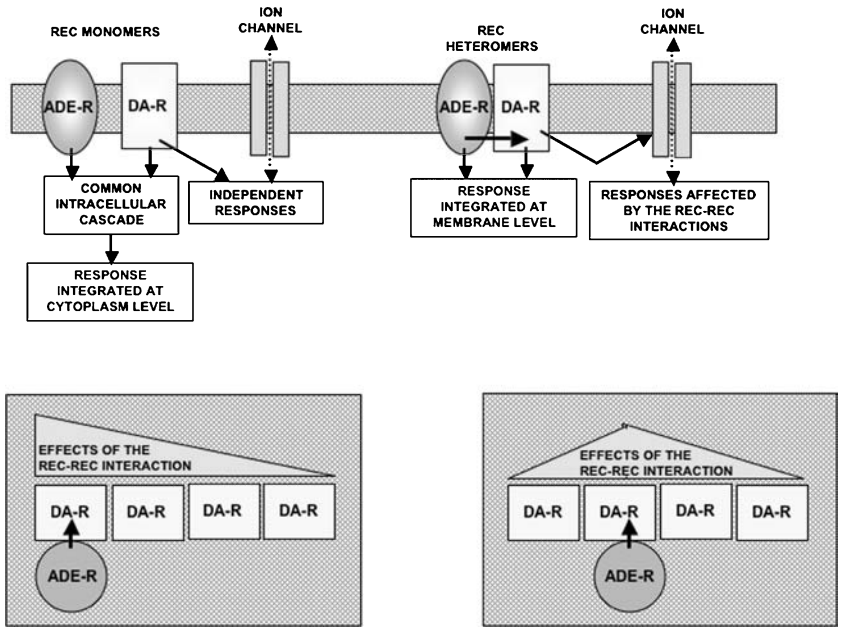


Fig. 15. Illustration of the role of stoichiometry and topology of adenosine/dopamine receptor–receptor interactions. As an example membrane integration of signals can take place via adenosine receptor regulation of DA receptor cooperativity (tetramer; RM1), which depends on the topology of the adenosine/dopamine receptor–receptor interaction

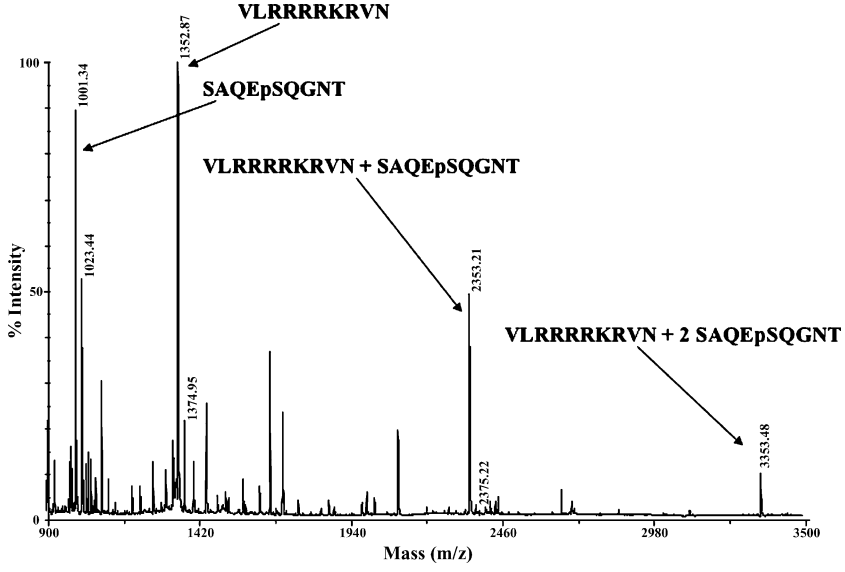


Fig. 16. Positive ion mode MALDI mass spectrum of an equimolar solution of the D2R epitope VLRRRRKRVN and A2A epitope SAQEpSQGNT show formation of noncovalent complexes between one epitope of D2 and one epitope of A2A and also between one epitope of D2 and 2 epitopes of A2A. This is probably due to the fact that 2 adjacent Arginines are sufficient for the interaction to take place, and the D2 epitope has 4 adjacent Arginines making the double interaction possible

interactions between positive charges in adjacent arginins in the N terminal part of the 3rd intracellular loop of the D2 and the negative charges in the A2A C-terminal involving especially a phosphorylated serine (guanidinium–phosphate interactions) (Woods, 2004). These electrostatic interactions may be a general mechanism in receptor–receptor interactions (Woods et al., 2005) and may reach a covalent-like stability (Woods and Ferré, 2005). It follows from this molecular mechanism that phosphorylation/dephosphorylation events will have a major modulation of the strength of the receptor–receptor interactions in the heteromers. In the case of the A2A receptor there exists a casein-kinase I consensus site in its epitope, which can increase the strength of the A2A/D2 interaction (Ciruela et al., 2004; Woods et al., 2005). The A2A/D2 heteromer is constitutive and is not disrupted by agonists. In fact, A2A and D2 agonists do not influence the BRET and FRET signal from the A2A/D2 heteromer (Canals et al., 2003). Instead the A2A agonist CGS 21680 causes a cointernalization of the A2A/D2 heteromer as does the D2 agonist quinpirole (Fig. 10) in neuroblastoma and CHO cells (Hillion et al., 2002; Torvinen et al., 2005b). The Agnati and Fuxe group has also made the important observation that caveolin-1 is involved in the internalization process of at least the majority of the A2A/D2 heteromers in CHO cells (Genedani et al., 2005). Colocation studies could be carried out with

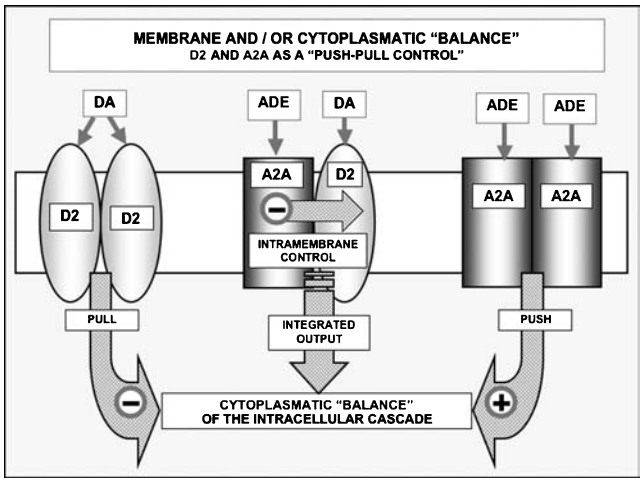


Fig. 17. Illustration of the balance between A2A homomers, A2A/D2 heteromers and D2 homomers at the membrane and cytoplasmatic level in the striatao-pallidal GABA neurons having a major impact on the firing, metabolism and gene expression of the striato-pallidal GABA neurons

a great deal of resolution thanks to a new computer-assisted image procedure developed by our group (Agnati et al., 2005b). Thus, in A2A/D2 cotransfected cells caveolin-1 colocalizes with both A2A and D2 receptors and CGS 21680 or quinpirole preferentially internalized A2A and D2 receptors colocated with caveolin-1. As illustrated in Fig. 10, quinpirole preferentially caused the disappearance of immunoreactive regions with high colocation of A2A and Caveolin IR (shown in red to white with the multiply method, see Genedani et al., 2005; Agnati et al., 2005b). A macrocomplex of A2A/D2/caveolin-1 IR therefore may exist, where Caveolin-1 may have role in the internalization process and thus Caveolin-1 may be involved in the control of the permanence of the heteromer on the surface membrane.

An interesting example of the integrated controls between horizontal molecular and vertical molecular networks through some crucial macromolecules is the demonstration that the A2A and D2 receptors not only directly interact at the level of the membrane, but also beyond the receptors at the cytoplasmatic level. Thus, besides the antagonistic intramembrane A2A/D2 receptor interactions within the heteromer there is the reciprocal crosstalk at the level of the adenylate cyclase (AC) with D2 via Gi/o inhibiting the A2A activated AC (Fig. 18). It is also illustrated how A2A via the receptor–receptor interaction can inhibit the D2 activation of protein phophatase 2B (calcineurin), leading to an increase in the Ca influx over the L-type voltage dependent Ca channels with increases in neuronal excitability (Fig. 18).

Multiple biochemical interactions also takes place in the vertical molecular networks as a result of changes in the activity of calcineurin and Ca/Calmodulin kinase (Agnati et al., 2003a).

