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Receptor–Receptor Interactions as an Integrative Mechanism in Nerve Cells

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Contents

Abstract

Several lines of evidence indicate that interactions among transmission lines can take place at the level of the cell membrane via interactions among macromolecules, integral or associated to the cell membrane, involved in signal recognition and transduction. The present view will focus on this last subject, i.e., on the interactions between receptors for chemical signals at the level of the neuronal membrane (receptor–receptor interaction).

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By receptor–receptor interaction we mean that a neurotransmitter or modulator, by binding to its receptor, modifies the characteristics of the receptor for another transmitter or modulator. Four types of interactions among transmission lines may be considered, but mainly intramembrane receptor–receptor interactions have been dealt with in this article, exemplified by the heteroregulation of D2 receptors via neuropeptide receptors and A2 receptors. The role of receptor–receptor interactions in the integration of signals is discussed, especially in terms of filtration of incoming signals, of integration of coincident signals, and of neuronal plasticity.

Index Entries: Transmitter; receptor; transmission line; heteroregulation; homoregulation.

Introduction

Interneuronal communication can take place via electrical or chemical signals reaching their target cells via either synaptic contacts or the extracellular fluid (ECF) (Agnati et al., 1986, 1992; Fuxe and Agnati, 1991). The neuronal membrane is capable of converting chemical into electrical signals and vice versa, as well as of transducing these signals into proper messages for the cellular biochemical machinery. Thus, thanks to this role as an interface between the ECF and the intracellular environment, the neuronal membrane subserves highly sophisticated informational tasks.

The neuronal membrane by means of receptors recognizes and transduces into proper intracellular signals a specific subset of the huge number of extracellular signals continuously impinging on it. Different transmission lines* allow the information to flow from the chemical signal–receptor complex down to the cytoplasmic target molecules and even to the genome. However, this is not a straight through process, since at several levels of the intracellular cascade feedback circuits can affect previous steps. Moreover, these parallel transmission lines can reciprocally interact at multiple levels, forming a complex network of signals stemming from the membrane and going back to it. Several lines of evidence indicate that interactions among transmission lines can take place at the level of the cell membrane via interactions among macromolecules, integral or associated to the cell membrane, involved in signal recognition and transduction. The present review will focus on this last subject, i.e., on the interactions between receptors for chemical signals at the level of the neuronal membrane ("receptor–receptor interaction").

By receptor–receptor interaction we mean that a neurotransmitter or a modulator, by binding to its receptor, modifies the characteristics of the receptor for another transmitter or modulator. This type of regulatory phenomenon is then part of the broader class of receptor heteroregulation phenomena. Whereas receptor homoregulation (e.g., sensitization and desensitization) represents mechanisms by which a receptor is modulated by the molecules belonging to its own transmission line (ligand, second messengers, and so forth), receptor heteroregulation represents a direct or indirect modulation of a certain receptor by molecules (including other receptors) belonging to different transmission lines or by other intracellular signals.

From theoretical considerations and experimental evidence, four types of interactions among transmission lines involving receptor regulation may be surmized (*see* Fig. 1):

A. Allosteric changes of a receptor macromolecular complex caused by the binding on it of a modulator.
B. Interactions between physically distinct but adjacent receptors involving the extracellular loops (i.e., taking place in the glycocalyx milieu, mechanism type B1), the transmembrane helices (i.e., taking place in the lipid bilayer milieu, mechanism type B2), or the intracytoplasmic loops (i.e., taking place at the interface between the membrane and the cytoplasm,

*Transmitter = molecule that activates a receptor; modulator = molecule that changes the activity of a transmitter on its receptor; transmission line = the cascade of molecular events at membrane, cytoplasmic and, possibly, nuclear levels triggered by the binding of a transmitter to its receptor.