It is important to underline that A2A receptors upon activation also causes neurite outgrowth in neuroblastoma cells and in striatal neuronal precursor cells (Canals et al., 2005). This process was associated with the induction of TrkB expression and the arrest of the cells in the G1 phase, suggesting the involvement of A2A receptors in key steps of neuronal differentiation. Activation of protein kinase A (PKA) by A2A is a crucial step in the molecular mechanism leading to the A2A induced neuritogenesis. PKA activation in turn leads to triggering of activity of the MEK/ERK pathway and to the activation of a PKC dependent pathway, which are both required for a full neuritogenesis (Canals et al., 2005). These results show a role of A2A receptors also in neuronal differentiation and in neuronal repair. Therefore, it seems possible that the A2A/D2 heteromer may also have an important role in differentia-

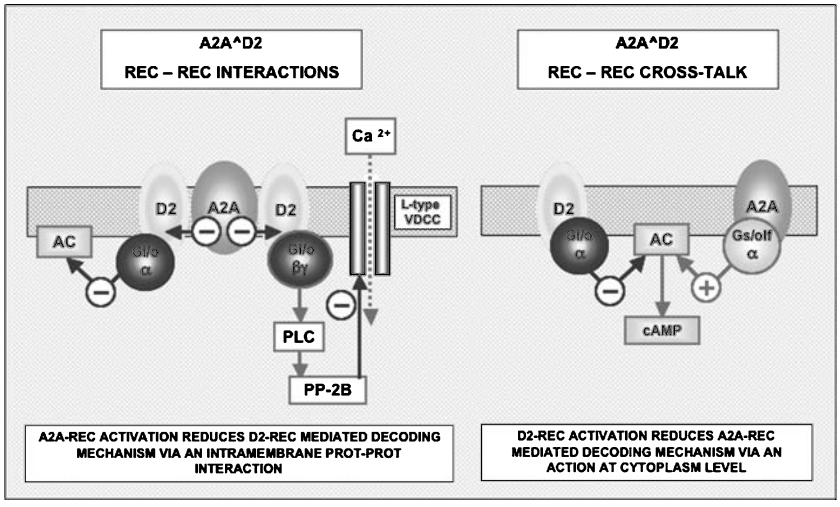


Fig. 18. Illustration of the antagonistic intramembrane A2A/D2 receptor interaction and the inhibitory D2/A2A crosstalk at the level of the adenylate cyclase (AC). The intramembrane receptor–receptor interaction makes it possible to antagonize D2 signaling to multiple effectors inter alia its inhibition of AC via Gi and its inhibition of the Ca influx over the L-type voltage dependent CA channels via activation of phopholipase C and protein phophatase-2B (calcineurin) with dephosphorylation of this Ca channel

tion and trophic mechanisms (Schwartzschild et al., 2003; Agnati et al., 2004c). Thus, the A2A/D2 heteromer could have a major role in development by integrating A2A and D2 signalling with an optimal control of differentiation. Furthermore, in neurodegenerative disease with demands for neuronal repair the A2A/D2 heteromer may be essential to obtain the balance in the A2A and D2 signaling necessary to reach an appropriate neurite outgrowth.

A2A/D3 heteromers

Arginine rich epitopes also exist in the N terminal part of the 3rd intracellular loop of the D3 receptors which could interact with the negatively charged epitopes of the carboxyl terminus of the A2A receptors (see Fuxe et al., 2005). In agreement with this possibility evidence for A2A/D3 heteromers has also been obtained in Hela cells transiently cotransfected with D3-GFP2 and A2A-YFP cDNAs. A significant FRET efficiency was found in A2A/D3 colocated membrane areas as studied by sensitized emission in living cells (Torvinen et al., 2005a). Furthermore, in A2A/D3 cotransfected CHO cells an antagonistic modulation by the A2A agonist CGS 21680 of 3H-DA binding to the D3 receptors was demonstrated as well as an A2A agonist counteraction of the DA inhibition of the forskolin induced increase of cAMP accumulation (Torvinen et al., 2005a). Thus, A2A/D3 heteromers may exist in the striatum especially in the nucleus accumbens rich in D3 receptors (Schwartz et al., 2000) provided they are expressed in the same nerve cells. In view of the existence of D3 tetramers (Nimchinsky et al., 1997) an A2A regulation of D3 cooperativity may take place in high order A2A/D3 heteromers (Torvinen et al., 2005).

Possible A2A/D4 heteromers

Again arginine rich epitopes also exist in the N terminal part of the 3rd intracellular loop of the D4 receptor (van Tol et al., 1991), which may interact with the A2A carboxyl terminus (see Fuxe et al., 2005). Thus, A2A/D4 heteromers may be present in the brain. However, there exists no evidence for their existence. Nevertheless we postulate that they may exist in the striatal islands which are rich in D4 (Rivera et al., 2002) but not in D2 receptors (Fuxe et al., 2006) and where also substantial A2A immunoreactivity may exist. Furthermore, in A2A/D4.4 cotransfected Hela cells D4 activation can counteract the CGS 21680 induced increase in cAMP accumulation, showing interactions at the AC level (Canals et al., unpublished data).

A2A/mGluR5 heteromeric receptor complexes

Colocalization of A2A and mGluR5 has been observed at the membrane level of non-permeabilized HEK-293 cells (Ferré et al., 2002) as well as in the soma and dendrites of striatal neurons in primary cultures (Fuxe et al., 2003). Coimmunoprecipitation experiments showed that A2A and mGluR5 formed heteromeric complexes both in membrane preparations from HEK-293 cells and in rat striatal membrane preparations (Ferré et al., 2002). The available results on A2A/D2 and mGluR5/D2 receptor interactions (see above) can be explained by the existence of a receptor mosaic of extrasynaptic mGluR5, A2A and D2 receptors on the dendritic spines of the striato-pallidal GABA neurons (Fig. 14), where synergistic interactions between A2A and mGluR5 counteract the D2 signalling. In contrast, in the glutamate synapse the mGluR5 forms a heteromeric receptor complex with the NMDA receptors where anchoring proteins link them together (Jeffrey Conn et al., 2005) and mGluR5 increases the NMDA receptor signalling and vice versa. The prejunctional extrasynaptic mosaic may

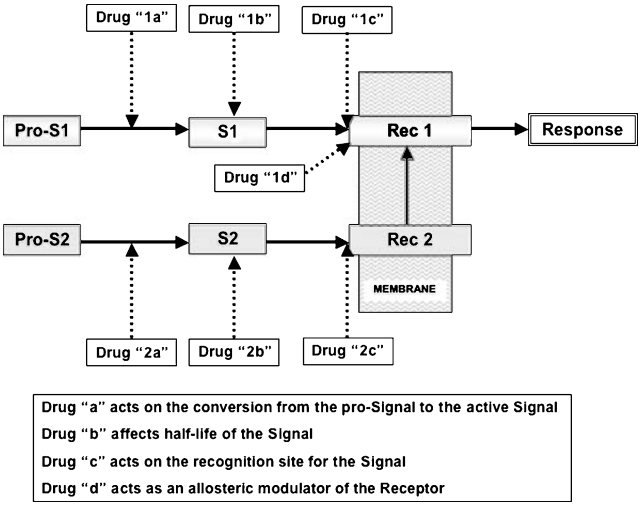


Fig. 19. Illustration of several targets for altering the receptor 1 signaling by drugs in all cell populations of the organism.This can be done not only via the transmitter binding pocket of receptor 1(1c) and via the transmitter binding pocket of receptor 2(2c) modulating receptor 1 via the intramembrane receptor–receptor interaction but also via allosteric sites (1d, 2d) located in both receptor 1 and 2 altering G protein coupling and signaling of receptor 1. This can be brought about via direct allosteric modulation (1d) or indirect allosteric modulation (2d) via receptor 2 with the conformational change in receptor 2 transferred to receptor 1. Thus, allosteric sites especially by modulating cooperativity development may have a major role in altering signaling in heteromeric receptor complexes, where the change in conformational state induced by the allosteric modulator in one receptor (receptor 1 or 2) can alter its modulation by the other receptors in the RM and thus its signaling. Drugs may also act on the conversion of the prosignal or on the half life of the signal to the respective receptors to modulate signaling in receptor 1(1a, 1b, 2a, 2b). This happens frequently in peptide transmission

involve mGluR5/A2A/D4? receptor mosaics (see above, under A2A antagonists).

Other receptor heteromers and their receptor–receptor interactions

The state of the art of receptor–receptor interactions especially among heptaspanning membrane receptors is found in a special issue of Journal of Molecular Neuroscience (Gozes, 2005), where also novel targets for drug development are outlined based on the receptor–receptor interactions within the receptor mosaics and their dynamics (Agnati and Fuxe, 2005). In Fig. 19 the role of allosteric modulators in controlling receptor–receptor interactions are outlined together with drugs affecting the halflife of the signal as well as its conversion from a prosignal to a true signal. For A1 heteromerization, see Franco et al. (this special issue) and for neuropeptide/ monoamine receptor–receptor interactions, see Tanganelli et al. and Narvaez et al. (this special issue).

On the impact of the receptor–receptor interactions in molecular medicine

The discovery of subtypes of somatostatin receptors forming functional homo and heteromers in cotransfected cell lines indicated that receptor–receptor interactions exist and play an important role in the endocrine system (Rocheville et al., 2000a). The formation of somatstatin receptor heteromers was shown to be subtype specific and agonist dependent (see also Patel et al., 2002). The heteromer sst5/sst1 made possible the internalization of the sst1 receptor and indicates the possibility that such a mechanism can help in the desensitization of the somatostatin receptors in the somatotrophes and thus play a role in GH release control (see Olias et al., 2004). The same may also be true for control of insulin release in the beta cells of the pancreas, since sst5 and sst1 are colocated in these cells. Subsequently, it has been shown that sst2 and sst3 also show homo and heterodimerization but in an agonist independent way (Pfeiffer et al., 2001). It is of interest that in the sst2A/sst3 heterodimer the sst3 signaling appears to be lost representing a novel mechanism for sst3 regulation. These heterdimers may exist both in the anterior lobe of the pituitary gland and in the islet cells of the pancreas.

Also sst5/D2 heteromers have been discovered (Rocheville et al., 2000b), triggered by agonists for somatostatin or D2 receptors leading to increased affinity at the two binding sites and increased signalling after combined activation. In analysis of human pituitary tumours indications were obtained that this heteromer may play a role since a combined D2/somatostain agonist had the highest efficacy to inhibit prolactin and growth hormone secretion (Saveanu et al., 2002). Finally, sst2A/u-opioid R(MOR1) heteromers have been discovered in cotransfected cell lines (Pfeiffer et al., 2002). It is not known to which extent this heteromer is involved in opioid addiction and in pain relief mediated by opioid agonists.

This analysis of receptor–receptor interactions within various somatostatin receptor heteromers in the endocrine and nervous system show that each heteromer provides a unique regulation of each of the participating receptors in terms of recognition, G-protein coupling and trafficking leading to highly specific signalling properties and function of these heteromers (see Olias et al., 2004).