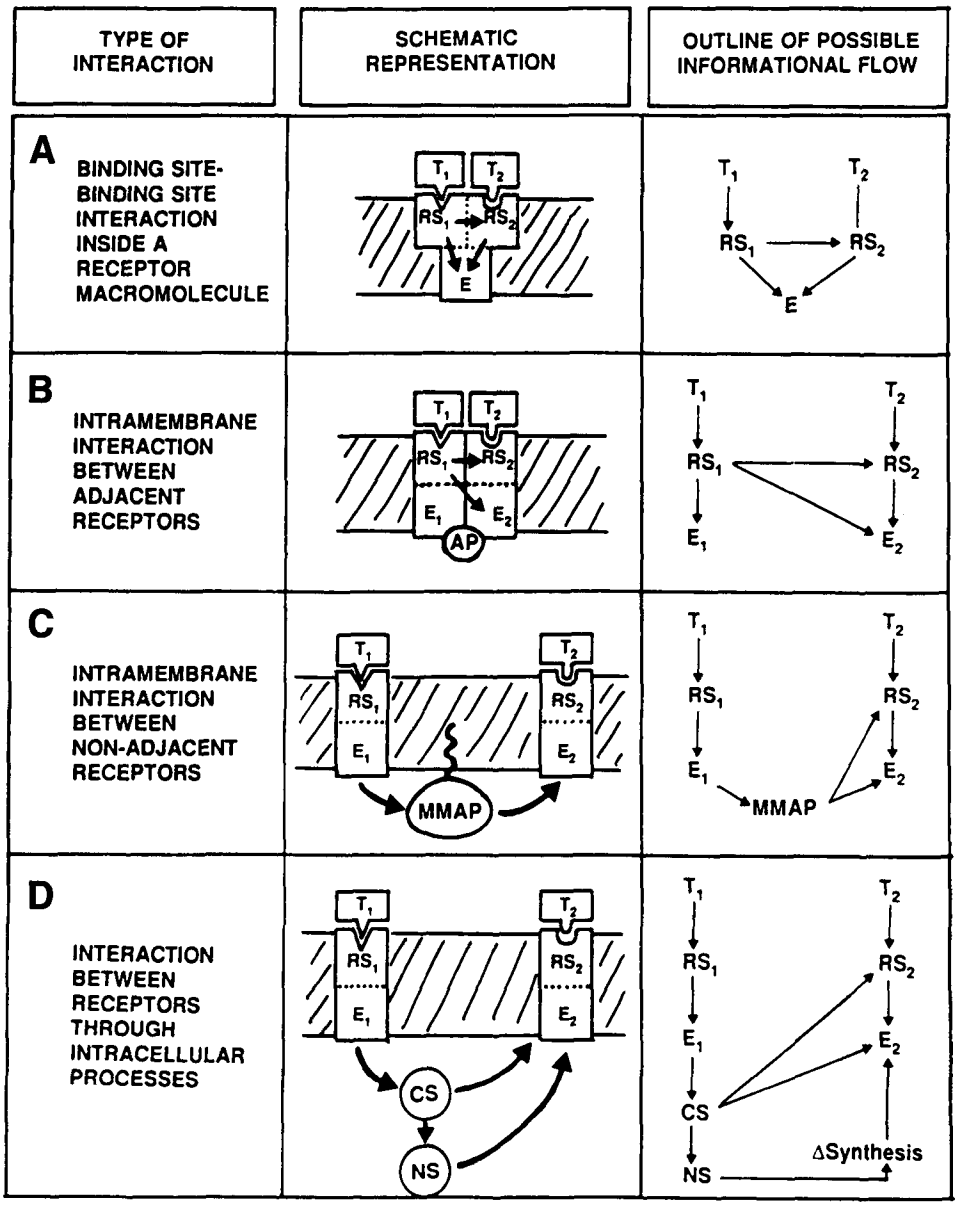

Fig. 1. Schematic representation of the possible types **(A–D)** of receptor–receptor interactions as outlined in the text. For the sake of simplicity, only unidirectional interactions are shown. Abbreviations: AP = associating protein, CS = cytoplasmic signals, E = effector portion of the receptor, MMAP = mobile membrane-associated protein (e.g., G protein), NS = nuclear signals, RS = recognition site, T = transmitter.

mechanism type B3) of the receptor molecules. These interactions either can be direct or can involve associated proteins.

C. Interactions between physically distinct and nonadjacent receptors involving the activation of mobile membrane-associated proteins, such as G proteins.

D. Interactions between physically distinct and nonadjacent receptors through intracellular processes, such as protein phosphorylation and protein biosynthesis (i.e., taking place in the internal milieu of the cell and often involving the gene level).

Interaction type A is an intramolecular phenomenon and can be defined as binding site–binding site interaction inside a receptor molecule. Interaction types B and C are true membrane

receptor–receptor interactions and represent a crosstalk between transmission lines at the level of the cellular membrane. Interaction type D represents a crosstalk between transmission lines through their postmembrane transductional mechanism, eventually resulting in modifications of membrane receptor molecules.

The existence of allosteric changes in a receptor molecule caused by the activation of a binding site for a modulator present on the receptor itself has been initially demonstrated for the γ-aminobutyric acid (GABA) A-benzodiazepine (BDZ) receptor. According to the previous classification, this phenomenon is an example of a type A interaction. The addition of GABA to crude membrane preparations increases the affinity of the BDZ binding site (Guidotti et al., 1978) and, conversely, BDZs facilitate the binding of GABA to its high-affinity binding sites (Tallman et al., 1978). More recently, it was found that the inhibitory neurotransmitter glycine can bind to the *N*-methyl-D-aspartate (NMDA)-type of glutamate receptors, thus facilitating the activation of the receptor by glutamate (Johnson and Ascher, 1987).

Soon after the first evidence of the GABA-BDZ interaction appeared, interactions were shown to occur between different receptor molecules not linked to each other in a supramolecular complex (Agnati et al., 1980, 1982; Fuxe et al., 1981a,b). These interactions, observed in brain membrane preparations, may occur via one or more types of interaction (B and C).