Early on it was also shown that gonadotrophin-releasing hormone (GnRH) agonists can cause microaggregation of GnRH receptors via promoting physical interactions between these receptors (see Janovick and Conn, 1996; Cornea et al., 2001). This is an early event in hormone action resistant to damage to the actin cytoskeleton and to the destabilization of the microfilaments unlike the slow macroaggregation of receptors involving clustering and internalization. By FRET analysis it has also been demonstrated that luteinizing hormone (LH) receptors are self-associated in the surface membrane the extent of which is dependent on agonist binding (Roess et al., 2000). Thus, receptor–receptor interactions appear to contribute to the function of LH receptors. Defined-function mutants have been used to give access to receptor–receptor interactions in LH receptors (Lee et al., 2002). The results show that the binding of LH to one LH receptor can also stimulate AC of an adjacent LH receptor via *trans*-activation (intermolecular activation) of its transmembrane domains but without the formation of a stable receptor dimer. Instead the results indicate the existence of transient interactions between LH receptor pairs. Finally the role of receptor–receptor interactions in the endocrine system is well illustrated by the demonstration that oxytocin and vasopressin (V1A and V2) receptors during biosynthesis can form homo and heterodimers (Terrillon et al., 1996).

In central cardiovascular regulation receptor–receptor interactions were early on suggested to play a major role involving especially neuropeptide Y R/alpha2 adrenergic R interactions (Agnati et al., 1983b; Fuxe et al., 1987; see Narvaez, this special issue). However, in peripheral cardiovascular regulation vasoconstrictor cooperation in vivo and in vitro between noradrenaline and Neuropeptide Y was not regarded as the result of a receptor–receptor

interaction but of a threshold synergism phenomenon (Wahlestedt et al., 1990). AT1 receptors play a major role in hypertension and related cardiovascular disorders. It is therefore of substantial interest that AT1 receptors exist both as homodimers and heterodimers (see AbdAlla et al., 2005). The most interesting heterodimer is the one between the AT1 and the bradykinin B2 receptor which show increased AT1 receptor signalling and altered internalization (AbdAlla et al., 2000, 2001b). The first indications for its existence was obtained by the discovery of intramembrane AT1/B2 receptor interactions in the nucleus tractus solitaries, an important cardiovascular region in the medulla oblongata (Fior et al., 1993). Evidence exists that increased AT1/B2 heterodimers exist in vessels and platelets in preeclampsia which via their increased AT1 signaling mediate the preeclampsia hypertension (AbdAlla et al., 2001b; Quitterer et al., 2004). Recently evidence has also been obtained that AT1/B2 heterodimers are involved in angiotensin II hypersensitivity in spontaneously hypertensive rats via increased AT1 signaling (AbdAlla et al., 2005). The heterodimers were found in high amounts on the renal mesangial cells and their increased signaling led to an increased secretion of endothelin1 from the mesangial cells. It has been suggested that the increased signalling of AT1/B2 heterodimers are involved in the pathogenesis of hypertensive renal disease with glomeroulsclerosis (AbdAlla et al., 2005). It should be considered that in contrast in the AT1/AT2 heterodimer the AT receptor signaling is reduced (AbdAlla et al., 2001a). It may therefore be that human hypertensive disease is related to a disbalance of AT1/B2 and AT1/AT2 heterodimers and their function in vascular beds with the AT1/B2 becoming dominant.

A functional role for receptor–receptor interactions in vivo in the cardiovascular system has also recently been obtained in an interesting paper by Rockman, Luttrell and Barki-Harrington involving analysis of cardiomyocytes (Barki-Harrington et al., 2003b). AT1 and beta adrenergic receptors were shown to form constitutive heteromeric complexes and form the structural basis for the observed transinhibitory actions of beta adrenergic and AT1 receptor antagonists brought about by receptor-G protein uncoupling in these heteromers. Dual inhibition of beta adrenergic and angiotensin II receptors can in this way be caused by one single antagonist. Thus, beta adrenergic antagonists may have a role in treatment of heart failure also by blocking AT1 receptor signaling which also may play a major role in heart failure. Similar types of observations have also been obtained in the study of crosstalk between B1 and B2 kinin receptors for proliferation in prostate cancer cells (Barki-Harrington et al., 2003a). Thus, receptor–receptor interactions among GPCR may have an impact also for cancer development and its treatment. Novel transmitter systems with their GPCRs have emerged in the regulation of vascular reactivity and cognate ligands have been identified for over 50 so called orphan receptors in the vascular system (Maguire and Davenport, 2005). With the probable existence of receptor–receptor interactions among them and with some orphan receptors potentially acting mainly as modulators of GPCR function via receptor–protein interactions (Agnati et al., 2004d; Lefkowitz, 2005) many undiscovered targets exist for drug development in treatment of hypertension and related disorders. It should also be noted that the receptor activity-modifying proteins (RAMP) regulate the pharmacology of the receptors for the calcitonin family of peptide hormones (McLatchie et al., 1998; see also Foord et al., 2005) like calcitonin gene-related peptide (CGRP), adrenomedullin (ADM) and intermedin (Roh et al., 2004). As an example the calcitonin receptor-like receptor (CRLR) requires a RAMP to reach the surface membrane and becomes a receptor for CGRP when coexpressed with RAMP1 or RAMP2, a receptor for ADM when coexpressed with RAMP3 and a receptor for intermedin when coexpressed with anyone of the RAMPs. Thus, a number of peptides can participate in cardiovascular, respiratory and gastrointestinal regulation by signalling via CRLR/RAMP receptor complexes, where multiple binding pockets in the CRLR may develop for CGRP, ADM or intermedin and so far undiscovered peptides. This research also underlines the importance of accessory proteins like the RAMPs for drug development.

In the immune system ligand induced chemokine receptor homo- and heterodimerization appears to play an important role in its physiological function and pathological processes (Rodriguez-Frade et al., 2001). The homodimerization was necessary for chemotaxis and Calcium flux and the heterodimer between CCR2 and CCR5 formed by their combined activation with the chemokines CCL2 and CCL5 made possible a distinct signalling response (Mellado et al., 2001). It involved the development of a high potency of the ligands to cause calcium responses, the recruitment of G alpha q11 and failure to undergo internalization and desensitization and a maintained phosphoinositide 3-kinase activation kinetics. The physiological relevance of this response appears to be activation of leucocyte adhesion to the endothelium and takes place at low concentration of the chemokines. At high concentrations as found e.g. in a perivascular inflammatory response homodimerization is instead favoured which will induce the migration of the leucocytes through the endothelium towards the inflammatory sites. In the tissue the heterodi-

mers will again be formed when the chemokine concentrations are low and again favour the leucocyte adherence and make them reside in these places (see Mellado et al., 2001). Thus, the chemokine receptor heterodimer gives rise to highly sensitive and dynamic responses in the leucocytes and would therefore be an interesting target for treatment of chronic inflammatory disease where chemokines play a major role. It is also of substantial interest that HIV-1 infection via CCR5 and CXCR4 could be blocked by acting in trans on the CCR2 chemokine receptor (Rodriguez-Frade et al., 2004) by use of an CCR2 monoclonal antibody activating the CCR2 receptor leading to oligomerization between CCR2/CCR5 and CCR2/CXCR4 receptors. Receptor–receptor interactions may therefore offer novel strategies for treatment of AIDS and its prevention without inflammatory side effects (Ward et al., 1998). It should be noticed that that human cytomegalo virus (HCMV) encoded GPCR are constitutively active and in this way reprogram the horizontal and vertical molecular networks in the membrane and cytoplasm respectively after infection (Vischer et al., 2006). They can also enhance the signaling of many GPCRs (Bakker et al., 2004). It seems likely that these modulatory actions involves receptor–receptor interactions via an oligomerization process and underscores the need of understanding how viral GPCR may participate in forming aberrant RM with pathological signalling that may lead to cell death (Agnati and Fuxe, in preparation). Such knowledge would help in developing novel strategies against viral diseases. Dimerization residues in transmembrane domains of CCR chemokine receptors are now also becoming identified (de Juan et al., 2005).

Many inactivating missense mutations of GPCRs are associated with a failure of expressing the mutant receptors in the surface membrane indicating a need for improved chaperone mechanisms (see Schöneberg et al., 2004). It may be that a receptor homo and/or heterodimer cannot be formed with the mutant protein with a failure to deliver the mutant receptor to the plasma membrane (see Bouvier, 2001). This is the reason why chemical chaperones are being developed to restore the native conformation allowing its insertion to the surface membrane.

Other inactivating mutations show reduced agonist binding affinity with unchanged maximal efficacy. Again this may inter alia be caused by altered receptor–receptor interactions known to be involved in regulating agonist affinity (see Agnati et al., 2003a). It is therefore possible that besides high doses of agonists (Scöneberg et al., 2004) treatment strategy can also be based on receptor–receptor interactions enhancing the agonist affinity of the mutant receptor. However, it is difficult to see how nonsense mutations of receptors resulting in truncated, non-functional receptor proteins can be helped by receptor–receptor interactions. Here instead the aminoglycoside antibiotics with reduced toxicity may be helpful by reducing the impact of premature stop codons (Schöneberg et al., 2004).

Inverse agonists are instead the preferred choice of treating diseases with activating mutations of GPCRs (see Lefkowitz et al., 1993; Schönebeerg et al., 2004). However, in this case the use of antagonistic receptor–receptor interactions may be an alternative strategy to cause the conformational changes in the mutant receptor leading to reduction in its constitutive activity with persistent activation of the G protein.

In conclusion, the intramembrane receptor–receptor interactions taking place via heterodimers and receptor mosaics (high order oligomers) appear to represent a new principle in molecular medicine making possible integration of signals already at the level of the surface membrane. They open up new targets for treatment of receptor dysfunction known to occur inter alia in neurological and mental disorders, and in diseases of the endocrine,cardiovascular and immune systems.

Acknowledgements

This work has been supported by a grant from the Swedish Research Council and by a grant from the European Commission (QLG3-CT-2001-01056) and from a Cofin grant (Ministero Ricerca Scientifica). Kjell Fuxe acknowledges the fundamental contributions of LF. Agnati to all his past work in the field of receptor–receptor interactions as well as to the present paper.