Leaving the membrane, and therefore the receptor crosstalk confined to this compartment, modulation of one receptor function can take place through mechanisms involving other cellular compartments. Several studies have shown that intracytoplasmic second messengers activated by a receptor can lead to the activation of a protein kinase and to the subsequent phosphorylation of a second receptor, thus modulating its function. Examples of these modulatory processes, which belong to the type D interaction of our classification, include the β-adrenergic receptor-prostaglandin E1 receptor interactions and the nicotinic acetylcholine (ACh)-calcitonin gene related peptide (CGRP) receptor interactions (*see* review by Huganir and Greengard, 1990).

Against this background we can now present some features of the receptor–receptor interactions and also some general aspects of the interactions among various transmission lines. Since several reviews are already available on points A and D, the main focus of this review will be on points B and C.

Different Levels of Receptor–Receptor Interactions

Type A Interactions—Interactions Between Binding Sites on the Same Receptor Molecule

The first demonstration of an allosteric modulator of a receptor was made in the analysis of $GABA_A$ receptors. It was shown early that BDZs could enhance GABA transmission (Costa et al., 1975; Haefley et al., 1975). A large number of papers have subsequently demonstrated that the BDZs bind to a high-affinity site located on the $GABA_A$ receptor, enhancing GABA receptor function through an increase in the frequency of channel opening. In addition, BDZs enhance the binding of GABA (Guidotti et al., 1978; Study and Barker, 1981; Sivilotti and Nistri, 1990; Haefley, 1992; Sieghart, 1992). It seems that, unlike barbiturates, BDZs alone are not able to open the Cl^- channel (Vicini et al., 1987). In turn, GABA in crude membrane preparations increases the affinity of the BDZ-binding site (Tallman et al., 1978). Cloning of the $GABA_A$ receptor subunits has shed light on some structural requirements for the BDZ action on the receptor. Although the presence of γ_2 subunits is necessary to confer sensitivity to BDZs, the properties of BDZ binding appear to be mainly dependent on the type of the α subunit present. Thus, the BZ_1 subtype is supposed to be constituted by the α_1 subunit, whereas the BZ_2 subtype by the α_2, α_3, and α_5 subunits and the low affinity BDZ receptors of cerebellar granule cells by the α_6 subunit (Lüddens and Wisden, 1991). Several other allosteric modulators of $GABA_A$ receptors have also

been characterized, including barbiturates, neurosteroids, and Zn^{2+} (Sieghart, 1992).

Also, the NMDA receptor complex contains a number of binding sites for allosteric modulators controlling the glutamate-binding site and the gating of the ion channel, including glycine, Zn^{2+}, and polyamines (Williams et al., 1990). As initially demonstrated by Johnson and Ascher (1987), the activation of the NMDA channel by glutamate is facilitated by the binding of glycine (Kemp and Leeson, 1993). After the cloning of the functional NMDA receptor, it has been possible to obtain direct evidence that a glycine-binding site is present in the same protein carrying the NMDA recognition site (Moriyoshi et al., 1991). In addition, different NMDA receptor subunits have distinct affinities for glycine (Kemp and Leeson, 1993). The case of glycine modulation of the NMDA receptor is, however, very peculiar. In fact, contrary to the other known allosteric modulators, the NMDA receptor cannot be activated by glutamate in the absence of glycine. Glycine should then be considered a coagonist at NMDA receptors (Kemp and Leeson, 1993).

As in the case of the GABA-BDZ interaction *(see below)*, binding of glycine appears to regulate the properties of glutamate binding and vice versa. Thus, glutamate enhances ^{3}H-glycine binding, and glycine enhances ^{3}H-glutamate binding (Kessler et al., 1989; Monaghan et al., 1988). The partial agonist for the glycine binding site HA-966 increases the dissociation rate of glutamate compared with the dissociation rate in the presence of glycine (Kemp and Priestley, 1991). HA-966 also noncompetitively inhibits ^{3}H-glutamate binding, while enhancing the binding of NMDA antagonists of the competitive type (Danysz et al., 1989). These data are at variance with electrophysiological experiments indicating that glycine and glutamate-binding sites are negatively coupled (Benveniste et al., 1990; Kemp and Leeson, 1993). It must, however, be remembered that equilibrium-binding experiments cannot discriminate among (multiple) open and desensitized states and do not reflect the fast kinetic changes observed in electrophysiological experiments.

Several modulators have been shown for muscle type nicotinic ACh receptor, another member of the ligand-gated ion channel superfamily (Léna and Changeux, 1993). Some evidence suggests that neuronal nicotinic ACh receptors also may have binding sites for allosteric modulators, such as substance P (SP), atrial natriuretic factor (ANF), and steroids. The ability of SP to inhibit nicotinic ACh receptor function in PC12 and chromaffin cells appears to be related to a stabilization of the desensitized state of the receptor (Boyd and Leeman, 1987). Structure–activity analysis revealed that the action of SP on nicotinic ACh receptors takes place at a binding site different from the G-protein-linked SP receptor (Geraghty et al., 1990), suggesting that the effect may be caused by the binding of SP to the nicotinic receptor. In addition, SP may directly block the flux of ions through the channel (Boyd and Leeman, 1987). In similar studies, it has been observed that ANF can also reduce nicotine-induced currents (Bormann et al., 1989). However, in this case it appears more likely that the gating of the ion channel of the nicotine receptor is brought about by an interaction between independent receptors (interaction types B or C). Further experiments using ANF and SP receptor antagonists and cloned neuronal nicotinic receptors will help differentiate between these two types of mechanistic models for peptide modulation of gating of nicotinic channels.