References

AbdAlla S, Abdel-Baset A, Lother H, el Massiery A, Quitterer U (2005) Mesangial AT1/B2 receptor heterodimers contribute to angiotensin II hyperresponsiveness in experimental hypertension. J Mol Neurosci 26: 185–192

AbdAlla S, Lother H, Abdel-tawab AM, Quitterer U (2001a) The angiotensin II AT2 receptor is an AT1 receptor antagonist. J Biol Chem 276: 39721–39726

AbdAlla S, Lother H, el Massiery A, Quitterer U (2001b) Increased AT(1) receptor heterodimers in preeclampsia mediate enhanced angiotensin II responsiveness. Nat Med 7: 1003–1009

AbdAlla S, Lother H, Quitterer U (2000) AT1-receptor heterodimers show enhanced G-protein activation and altered receptor sequestration. Nature 407: 94–98

Ackers GK, Doyle ML, Myers D, Daugherty MA (1992) Molecular code for cooperativity in hemoglobin. Science 255: 54–63

Agnati L, Fuxe K, Zini I, Lenzi P, Hökfelt T (1980) Aspects on receptor regulation and isoreceptor identification. Med Biol 58: 182–187

Agnati L, Fuxe K, Zoli M, Rondanini C, Ögren S-O (1982) New vistas on synaptic plasticity: mosaic hypothesis on the engram. Med Biol 60: 183–190

Agnati LF, Fuxe K, Ferri M, Benfenati F, Ögren S-O (1981) A new hypothesis on memory. A possible role of local circuits in the formation of the memory trace. Med Biol 59: 224–229

Agnati LF, Ferré S, Burioni R, Woods A, Genedani S, Franco R, Fuxe K (2005a) Existence and theoretical aspects of homomeric and heteromeric dopamine receptor complexes and their relevance for neurological diseases. Neuromol Med 7: 61–78

Agnati LF, Ferré S, Genedani S, Franzini C, Woods AS, Franco R, Yang S-N, Fuxe K (2004a) The neurobiological bases of consciousness the multiple mirror network hypothesis. Acc Naz Sci Lett Arti di Modena Sez VIII, v. VII fasc. II: 317–374

Agnati LF, Ferré S, Leo G, Lluis C, Canela EI, Franco R, Fuxe K (2004b) On the molecular basis of the receptor mosaic hypothesis of the engram. Cell Mol Neurobiol 24: 501–516

Agnati LF, Ferré S, Lluis C, Franco R, Fuxe K (2003a) Molecular mechanisms and therapeutical implications of intramembrane receptor/receptor interactions among heptahelical receptors with examples from the striatopallidal GABA neurons. Pharmacol Rev 55: 509–550

Agnati LF, Franzen O, Ferré S, Leo G, Franco R, Fuxe K (2003b) Possible role of intramembrane receptor–receptor interactions in memory and learning via formation of long-lived heteromeric complexes: focus on motor learning in the basal ganglia. J Neural Transm Suppl 65: 1–28

Agnati LF, Fuxe K (1984) New concepts on the structure of the neuronal networks: the miniaturization and hierarchical organization of the central nervous system. (Hypothesis). Biosci Rep 4: 93–98

Agnati LF, Fuxe K (2000) Volume transmission as a key feature of information handling in the central nervous system possible new interpretative value of the Turing's B-type machine. Prog Brain Res 125: 3–19

Agnati LF, Fuxe K (2005) Concluding remarks. J Mol Neurosci 26: 299–302

Agnati L, Fuxe K, Andersson K, Hökfelt T, Skirboll L, Benfenati F, Battistini N, Calza L (1983a) Possible functional meaning of the coexistence of monoamines a dn peptides in the same neurons. A study on the interactions between cholecystokini-8 and dopamine in the brain. In: Biggio G, Costa E, Gessa GL, Spano PF (eds) Receptors as supramoledcular entities. Pergamon Press, New York, pp 61–70

Agnati LF, Fuxe K, Benfenati F, Battistini N, Harfstrand A, Tatemoto K, Hokfelt T, Mutt V (1983b) Neuropeptide Y in vitro selectivity increases the number of alpha 2-adrenergic binding sites in membranes of the medulla oblongata of the rat. Acta Physiol Scand 118: 293–295

Agnati L, Fuxe K, Benfenati F, Calza L, Battistini N, Ögren S (1983c) Receptor–receptor interactions: possible new mechanisms for the action of some antidepressant drugs. In: Usdin E, Goldstein M, Friedhoff A, Gergotas A (eds) Frontiers in neuropsychiatric research. Macmillan Press, London, pp 301–318

Agnati LF, Fuxe K, Benfenati F, Zini I, Hokfelt T (1983d) On the functional role of coexistence of 5-HT and substance P in bulbospinal 5-HT neurons. Substance P reduces affinity and increases density of 3H-5-HT binding sites. Acta Physiol Scand 117: 299–301

Agnati LF, Fuxe K, Ferré S (2005a) How receptor mosaics decode transmitter signals. Possible relevance of cooperativity. Trends Biochem Sci 30: 188–193

Agnati LF, Fuxe K, Ferri M, Benfenati F, Ögren S-O (1981) A new hypothesis on memory. A possible role of local circuits in the formation of the memory trace. Med Biol 59: 224–229

Agnati LF, Fuxe K, Torvinen M, Genedani S, Franco R, Watson S, Nussdorfer GG, Leo G, Guidolin D (2005b) New methods to evaluate colocalization of fluorophores in immunocytochemical preparations as exemplified by a study on A2A and D2 receptors in Chinese hamster ovary cells. J Histochem Cytochem 53: 941–953

Agnati LF, Fuxe K, Zoli M, Pich EM, Benfenati F, Zini I, Goldstein M (1986) Aspects on the information handling by the central nervous system: focus on cotransmission in the aged rat brain. Prog Brain Res 68: 291–301

Agnati LF, Guidolin D, Genedani S, Ferré S, Bigiani A, Woods AS, Fuxe K (2005c) How proteins come together in the plasma membrane and function in macromolecular assemblies: focus on receptor mosaics. J Mol Neurosci 26: 133–154

Agnati LF, Leo G, Vergoni AV, Martinez E, Hockemeyer J, Lluis C, Franco R, Fuxe K, Ferré S (2004c) Neuroprotective effect of L-DOPA co-administered with the adenosine A2A receptor agonist CGS 21680 in an animal model of Parkinson's disease. Brain Res Bull 64: 155–164

Agnati LF, Santarossa L, Benfenati F, Ferri M, Morpurgo A, Apolloni B, Fuxe K (2002) Molecular basis of learning and memory: modelling based on receptor mosaics. In: Apolloni B, Kurfes F (eds) From synapses to rules. Kluwer Academic/Plenum Publishers, New York, pp 165–196

Agnati L, Santarossa L, Genedani S, Canela EI, Leo G, Franco R, Fuxe K (2004d) On the nested hierarchical organization of CNS: basic characteristics of neuronal molecular networks. In: Erdi P, Esposito A, Marinaro M, Scarpette S (eds) Computational neuroscience: cortical dynamics. Lecture Notes in Computer Science. Springer, Berlin, Heidelberg New York, pp 24–54

Agnati LF, Tarakanov AO, Ferré S, Fuxe K, Guidolin D (2005d) Receptor–receptor interactions, receptor mosaics, and basic principles of molecular network organization: possible implications for drug development. J Mol Neurosci 26: 193–208

Agnati L, Zoli M, Merlo-Pich E, Benfenati F, Fuxe K (1990) Aspects of neural plasticity in the central nervous system. VIITheoretical aspects of brain communication and communication. Neurochem Int 16: 479–500

Aguirre JA, Kehr J, Yoshitake T, Liu FL, Rivera A, Fernandez-Espinola S, Andbjer B, Leo G, Medhurst AD, Agnati LF, Fuxe K (2005) Protection but maintained dysfunction of nigral dopaminergic nerve cell bodies and striatal dopaminergic terminals in MPTP-lesioned mice after acute treatment with the mGluR5 antagonist MPEP. Brain Res 1033: 216–220

Andén N-E, Butcher SG, Corrodi H, Fuxe K, Ungerstedt U (1970) Receptor activity and turnover of dopamine and noradrenaline after neuroleptics. Eur J Pharmcol 11: 303–314

Anden NE, Dahlstrom A, Fuxe K, Larsson K (1966) Functional role of the nigro-neostriatal dopamine neurons. Acta Pharmacol Toxicol (Copenh) 24: 263–274

Andersen MB, Fuxe K, Werge T, Gerlach J (2002) The adenosine A2A receptor agonist CGS 21680 exhibits antipsychotic-like activity in Cebus apella monkeys. Behav Pharmacol 13: 639–644

Angers S, Salahpour A, Bouvier M (2002) Dimerization: an emerging concept for G protein-coupled receptor ontogeny and function. Annu Rev Pharmacol Toxicol 42: 409–435

Antonelli T, Fuxe K, Agnati L, Mazzoni E, Tanganelli S, Tomasini MC, Ferraro L (2006) Experimental studies and theoretical aspects on A2A/D2 receptor interactions in a model of Parkinson's disease. Relevance for l-dopa induced dyskinesias. J Neurol Sci Jun 7; [Epub ahead of print]

Armstrong D, Strange PG (2001) Dopamine D2 receptor dimer formation: evidence from ligand binding. J Biol Chem 276: 22621–22629

Bakker RA, Casarosa P, Timmerman H, Smit MJ, Leurs R (2004) Constitutively active Gq/11-coupled receptors enable signaling by co-expressed G(i/o)-coupled receptors. J Biol Chem 279: 5152–5161

Bara-Jimenez W, Sherzai A, Dimitrova T, Favit A, Bibbiani F, Gillespie M, Morris MJ, Mouradian MM, Chase TN (2003) Adenosine A(2A) receptor antagonist treatment of Parkinson's disease. Neurology 61: 293–296