Receptor changes on agonist and modulator binding have been interpreted (Changeux, 1990) according to the model of allosteric transitions between conformational states proposed by Monod et al. (1965). The allosteric modulators bind to sites different from that of the agonist and can affect the equilibrium and/or the kinetics of the transitions between the conformational states. It is assumed that changes in the properties of the conformational states caused by the modulators are of negligible entity when compared with the differences existing between the properties of the main conformational states themselves (for a detailed discussion, *see* Léna and Changeux, 1993). This theory has been successfully applied to the explanation of the properties of muscle nicotinic ACh receptor on binding of ACh and some modulators (Heidmann and Changeux, 1979a,b). Recent studies are starting to charac-

terize conformational states and their transitions in the presence of the agonist or modulators for the other ligand-gated ion channels (Macdonald et al. 1989; Weiss and Magleby, 1989; Ito et al., 1990; Sieghart, 1992).

Since discussed above, a wealth of allosteric modulators have been demonstrated for ligand-gated ion channels. Although the pharmacological actions of these modulators are clearcut (*see*, e.g., the case of BDZs and the search for allosteric antagonists of glutamate receptors), their physiological role is still debated. Some questions seem to be relevant in this respect:

1. Are there endogenous substances capable of binding to the allosteric modulator site in the effective range of concentrations?
2. Are there physiological mechanisms for regulating the concentration of the endogenous ligands? If this is the case, it is interesting to know if the substance can be released by neurons, i.e., acting like a neuromodulator or derive from other sources.
3. Since the same modulator can affect several receptor types present on a postsynaptic cell how can its action be differently regulated?

Several claims have been made for the existence of endogenous BDZs, or endozepins (*see*, e.g., Barbaccia et al., 1988; Rothstein et al., 1992). Evidence for their physiological roles is, however, not conclusive. Among others, a strong case for an action as a neuromodulator has been made for Zn^{2+}. This molecule is able to modulate NMDA, AMPA/kainate, and GABA receptor gating properties (Mayer et al., 1989; Legendre and Westbrook, 1991). Interestingly, Zn^{2+} is highly enriched in the synaptic vesicles of the hippocampal mossy fibers, and can be released during neuronal activity (Perez-Clausell and Danscher, 1985; Assaf and Chung, 1984; Howell et al., 1984). Also, polyamines (positive allosteric modulators of glutamate binding on NMDA receptors; Williams et al., 1991) have been detected in the brain ECF (Grimaldi et al., 1991). In particular, they have been shown to increase after lesion (Desiderio et al., 1988; Paschen et al., 1988), which led to the suggestion that they may be involved in modulating glutamate receptor neurotoxicity (Paschen et al., 1988; Zoli et al., 1993). Finally, cytoskeletal proteins, such as fodrin, should be considered as a regulator (Simon et al., 1985).

The mechanism proposed for the physiological action of glycine on the NMDA receptor is peculiar. Glycine levels in the ECF are supposed to be sufficient to saturate the glycine site in basal conditions (Kemp and Leeson, 1993). The NMDA receptor would therefore be always activatable by the endogenous ligand. However, the glycine modulation of NMDA receptor function may occur through a local decrease in glycine extracellular levels via a neuron-specific glycine transporter, a protein highly enriched in areas containing high levels of NMDA receptors (D'Angelo et al., 1990; Smith et al., 1992). This view is supported by electrophysiological evidence showing that glycine can potentiate some glutamate responses in vivo (Kemp and Leeson, 1993).

Type B and C Interactions—The Membrane Level of Receptor-Receptor Interactions: G Protein-Mediated and G Protein-Independent Mechanisms

A huge amount of experimental evidence has accumulated in the last 10 yr on the existence of changes in receptor characteristics induced by the administration of a modulator in crude membrane preparations (for reviews *see* Fuxe and Agnati, 1985, 1987). Intramembrane modulatory processes between neuropeptide and monoamine receptors were early described in the central nervous system (CNS), including interactions between SP/5-HT_1 receptors, vasoactive intestinal polypeptide (VIP)/5-HT_1 receptors, cholecystokinin (CCK)/dopamine (DA) D2 receptors, neurotensin (NT)/DA D2 receptors, and the neuropeptide (NPY)/α2 adrenergic receptor interactions (Agnati et al., 1980, 1982, 1983a,b,c, d,e, 1984; Agnati and Fuxe, 1983; Fuxe et al., 1981a,b, 1983c; Rostene et al., 1983a,b). Similar modulatory events have been described for interactions between monoamine receptor subtypes (Seeman

et al., 1989), peptide receptors (Rothman et al., 1988), monoamine and adenosine receptors (Ferré et al., 1991d; Murayama et al., 1990), glutamate receptor subtypes (Fuxe et al., 1983b), and monoamine or peptide and ligand-gated ion channels (Fuxe et al., 1983a, 1984b, 1989b).