Barki-Harrington L, Bookout AL, Wang G, Lamb ME, Leeb-Lundberg LM, Daaka Y (2003a) Requirement for direct cross-talk between B1 and B2 kinin receptors for the proliferation of androgen-insensitive prostate cancer PC3 cells. Biochem J 371: 581–587

Barki-Harrington L, Luttrell LM, Rockman HA (2003b) Dual inhibition of beta-adrenergic and angiotensin II receptors by a single antagonist: a

functional role for receptor–receptor interaction in vivo. Circulation 108: 1611–1618

Battaglia G, Busceti CL, Molinaro G, Biagioni F, Storto M, Fornai F, Nicoletti F, Bruno V (2004) Endogenous activation of mGlu5 metabotropic glutamate receptors contributes to the development of nigro-striatal damage induced by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine in mice. J Neurosci 24: 828–835

Bellido I, Diaz-Cabiale Z, Jimenez-Vasquez PA, Andbjer B, Mathe AA, Fuxe K (2002) Increased density of galanin binding sites in the dorsal raphe in a genetic rat model of depression. Neurosci Lett 317: 101–105

Bourne HR, Nicoll R (1993) Molecular machines integrate coincident synaptic signals. Cell Suppl 72: 65–75

Bouvier M (2001) Oligomerization of G-protein-coupled transmitter receptors. Nat Rev Neurosci 2: 274–286

Canals M, Angulo E, Casado V, Canela EI, Mallol J, Vinals F, Staines W, Tinner B, Hillion J, Agnati L, Fuxe K, Ferré S, Lluis C, Franco R (2005) Molecular mechanisms involved in the adenosine A and A receptor-induced neuronal differentiation in neuroblastoma cells and striatal primary cultures. J Neurochem 92: 337–348

Canals M, Burgueno J, Marcellino D, Cabello N, Canela EI, Mallol J, Agnati L, Ferré S, Bouvier M, Fuxe K, Ciruela F, Lluis C, Franco R (2004) Homodimerization of adenosine A2A receptors: qualitative and quantitative assessment by fluorescence and bioluminescence energy transfer. J Neurochem 88: 726–734

Canals M, Marcellino D, Fanelli F, Ciruela F, de Benedetti P, Goldberg SR, Neve K, Fuxe K, Agnati LF, Woods AS, Ferré S, Lluis C, Bouvier M, Franco R (2003) Adenosine A2A-dopamine D2 receptor–receptor heteromerization: qualitative and quantitative assessment by fluorescence and bioluminescence energy transfer. J Biol Chem 278: 46741–46749

Carlsson A, Lindqvist M (1963) Effect of chlorpromazine or haloperidol on formation of 3-methoxytyramine and normetanephrine in mouse brain. Acta Pharmacol Toxicol (Copenh) 20: 140–144

Carr DB, Sesack SR (2000) Projections from the rat prefrontal cortex to the ventral tegmental area: target specificity in the synaptic associations with mesoaccumbens and mesocortical neurons. J Neurosci 20: 3864–3387

Changeux JP, Edelstein SJ (2005) Allosteric mechanisms of signal transduction. Science 308: 1424–1428

Chase TN, Bibbiani F, Bara-Jimenez W, Dimitrova T, Oh-Lee JD (2003) Translating A2A antagonist KW6002 from animal models to parkinsonian patients. Neurology 61: S107–S111

Ciruela F, Burgueno J, Casado V, Canals M, Marcellino D, Goldberg SR, Bader M, Fuxe K, Agnati LF, Lluis C, Franco R, Ferré S, Woods AS (2004) Combining mass spectrometry and pull-down techniques for the study of receptor heteromerization. Direct epitope–epitope electrostatic interactions between adenosine A2A and dopamine D2 receptors. Anal Chem 76: 5354–5363

Coccurello R, Breysse N, Amalric M (2004) Simultaneous blockade of adenosine A2A and metabotropic glutamate mGlu5 receptors increase their efficacy in reversing Parkinsonian deficits in rats. Neuropsychopharmacology 29: 1451–1461

Conn PJ, Battaglia G, Marino MJ, Nicoletti F (2005) Metabotropic glutamate receptors in the basal ganglia motor circuit. Nat Rev Neurosci 6: 787–798

Cornea A, Janovick JA, Maya-Nunez G, Conn PM (2001) Gonadotropin-releasing hormone receptor microaggregation. Rate monitored by fluorescence resonance energy transfer. J Biol Chem 276: 2153–2158

de Juan D, Mellado M, Rodriguez-Frade JM, Hernanz-Falcon P, Serrano A, Del Sol A, Valencia A, Martinez AC, Rojas AM (2005) A framework for computational and experimental methods: Identifying dimerization residues in CCR chemokine receptors. Bioinformatics 21 Suppl 2: ii13–ii18

Diaz-Cabiale Z, Hurd Y, Guidolin D, Finnman UB, Zoli M, Agnati LF, Vanderhaeghen JJ, Fuxe K, Ferré S (2001) Adenosine A2A agonist CGS 21680 decreases the affinity of dopamine D2 receptors for dopamine in human striatum. Neuroreport 12: 1831–1834

Diaz-Cabiale Z, Vivo M, Del Arco A, O'Connor WT, Harte MK, Muller CE, Martinez E, Popoli P, Fuxe K, Ferré S (2002) Metabotropic glutamate mGlu5 receptor-mediated modulation of the ventral striopallidal GABA pathway in rats. Interactions with adenosine A(2A) and dopamine D(2) receptors. Neurosci Lett 324: 154–158

Fenu S, Pinna A, Ongini E, Morelli M (1997) Adenosine A2A receptor antagonism potentiates L-DOPA-induced turning behaviour and c-fos expression in 6-hydroxydopamine-lesioned rats. Eur J Pharmacol 321: 143–147

Ferré S, Ciruela F, Canals M, Marcellino D, Burgueno J, Casado V, Hillion J, Torvinen M, Fanelli F, Benedetti Pd P, Goldberg SR, Bouvier M, Fuxe K, Agnati LF, Lluis C, Franco R, Woods A (2004) Adenosine A2A-dopamine D2 receptor–receptor heteromers. Targets for neuropsychiatric disorders. Parkinsonism Relat Disord 10: 265–271

Ferré S, Karcz-Kubicha M, Hope BT, Popoli P, Burgueno J, Gutierrez MA, Casado V, Fuxe K, Goldberg SR, Lluis C, Franco R, Ciruela F (2002) Synergistic interaction between adenosine A2A and glutamate mGlu5 receptors: implications for striatal neuronal function. Proc Natl Acad Sci USA 99: 11940–11945

Ferré S, Fredholm B, Morelli M, Popoli P, Fuxe K (1997) Adenosine-dopamine receptor–receptor interactions as an integrative mechanism in the basal ganglia. Trends Neurosci 20: 482–487

Ferré S, O'Connor W, Fuxe K, Ungerstedt U (1993) The striopallidal neuron: a main locus for adenosine-dopamine interactions in the brain. J Neurosci 13: 5402–5406

Ferré S, O'Connor W, Snaprud P, Ungerstedt U, Fuxe K (1994) Antagonistic interaction between adenosine A2A receptors and dopamine D2 receptors in the ventral striopallidal system. Implications for the treatment of schizophrenia. Neuroscience 63: 765–773

Ferré S, Popoli P, Giménez-Llort L, Rimondini R, Müller CE, Strömberg I, Ögren SO, Fuxe K (2001) Adenosine/dopamine interaction: implications for the treatment of Parkinson's disease. Parkinsonism Rel Disord 7: 235–241

Ferré S, Popoli P, Rimondini R, Reggio R, Kehr J, Fuxe K (1999) Adenosine A2A and group I metabotropic glutamate receptors synergistically modulate the binding characteristics of dopamine D2 receptors in the rat striatum. Neuropharmacology 38: 129–140

Ferré S, von Euler G, Johansson B, Fredholm B, Fuxe K (1991) Stimulation of high affinity adenosine A2 receptors decreases the affinity of dopamine D2 receptors in rat striatal membranes. Proc Natl Acad Sci USA 88: 7238–7241

Fior D, Hedlund P, Fuxe K (1993) Autoradiographic evidence for a bradykinin/angiotensin II receptor–receptor interactions in the rat brain. Neurosci Lett 163: 58–62

Foord SM, Topp SD, Abramo M, Holbrook JD (2005) New methods for researching accessory proteins. J Mol Neurosci 26: 265–276

Franco R, Casado V, Mallol J, Ferré S, Fuxe K, Cortes A, Ciruela F, Lluis C, Canela EI (2005) Dimer-based model for heptaspanning membrane receptors. Trends Biochem Sci 30: 360–366

Franco R, Ferré S, Agnati L, Torvinen M, Gines S, Hillion J, Casado V, Lledo P, Zoli M, Lluis C, Fuxe K (2000) Evidence for adenosine/dopamine receptor interactions: indications for heteromerization. Neuropsychopharmacology 23: S50–S59

Fraser CM, Venter JC (1982) The size of the mammalian lung beta 2-adrenergic receptor as determined by target size analysis and immunoaffinity chromatography. Biochem Biophys Res Commun 109: 21–29

Fredholm BB, Fuxe K, Agnati L (1976) Effect of some phosphodiesterase inhibitors on central dopamine mechanisms. Eur J Pharmacol 38: 31–38

Fredholm BB, Svenningsson P (2003) Adenosine-Dopamine interactions. Development of a concept and some comments on therapeutic possibilities. Neurology 61 Suppl 6: S5–S9

Fuxe K, Agnati L (1987) Receptor–receptor interactions. A new intramembrane integrative mechanism. Macmillan Press, London

Fuxe K, Agnati L, Benfenati F, Cimmino M, Algeri S, Hökfelt T, Mutt V (1981) Modulation by cholecystokinin of [^{3}H]-spiroperidol binding in rat striatum: Evidence for increased affinity and reduction in the number of binding sites. Acta Physiol Scand 113: 567–569

Fuxe K, Agnati LF (1985) Receptor–receptor interactions in the central nervous system. A new integrative mechanism in synapses. Med Res Rev 5: 441–482

Fuxe K, Agnati LF, Benfenati F, Celani MF, Zini I, Zoli M, Mutt V (1983a) Evidence for the existence of receptor–receptor interactions in the central nervous system. Studies on the regulation of monoamine receptors by neuropeptides. J Neural Transm 18: 165–179

Fuxe K, Agnati L, Celani MF, Benfenati F, Andersson K, Collins J (1983b) Studies on excitatory amino acid receptors and their interactions and regulation of pre and postsynaptic dopaminergic mechanisms in the rat telencephalon. In: Fuxe K, Roberts P, Schwarcz R (eds) Excitotoxins. MacMillan Press, London, pp 138–156

Fuxe K, Agnati LF, Harfstrand A, Andersson K, Mascagni F, Zoli M, Kalia M, Battistini N, Benfenati F, Hokfelt T et al. (1986a) Studies on peptide comodulator transmission. New perspective on the treatment of disorders of the central nervous system. Prog Brain Res 66: 341–368

Fuxe K, Agnati L, Härfstrand A, Janson A, Neumeyer A, Andersson K, Ruggeri M, Zoli M, Goldstein M (1986b) Morphofunctional studies on the neuropeptide Y/adrenaline costoring terminal systems in the dorsal cardiovascular region of the medulla oblongata. Focus on receptor–receptor interactions in cotransmission. In: Hökfelt T, Fuxe K, Pernow B (eds) Prog in Brain Res, Vol. 68. Elsevier, Amsterdam, pp 303–320

Fuxe K, Agnati LF, Jacobsen K, Hillion J, Canals M, Torvinen M, Tinner-Staines B, Staines W, Rosin D, Terasmaa A, Popoli P, Leo G, Vergoni V, Lluis C, Ciruela F, Franco R, Ferré S (2003) Receptor heteromerization in adenosine A2A receptor signaling: relevance for striatal function and Parkinson's disease. Neurology 61: S19–S23

Fuxe K, Agnati L, Ögren S, Andersson K, Benfenati F (1983c) Neurobiology of central monoamine neurotransmission: Functional neuroanatomy and noradrenaline and 5-hydroxytryptamine involvement in learning and memory. In: Caputto R, Marsan C (eds) Neural transmission, learning and memory. Raven Press, New York, pp 237–255

Fuxe K, Celani MF, Martire M, Zini I, Zoli M, Agnati LF (1984) l-Glutamate reduces the affinity of 3H-N-propylnorapomorphine binding sites in striatal membranes. Eur J Pharmacol 100: 127–130

Fuxe K, Ferré S, Canals M, Torvinen M, Terasmaa A, Marcellino D, Goldberg SR, Staines W, Jacobsen KX, Lluis C, Woods AS, Agnati LF, Franco R (2005) Adenosine A2A and dopamine D2 heteromeric receptor complexes and their function. J Mol Neurosci 26: 209–220

Fuxe K, Ferré S, Franco R, Agnati L (2006) Adenosine receptor-dopamine receptor interactions in the basal ganglia and their relevance for brain function. Physiol Behav (in press)

Fuxe K, Ferré S, Zoli M, Agnati LF (1998) Integrated events in central dopamine transmission as analyzed at multiple levels. Evidence for intramembrane adenosine A2A/dopamine D2 and adenosine A1/dopamine D1 receptor interactions in the basal ganglia. Brain Res Rev 26: 258–273

Fuxe K, Harfstrand A, Agnati L, Kalia M, Fredholm B, Ganten D (1987) Central catecholamine-neuropeptide Y interactions at the pre- and post-synaptic level in cardiovascular centers. J Cardiovasc Pharmacol 10: 1–13

Fuxe K, Hedlund P, Euler Gv, Lundgren K, Martire M, Ögren S, Eneroth P, Agnati L (1991) Galanin/5-HT interactions in the rat central nervous system. Relevance for depression. In: Hökfelt T, Bartfai T, Jacobowitz D, Ottoson D (eds) Galanin. A new multifunctional peptide in the neuroendocrine system, Vol. 58. Macmillan, London, pp 221–235

Fuxe K, Manger P, Genedani S, Agnati L (2006) The nigrostriatal DA pathway and Parkinson's disease. J Neural Transm Suppl 70: 71–83

Fuxe K, Strömberg I, Popoli P, Rimondini-Giorgini R, Torvinen M, Ögren SO, Franco R, Agnati LF, Ferré S (2001) Adenosine receptors and Parkinson's Disease. Relevance of antagonistic adenosine and dopamine receptor interactions in the striatum. In: Calne D, Calne S (eds) Parkinson's disease, Vol. 86. Lippincott Williams & Wilkins, Philadelphia, pp 345–353

Fuxe K, Ungerstedt U (1974) Action of caffeine and theophyllamine on supersensitive dopamine receptors: Considerable enhancement of receptor response to treatment with dopa and dopamine receptor agonists. Med Biol 52: 48–54

Fuxe K, Ungerstedt U (1976) Antiparkinsonian drugs and dopaminergic neostriatal mechanisms: studies in rats with unilateral 6-hydroxydopamine (= 6-OH–DA)-induced degeneration of the nigro-neostriatal DA pathway and quantitative recording of rotational behaviour. Pharmacol Ther [B] 2: 41–47

Fuxe K, von Euler G, Agnati LF, Ögren SO (1988a) Galanin selectively modulates 5-hydroxytryptamine 1A receptors in the rat ventral limbic cortex. Neurosci Lett 85: 163–167

Fuxe K, Ögren S, Agnati L, Gustafsson J-Å, Jonsson G (1977) On the mechanism of action of the antidepressant drugs amitriptyline and nortriptyline. Evidence for 5-hydroxytryptamine receptor blocking activity. Neurosci Lett 6: 339–343

Fuxe K, Ögren S, Jansson A, Cintra A, Härfstrand A, Agnati L (1988b) Intraventricular injections of galanin reduces 5-HT metabolism in the ventral limbic cortex, the hippocampal formation and the fronto-parietal cortex of the male rat. Acta Physiol Scand 133: 579–581

Goldman-Rakic PS, Castner SA, Svensson TH, Siever LJ, Williams GV (2004) Targeting the dopamine D1 receptor in schizophrenia: insights for cognitive dysfunction. Psychopharmacology (Berl) 174: 3–16

Gozes I (ed) (2005) Receptor–receptor interactions among heptaspanning membrane receptors: From structure to function. J Mol Neurosci 26: 109–303

Graybiel AM (2005) The basal ganglia: learning new tricks and loving it. Curr Opin Neurobiol 15: 638–644

Hauber W, Koch M (1997) Adenosine A2A receptors in the nucleus accumbens modulate prepulse inhibition of the startle response. Neuroreport 8: 1515–1518

Hauser RA, Hubble JP, Truong DD (2003) Randomized trial of the adenosine A(2A) receptor antagonist istradefylline in advanced PD. Neurology 61: 297–303

Hawkins RD, Kandel ER, Sigelbaum SA (1993) Learning to modulate transmitter release: Themes and variations in synaptic plasticity. Ann Rev Neurosci 16: 625–665

Hebb D (1949) The organization of behavior. Wiley, New York

Hedlund P, Finnman U-B, Yanaihara N, Fuxe K (1994) Galanin-(1–15), but not galanin-(1–29), modulates 5-HT1A receptors in the dorsal hippocampus of the rat brain: possible existence of galanin receptor subtypes. Brain Res 634: 163–167

Hedlund P, Fuxe K (1991) Chronic imipramine treatment increases the affinity of [^{125}I]galanin binding sites in the tel- and diencephalon of the rat and alters the 5-HT1A/galanin receptor interaction. Acta Physiol Scand 141: 137–138

Hedlund P, von Euler G, Fuxe K (1991) Activation of 5-hydroxytryptamine 1A receptors increases the affinity of galanin receptors in di- and telencephalic areas of the rat. Brain Res 560: 251–259

Hedlund P, Yanaihara N, Fuxe K (1992) Evidence for specific N-terminal galanin fragment binding sites in the rat brain. Eur J Pharmacol 224: 203–205

Hedlund PB, Huitron-Resendiz S, Henriksen SJ, Sutcliffe JG (2005) 5-HT7 receptor inhibition and inactivation induce antidepressantlike behavior and sleep pattern. Biol Psychiatry 58: 831–837

Heisler LK, Chu HM, Brennan TJ, Danao JA, Bajwa P, Parsons LH, Tecott LH (1998) Elevated anxiety and antidepressant-like responses in

serotonin 5-HT1A receptor mutant mice. Proc Natl Acad Sci USA 95: 15049–15054

Hillion J, Canals M, Torvinen M, Casado V, Scott R, Terasmaa A, Hansson A, Watson S, Olah ME, Mallol J, Canela EI, Zoli M, Agnati LF, Ibanez CF, Lluis C, Franco R, Ferré S, Fuxe K (2002) Coaggregation, cointernalization, and codesensitization of adenosine A2A receptors and dopamine D2 receptors. J Biol Chem 277: 18091–18097

Jaffe EK (2005) Morpheeins – a new structural paradigm for allosteric regulation. Trends Biochem Sci 30: 490–497

Janovick JA, Conn PM (1996) Gonadotropin releasing hormone agonist provokes homologous receptor microaggregation: an early event in seven-transmembrane receptor mediated signaling. Endocrinology 137: 3602–3605

Jentsch JD, Roth RH (1999) The neuropsychopharmacology of phencyclidine: from NMDA receptor hypofunction to the dopamine hypothesis of schizophrenia. Neuropsychopharmacology 20: 201–225