Since these interactions have been found in crude membrane preparations, they do not involve cytoplasmic loops (second messengers, phosphorylations, and so forth). These phenomena have therefore been explained on the basis of an interaction between receptor molecules, possibly including G proteins and other molecules associated with the membrane.

Direct binding of the modulator to the receptor cannot be, in principle, ruled out. As already discussed, a huge number of modulatory sites has been shown in ligand-gated ion channels and it cannot be excluded that similar sites are present in G-protein-linked receptors. However, several lines of evidence indicate that the interaction takes place between independent receptor molecules. Independent receptors for the modulatory substances have been demonstrated biochemically and sometimes also cloned. These receptors are known to be present in the areas, and sometimes even in the same cells, where the modulation is demonstrated. The effects are present when the modulator is administered at concentrations close to the K_d of the known modulator receptors. Experiments on reconstituted systems will help clarify the possible existence of modulatory sites on the receptors.

In a number of studies it has been shown that receptor–receptor interactions are specific for receptor isotypes (*see* Table 1). For instance, several modulators (CCK, NT, adenosine) administered in the nanomolar range are able to change the binding characteristics of D2, but not D1 dopamine receptors in the basal ganglia *(see below)*. On the other hand, NT in the micromolar range affects D1 binding. Interestingly, the NT effect on D2 receptors appears to be G protein-independent (von Euler, 1991), whereas that on D1 receptors is G protein-dependent (Miyoshi et al., 1989). Since both D1 and D2 receptors are highly enriched in striatum, the selective changes in the binding characteristics of one dopamine receptor subtype could, in different physiological states, switch DA transmission from one to the other receptor isotype (Fig. 2).

The evidence of intramembrane receptor–receptor interactions in vivo is still indirect. In several cases, the same modulation of receptor characteristics has been demonstrated in vitro and after intracerebroventricular (icv) administration of the modulator (*see* Table 1). Colocalization of the two interacting receptors is usually prominent in areas where the interaction is demonstrated. Finally, in many instances receptor crosstalk has been correlated with functional changes at neurochemical and/or behavioral levels (GAL [Fuxe et al., 1988a; Hedlund et al., 1991a,b], NPY [Härfstrand et al., 1989; Aguirre et al., 1990], and A1–D2; *see* following article on DA receptor modulation).

For instance, a powerful inhibitory interaction has been shown between the NPY receptor (Wahlenstedt and Håkanson, 1987) and one adrenergic receptor subtype, the α2 receptor, in the dorsal medulla oblongata, an area where the two receptors are highly concentrated (Fuxe et al., 1989a). The modulation was not present when other adrenergic receptor subtypes were tested. The interaction was first observed in vitro and then on icv injections of the peptide (Agnati et al., 1989). Receptor autoradiography experiments confirmed that the site of interaction was in the subregions of the nucleus tractus solitarius (nTS) where the distribution of the two receptors markedly overlaps (Härfstrand et al., 1989). Finally, NPY and clonidine injected icv have a vasodepressor action that is diminished when the two substances are coadministered. Overall, these data indicate that NPY receptors have an antagonistic interaction with the α2 transmission in the dorsal medulla oblongata and that an interaction between NPY and α2 receptors is likely to be the mechanism mediating this action.

Although the presence of membrane receptor–receptor interactions can give a functional counterpart to the coexistence of two or more substances in the nerve terminals, these interactions are not restricted to cases of coexistence (*see*, e.g., the interaction between NT and D2 receptors in the basal ganglia, and Table 1). It can then be

Table 1
Main Characteristics of Some Intramembrane (Types B and C) Receptor–Receptor Interactions in the Central Nervous System