Kachroo A, Orlando LR, Grandy DK, Chen JF, Young AB, Schwarzschild MA (2005) Interactions between metabotropic glutamate 5 and adenosine A2A receptors in normal and parkinsonian mice. J Neurosci 25: 10414–10419

Kamiya T, Saitoh O, Yoshioka K, Nakata H (2003) Oligomerization of adenosine A2A and dopamine D2 receptors in living cells. Biochem Biophys Res Commun 306: 544–549

Kanda T, Jackson MJ, Smith LA, Pearce RK, Nakamura J, Kase H, Kuwana Y, Jenner P (1998) Adenosine A2A antagonist: a novel antiparkinsonian agent that does not provoke dyskinesia in parkinsonian monkeys. Ann Neurol 43: 507–513

Kapur S, Mamo D (2003) Half a century of antipsychotics and still a central role for dopamine D2 receptors. Prog Neuropsychopharmacol Biol Psychiatry 27: 1081–1090

Kaufmann A (1993) The origins of order. Oxford University Press, New York

Kearney JA, Albin RL (1995) Adenosine A2 receptor-mediated modulation of contralateral rotation induced by metabotropic glutamate receptor activation. Eur J Pharmacol 287: 115–120

Kehr J, Yoshitake T, Wang FH, Razani H, Gimenez-Llort L, Jansson A, Yamaguchi M, Ogren SO (2002) Galanin is a potent in vivo modulator of mesencephalic serotonergic neurotransmission. Neuropsychopharmacology 27: 341–356

Kenakin T (2003) Ligand-selective receptor conformations revisited: the promise and the problem. Trends Pharmacol Sci 24: 346–354

Le Moine C, Svenningsson P, Fredholm BB, Bloch B (1997) Dopamine-adenosine interactions in the striatum and the globus pallidus: inhibition of striatopallidal neurons through either D2 or A2A receptors enhances D1 receptor-mediated effects on c-fos expression. J Neurosci 17: 8038–8040

Lee C, Ji IJ, Ji TH (2002) Use of defined-function mutants to access receptor–receptor interactions. Methods 27: 318–323

Lee FJ, Xue S, Pei L, Vukusic B, Chery N, Wang Y, Wang YT, Niznik HB, Yu XM, Liu F (2002) Dual regulation of NMDA receptor functions by direct protein–protein interactions with the dopamine D1 receptor. Cell 111: 219–230

Lee SP, So CH, Rashid AJ, Varghese G, Cheng R, Lanca AJ, O'Dowd BF, George SR (2004) Dopamine D1 and D2 receptor Co-activation generates a novel phospholipase C-mediated calcium signal. J Biol Chem 279: 35671–35678

Lee SP, Xie Z, Varghese G, Nguyen T, O'Dowd BF, George SR (2000) Oligomerization of dopamine and serotonin receptors. Neuropsychopharmacology 23: S32–S40

Lefkowitz RJ (2004) Historical review: a brief history and personal retrospective of seven-transmembrane receptors. Trends Pharmacol Sci 25: 413–422

Lefkowitz RJ (2005) Summary of Wenner-Gren international symposium receptor–receptor interactions among heptaspanning membrane receptors: from structure to function. J Mol Neurosci 26: 293–294

Lefkowitz RJ, Cotecchia S, Samama P, Costa T (1993) Constitutive activity of receptors coupled to guanine nucleotide regulatory proteins. Trends Pharmacol Sci 14: 303–307

Limbird LE, Lefkowitz RJ (1976) Negative cooperativity among beta-adrenergic receptors in frog erythrocyte membranes. J Biol Chem 251: 5007–5014

Limbird LE, Meyts PD, Lefkowitz RJ (1975) Beta-adrenergic receptors: evidence for negative cooperativity. Biochem Biophys Res Commun 64: 1160–1168

Liu F, Wan Q, Pristupa ZB, Yu XM, Wang YT, Niznik HB (2000) Direct protein–protein coupling enables cross-talk between dopamine D5 and gamma-aminobutyric acid A receptors. Nature 403: 274–280

Maguire JJ, Davenport AP (2005) Regulation of vascular reactivity by established and emerging GPCRs. Trends Pharmacol Sci 26: 448–454

Marino MJ, Valenti O, Conn PJ (2003) Glutamate receptors and Parkinson's disease: opportunities for intervention. Drugs Aging 20: 377–397

Marshall FH, Jones KA, Kaupmann K, Bettler B (1999) GABAB receptors – the first 7TM heterodimers. Trends Pharmacol Sci 20: 396–399

McLatchie LM, Fraser NJ, Main MJ, Wise A, Brown J, Thompson N, Solari R, Lee MG, Foord SM (1998) RAMPs regulate the transport and ligand specificity of the calcitonin-receptor-like receptor. Nature 393: 333–339

Mellado M, Rodriguez-Frade JM, Vila-Coro AJ, Fernandez S, Martin de Ana A, Jones DR, Toran JL, Martinez AC (2001) Chemokine receptor homo- or heterodimerization activates distinct signaling pathways. EMBO J 20: 2497–2507

Meyer-Lindenberg A, Miletich RS, Kohn PD, Esposito G, Carson RE, Quarantelli M, Weinberger DR, Berman KF (2002) Reduced prefrontal activity predicts exaggerated striatal dopaminergic function in schizophrenia. Nat Neurosci 5: 267–271

Milligan G (2004) G protein-coupled receptor dimerization: function and ligand pharmacology. Mol Pharmacol 66: 1–7

Morelli M, Wardas J (2001) Adenosine A(2A) receptor antagonists: potential therapeutic and neuroprotective effects in Parkinson's disease. Neurotox Res 3: 545–556

Murase S, Mathe JM, Grenhoff J, Svensson TH (1993) Effects of dizocilpine (MK-801) on rat midbrain dopamine cell activity: differential actions on firing pattern related to anatomical localization. J Neural Transm Gen Sect 91: 13–25

Nakata H, Yoshioka K, Kamiya T, Tsuga H, Oyanagi K (2005) Functions of heteromeric association between adenosine and P2Y receptors. J Mol Neurosci 26: 233–238

Ng GY, O'Dowd BF, Caron M, Dennis M, Brann MR, George SR (1994) Phosphorylation and palmitoylation of the human D2L dopamine receptor in Sf9 cells. J Neurochem 63: 1589–1595

Nicola SM, Surmeier J, Malenka RC (2000) Dopaminergic modulation of neuronal excitability in the striatum and nucleus accumbens. Annu Rev Neurosci 23: 185–215

Nimchinsky EA, Hof PR, Janssen WG, Morrison JH, Schmauss C (1997) Expression of dopamine D3 receptor dimers and tetramers in brain and in transfected cells. J Biol Chem 272: 29229–29237

Ögren S-O, Fuxe K, Agnati LF, Gustafsson J-Å, Jonsson G, Holm AC (1979) Reevaluation of the indoleamine hypothesis of depression. Evidence for a reduction of functional activity of central 5-HT systems by antidepressant drugs. J Neural Transm 46: 85–103

Olias G, Viollet C, Kusserow H, Epelbaum J, Meyerhof W (2004) Regulation and function of somatostatin receptors. J Neurochem 89: 1057–1091

Ossowska K, Konieczny J, Wolfarth S, Wieronska J, Pilc A (2001) Blockade of the metabotropic glutamate receptor subtype 5 (mGluR5) produces antiparkinsonian-like effects in rats. Neuropharmacology 41: 413–420

Paglin S, Jamieson JD (1982) Covalent crosslinking of angiotensin II to its binding sites in rat adrenal membranes. Proc Natl Acad Sci USA 79: 3739–3743

Parks CL, Robinson PS, Sibille E, Shenk T, Toth M (1998) Increased anxiety of mice lacking the serotonin1A receptor. Proc Natl Acad Sci USA 95: 10734–10739

Patel RC, Kumar U, Lamb DC, Eid JS, Rocheville M, Grant M, Rani A, Hazlett T, Patel SC, Gratton E, Patel YC (2002) Ligand binding to somatostatin receptors induces receptor-specific oligomer formation in live cells. Proc Natl Acad Sci USA 99: 3294–3299

Peroutka SJ, Snyder SH (1979) Multiple serotonin receptors: differential binding of [3H]5-hydroxytryptamine, [3H]lysergic acid diethylamide and [3H]spiroperidol. Mol Pharmacol 16: 687–699

Pfeiffer M, Koch T, Schroder H, Klutzny M, Kirscht S, Kreienkamp HJ, Hollt V, Schulz S (2001) Homo- and heterodimerization of somatostatin receptor subtypes. Inactivation of sst(3) receptor function by heterodimerization with sst(2A). J Biol Chem 276: 14027–14036

Pfeiffer M, Koch T, Schroder H, Laugsch M, Hollt V, Schulz S (2002) Heterodimerization of somatostatin and opioid receptors cross-modulates phosphorylation, internalization, and desensitization. J Biol Chem 277: 19762–19772

Pinna A, di Chiara G, Wardas J, Morelli M (1996) Blockade of A2A adenosine receptors positively modulates turning behaviour and c-Fos expression induced by D1 agonists in dopamine-denervated rats. Eur J Neurosci 8: 1176–1181

Pinna A, Wardas J, Cozzolino A, Morelli M (1999) Involvement of adenosine A2A receptors in the induction of c-fos expression by clozapine and haloperidol. Neuropsychopharmacology 20: 44–51

Pintor A, Quarta D, Pezzola A, Reggio R, Popoli P (2001) SCH 58261 (an adenosine A(2A) receptor antagonist) reduces, only at low doses, K(+)-evoked glutamate release in the striatum. Eur J Pharmacol 421: 177–180

Pollack AE, Fink JS (1996) Synergistic interaction between an adenosine antagonist and a D1 dopamine agonist on rotational behavior and striatal c-Fos induction in 6-hydroxydopamine-lesioned rats. Brain Res 743: 124–130