Receptors involved	Cerebral region	Coexistence of endogenous ligands	Interaction in vitro membrane			Interaction in vivo	Selectivity[a]	G-protein dependence	References
			K_d	B_{max}	Section				
NPY → α2	dMO	A or NA/NPY	↑	↑	↓	↓	α1, β	yes	Agnati et al. 1983e, 1989; Härfstrand et al. 1989; von Euler et al. 1989
α2 → NPY	dMO	A or NA/NPY	nt	nt	↓	nt	nt	nt	Härfstrand et al. 1989
ANG → α2	dMO	no	↑	≈	nt	nt	nt	nt	Fuxe et al. 1988b
ANG → NPY	dMO	no	nt	nt	↑	nt	nt	nt	Aguirre et al. 1991
GAL → 5HT1a	BF	GAL/5HT	↑	≈	↓	nt	5HT1b, 5HT2	nt	Fuse et al. 1988a
5HT1a → GAL	BF	GAL/5HT	↓	≈	↑	↑	nt	no	Hedlund et al. 1991a,b
SP → 5HT1	SC	SP/5HT	↑	↑	nt	nt	nt	nt	Agnati et al. 1983d
VIP → 5HT1	Hip	no	≈	↑	↑	nt	Secr, Gluc, 5HT2	nt	Rotene et al. 1983a,b
D1 → D2	BG	—	↑	≈	nt	nt	5HT, α1	yes	Seeman et al. 1989
NT → D2ago	BG	no	↑	≈	nt	↓	D2 antago, D1	no	Agnati et al. 1983c, 1990b; von Euler 1991; von Euler et al. 1991
NT → D1	BG	no	↑	≈	nt	nt	nt	yes	Miyoshi et al. 1989
CCK-B → D2ago	BG	no[b]	↑	≈	nt	↑	CCK-A, D1	no	Agnati et al. 1983b, 1985b; Li et al. 1993c
Glu → D2	BG	no	↑	≈	nt	nt	NMDA, Quis	no	Fuxe et al. 1984b
A2 → D2	BG	no	↑	≈	nt	nt	A1, D1	no	Ferré et al. 1991b,d
D2 → A1	Cx	no	↑	≈	nt	nt	M1	yes	Murayama et al. 1990

[a]Related modulators or receptors for which the interaction is not present.

[b]In dorsal striatum CCK is largely not costored in DA terminals; however, in the caudal part of ventral striatum coexistence between CCK and DA is extensive.

Abbreviations: A = adrenaline, ANG = angiostenin, BF = basal forebrain, BG = basal ganglia, CCK = cholecystokinin, Cx = cortex, DA = dopamine, GAL = galanine, Glu = glutamate, Gluc = glucagon, 5HT = serotonin, dMO = dorsal medulla oblongata, nt = not tested, NA = noradrenaline, NMDA = *N*-methyl-D-aspartate, NPY = neuropeptide Y, NTS = nucleus tractus solitarius, Quis = quisqualate, SC = Spinal cord, Secr = secretin, SP = substance P, VIP = vasoactive intestinal polypeptide.

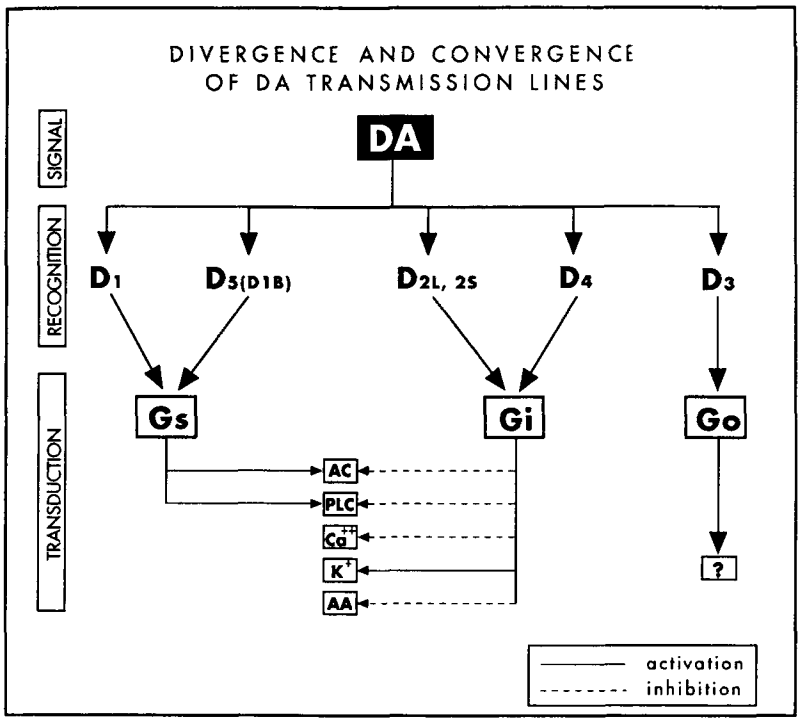

Fig. 2. Divergence and convergence of dopamine transmission lines at the level of receptor subtypes, G proteins and second messenger systems. Abbreviations: AA = arachidonic acid, AC = adenylate cyclase, PLC = phospholipase C.

hypothesized that receptors interacting with each other belong to the same synapse or to adjacent synapses. A further possibility is that interacting receptors may be located extrasynaptically, activated by transmitters diffusing in the ECF (Fig. 3) (volume transmission, Fuxe and Agnati, 1991).

Mechanistic Aspects

At the present time it is only possible to speculate about what may be the mechanisms for interactions between the G-protein coupled receptors within the membrane. Two main mechanistic models of intramembrane interactions may be discussed. One mechanism is represented by direct interactions between physically distinct but adjacent receptors, possibly also involving some associated proteins (type B). The other mechanism is based on the use of mobile proteins associated to the membrane, such as G proteins, as messengers for the interactions between physically distinct receptors (type C). However, multiple types of interaction are likely to be present for each receptor.

The available data give support to the existence of both types of mechanisms. For instance, NT modulation of the D2 receptor binding characteristics in membrane preparations of the neostriatum is maintained unchanged after a marked inactivation of G_i and G_o proteins induced by *N*-ethylmaleimide (NEM) or pertussis toxin (PTX) treatment (von Euler et al., 1991). PTX-sensitive G_i and G_o proteins are coupled to D2 receptors and are known to mediate its physiological effects (Cote et al., 1986; Fujita et al., 1985). Furthermore, an A2 agonist can counteract the action of GTP at D2 receptors at an independent site of action (for a detailed explanation, *see below*) (Ferré et al., 1993). On the other hand, many receptor–receptor interactions observed in membrane preparations seem to involve a G protein as an essential link (*see*, e.g., von Euler, 1989, Miyoshi et al., 1989; Murayama et al., 1990, and Table 1). For instance, G_i/G_o proteins seem to be involved in the reduction of affinity of α_2 receptors induced by NPY in membranes of the medulla oblongata (von Euler et al., 1989; Fuxe

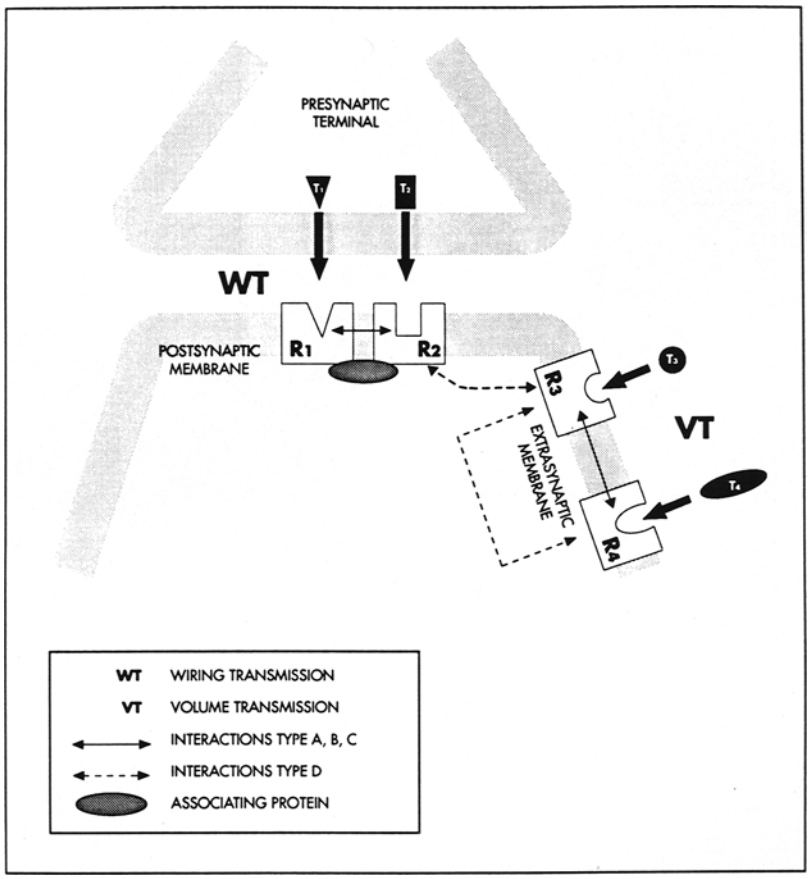

Fig. 3. Schematic drawing showing receptor–receptor interactions in synaptic (wiring transmission, WT) and nonsynaptic (volume transmission, VT) sites on neuronal membranes.

et al., 1989a). NPY effects were, in fact, blocked by prior intracisternal treatment of the animals with PTX. This treatment also counteracted the cardiovascular actions of both NPY and clonidine (an α2 agonist) on intracisternal injection. The blockade was not caused by a loss of high-affinity ^{125}I-NPY 1–36 binding sites by the PTX treatment, since this treatment rather produced an increase in ^{125}I-NPY binding. Another example of G protein mediation of receptor crosstalk is the interaction between adenosine A1 and D2 receptors in rat neocortex. PTX treatment was able to prevent the DA agonist apomorphine-induced reduction of adenosine A1 binding (Murayama et al., 1990).

MECHANISM TYPE B

A possible basis for interactions between receptors is dimerization of structurally different receptors (heterologous dimerization; Fig. 4). Dimerization (and oligomerization) of receptor molecules on activation by the agonist seems to be a general phenomenon (Hollenberg, 1991). According to the type of receptor, dimerization would be a necessary condition for activation or a means leading to response potentiation or metabolic stabilization of the receptors.