Popoli P, Pezzola A, Torvinen M, Reggio R, Pintor A, Scarchilli L, Fuxe K, Ferré S (2001) The selective mGlu(5) receptor agonist CHPG inhibits quinpirole-induced turning in 6-hydroxydopamine-lesioned rats and modulates the binding characteristics of dopamine D(2) receptors in the rat striatum: interactions with adenosine A(2A) receptors. Neuropsychopharmacology 25: 505–513

Quitterer U, Lother H, Abdalla S (2004) AT1 receptor heterodimers and angiotensin II responsiveness in preeclampsia. Semin Nephrol 24: 115–119

Razani H, Diaz-Cabiale Z, Fuxe K, Ögren SO (2000) Intraventricular galanin produces a time-dependent modulation of 5-HT1A receptors in the dorsal raphe of the rat. NeuroReport 11: 3943–3948

Rimondini R, Ferré S, Giménez-Llort L, Ögren SO, Fuxe K (1998) Differential effects of selective adenosine A1 and A2A receptor agonists on dopamine receptor agonist-induced behavioural responses in rats. Eur J Pharmacol 347: 153–158

Rimondini R, Ferré S, Ögren SO, Fuxe K (1997) Adenosine A2A agonists: A potential new type of atypical antipsychotic. Neuropsychopharmacology 17: 82–91

Rivera A, Cuellar B, Giron FJ, Grandy DK, de la Calle A, Moratalla R (2002) Dopamine D4 receptors are heterogeneously distributed in the striosomes/matrix compartments of the striatum. J Neurochem 80: 219–229

Rocheville M, Lange DC, Kumar U, Patel SC, Patel RC, Patel YC (2000a) Receptors for dopamine and somatostatin: formation of hetero-oligomers with enhanced functional activity. Science 288: 154–157

Rocheville M, Lange DC, Kumar U, Sasi R, Patel RC, Patel YC (2000b) Subtypes of the somatostatin receptor assemble as functional homo- and heterodimers. J Biol Chem 275: 7862–7869

Rodrigues RJ, Alfaro TM, Rebola N, Oliveira CR, Cunha RA (2005) Co-localization and functional interaction between adenosine A(2A) and metabotropic group 5 receptors in glutamatergic nerve terminals of the rat striatum. J Neurochem 92: 433–441

Rodriguez-Frade JM, del Real G, Serrano A, Hernanz-Falcon P, Soriano SF, Vila-Coro AJ, de Ana AM, Lucas P, Prieto I, Martinez AC, Mellado M (2004) Blocking HIV-1 infection via CCR5 and CXCR4 receptors by acting in trans on the CCR2 chemokine receptor. EMBO J 23: 66–76

Rodriguez-Frade JM, Mellado M, Martinez AC (2001) Chemokine receptor dimerization: two are better than one. Trends Immunol 22: 612–617

Roess DA, Horvat RD, Munnelly H, Barisas BG (2000) Luteinizing hormone receptors are self-associated in the plasma membrane. Endocrinology 141: 4518–4523

Roh J, Chang CL, Bhalla A, Klein C, Hsu SY (2004) Intermedin is a calcitonin/calcitonin gene-related peptide family peptide acting through the calcitonin receptor-like receptor/receptor activity-modifying protein receptor complexes. J Biol Chem 279: 7264–7274

Saveanu A, Lavaque E, Gunz G, Barlier A, Kim S, Taylor JE, Culler MD, Enjalbert A, Jaquet P (2002) Demonstration of enhanced potency of a chimeric somatostatin-dopamine molecule, BIM-23A387, in suppressing growth hormone and prolactin secretion from human pituitary somatotroph adenoma cells. J Clin Endocrinol Metab 87: 5545–5552

Scarselli M, Novi F, Schallmach E, Lin R, Baragli A, Colzi A, Griffon N, Corsini GU, Sokoloff P, Levenson R, Vogel Z, Maggio R (2001) D2/D3 dopamine receptor heterodimers exhibit unique functional properties. J Biol Chem 276: 30308–30314

Schöneberg T, Schulz A, Biebermann H, Hermsdorf T, Rompler H, Sangkuhl K (2004) Mutant G-protein-coupled receptors as a cause of human diseases. Pharmacol Ther 104: 173–206

Schwartz JC, Diaz J, Pilon C, Sokoloff P (2000) Possible implications of the dopamine D(3) receptor in schizophrenia and in antipsychotic drug actions. Brain Res Brain Res Rev 31: 277–287

Schwarzschild MA, Xu K, Oztas E, Petzer JP, Castagnoli K, Castagnoli N Jr, Chen JF (2003) Neuroprotection by caffeine and more specific A2A receptor antagonists in animal models of Parkinson's disease. Neurology 61: S55–S61

Shiozaki S, Ichikawa S, Nakamura J, Kitamura S, Yamada K, Kuwana Y (1999) Actions of adenosine A2A receptor antagonist KW-6002 on drug-induced catalepsy and hypokinesia caused by reserpine or MPTP. Psychopharmacology (Berl) 147: 90–95

Strömberg I, Popoli P, Müller CE, Ferré S, Fuxe K (2000) Electrophysiological and behavioural evidence for an antagonistic modulatory role of adenosine A2A receptors in dopamine D2 receptor regulation in the rat dopamine-denervated striatum. Eur J Neurosci 12: 4033–4037

Svensson TH (2000) Dysfunctional brain dopamine systems induced by psychotomimetic NMDA-receptor antagonists and the effects of antipsychotic drugs. Brain Res Brain Res Rev 31: 320–329

Swanson CJ, Blackburn TP, Zhang X, Zheng K, Xu ZQ, Hokfelt T, Wolinsky TD, Konkel MJ, Chen H, Zhong H, Walker MW, Craig DA, Gerald CP, Branchek TA (2005) Anxiolytic- and antidepressant-like profiles of the galanin-3 receptor (Gal3) antagonists SNAP 37889 and SNAP 398299. Proc Natl Acad Sci USA 102: 17489–17494

Tanganelli S, Sandager Nielsen K, Ferraro L, Antonelli T, Kehr J, Franco R, Ferré S, Agnati LF, Fuxe K, Scheel-Kruger J (2004) Striatal plasticity at the network level. Focus on adenosine A2A and D2 interactions in models of Parkinson's Disease. Parkinsonism Relat Disord 10: 273–280

Terrillon S, Durroux T, Mouillac B, Breit A, Ayoub MA, Taulan M, Jockers R, Barberis C, Bouvier M (2003) Oxytocin and vasopressin V1A and V2 receptors form constitutive homo- and heterodimers during biosynthesis. Mol Endocrinolopy 17: 677–691

Torvinen M, Kozell LB, Neve KA, Agnati LF, Fuxe K (2004) Biochemical identification of the dopamine D2 receptor domains interacting with the adenosine A2A receptor. J Mol Neurosci 24: 173–180

Torvinen M, Marcellino D, Canals M, Agnati LF, Lluis C, Franco R, Fuxe K (2005a) Adenosine A2A receptor and dopamine D3 receptor interac-

tions: evidence of functional A2A/D3 heteromeric complexes. Mol Pharmacol 67: 400–407

Torvinen M, Torri C, Tombesi A, Marcellino D, Watson S, Lluis C, Franco R, Fuxe K, Agnati LF (2005b) Trafficking of adenosine A2A and dopamine D2 receptors. J Mol Neurosci 25: 191–200

van Holde KE, Miller KI, van Olden E (2000) Allostery in very large molecular assemblies. Biophys Chem 86: 165–172

van Tol HH, Bunzow JR, Guan HC, Sunahara RK, Seeman P, Niznik HB, Civelli O (1991) Cloning of the gene for a human dopamine D4 receptor with high affinity for the antipsychotic clozapine. Nature 350: 610–614

Vischer HF, Leurs R, Smit MJ (2006) HCMV-encoded G-protein-coupled receptors as constitutively active modulators of cellular signaling networks. Trends Pharmacol Sci 69: 56–63

Wahlestedt C, Hakanson R, Vaz CA, Zukowska-Grojec Z (1990) Norepinephrine and neuropeptide Y: vasoconstrictor cooperation in vivo and in vitro. Am J Physiol 258: R736–R742

Ward SG, Bacon K, Westwick J (1998) Chemokines and T lymphocytes: more than an attraction. Immunity 9: 1–11

Woods AS (2004) The mighty arginine, the stable quaternary amines, the powerful aromatics, and the aggressive phosphate: their role in the noncovalent minuet. J Proteome Res 3: 478–484

Woods AS, Ciruela F, Fuxe K, Agnati LF, Lluis C, Franco R, Ferré S (2005) Role of electrostatic interaction in receptor–receptor heteromerization. J Mol Neurosci 26: 125–132

Woods AS, Ferré S (2005) Amazing stability of the arginine-phosphate electrostatic interaction. J Proteome Res 4: 1397–1402

Xu K, Bastia E, Schwarzschild M (2005) Therapeutic potential of adenosine A(2A) receptor antagonists in Parkinson's disease. Pharmacol Ther 105: 267–310

Xu ZQ, Zhang X, Pieribone VA, Grillner S, Hokfelt T (1998) Galanin-5-hydroxytryptamine interactions: electrophysiological, immunohistochemical and in situ hybridization studies on rat dorsal raphe neurons with a note on galanin R1 and R2 receptors. Neuroscience 87: 79–94

Zangen A, Overstreet DH, Yadid G (1997) High serotonin and 5-hydroxyindoleacetic acid levels in limbic brain regions in a rat model of depression: normalization by chronic antidepressant treatment. J Neurochem 69: 2477–2483

Zoli M, Agnati LF, Hedlund PB, Li XM, Ferré S, Fuxe K (1993) Receptor–receptor interactions as an integrative mechanism in nerve cells. Mol Neurobiol 7: 293–334

Zoli M, Guidolin D, Fuxe K, Agnati LF (1996) The receptor mosaic hypothesis of the engram: possible relevance of Boolean network modeling. Int J Neural Syst 7: 363–368