The best known case of receptor dimerization is that of the tyrosine kinase receptor superfamily (Schlessinger, 1988; Ullrich and Schlessinger, 1990). This property, which is present both in preparations of purified receptors and in living cells, is essential for signal transduction, including autophosphorylation. In addition, dimerization increases the affinity for the agonist. Although for some receptors, like insulin and IGF-1 receptors, stable dimerization is attained

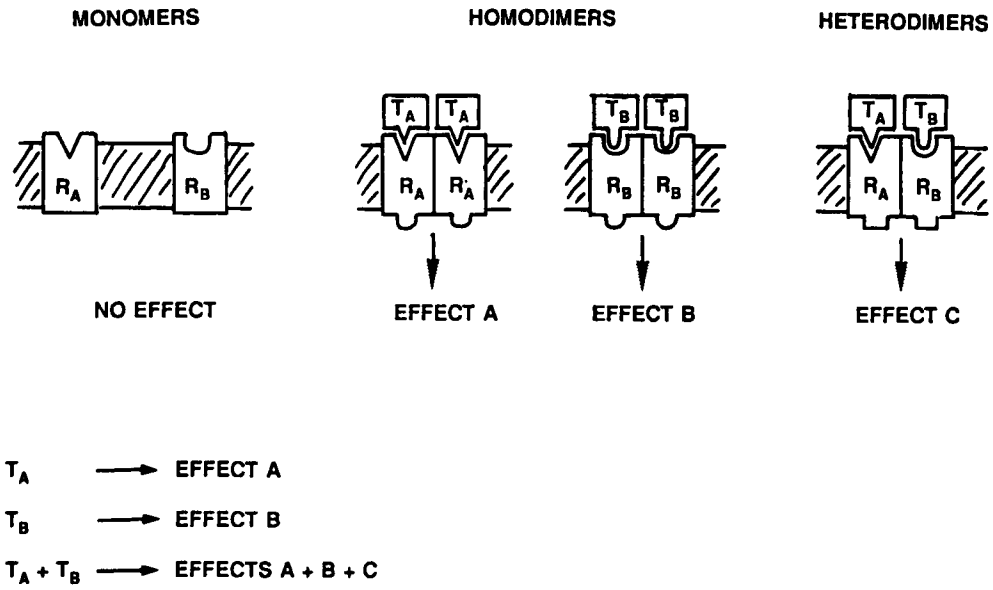

Fig. 4. Homo- and heterodimerization of membrane receptors. Heterodimers induce a cellular effect different from homodimers. The relative proportion of the two transmitters, the concentration of the two receptor populations, and the characteristics of receptor–receptor interactions will determine the amount of homo- vs heterodimers and thus the overall effect on the target cell.

through disulfide bridges, in most cases transient dimerization is induced by conformational changes on agonist binding.

Some evidence of dimerization and oligomerization is also available for other types of receptors. In the family of ligand-gated ion channels, the occurrence of functional dimers as the prevalent active form in vivo has been demonstrated for nicotinic receptors in both rat myotubes (Yeramian et al., 1986) and *Torpedo californica* electric organ (Schindler et al., 1984). Again, although in some instances receptors can be stably dimerized through covalent SH bridges, in other cases the interaction is noncovalent. Formation of large clusters of nicotinic receptors occurs during maturation of the neuromuscular junction, involving changes in receptor metabolism and function (Laufer and Changeux, 1989). In this case, proteins associated with nicotinic receptors, such as the 43K protein, may have an important role (Hill, 1992).

Functional G protein-linked receptors also appear to be in a dimeric form. Purification of several receptors of this family demonstrated that the native receptor protein is a dimer (*see*, e.g., Venter and Fraser, 1983; Roche and Ryan, 1989). Interestingly, studies on the activation of the luteinizing hormone-releasing hormone (LHRH) receptor showed that dimerization is a necessary step in receptor function and that an antagonist is transformed into an agonist when it is capable of bringing two receptor molecules within a critical distance (Conn et al., 1982).

If molecular mechanisms for dimerization are conserved within receptor superfamilies, the possibility exists that dimerization occurs between different members of the family (i.e., heterodimerization vs homodimerization). Heterologous dimerization has been clearly demonstrated for tyrosine kinase receptors, even if its physiological properties remain to be clarified (Ullrich and Schlessinger, 1990). Recent work on opiate receptors, belonging to the family of G protein-linked receptors, demonstrated that certain subtypes of μ- and ∂-binding sites are functionally and physically associated (Rothmann et al., 1988; Schoffelmeer et al., 1990). This phenomenon has not yet been explained on a molecular basis. However, association of independent μ and ∂ receptors (mechanism type B) remains a distinct

possibility as an alternative to the presence of a single receptor complex with two binding sites (mechanism type A).

In conclusion, several cases of receptor–receptor interactions in crude membrane preparations may be based on a process of heterodimerization. The density of the two or more interacting receptors and the number of receptors activated by the agonist in each population would then determine the proportion of homo- vs heterodimerizations and, then, the overall effect on target cell function (Fig. 4).

In the case of tyrosine kinase receptors, dimerization occurs through an interaction between the glycosilated extracellular domains (mechanism type B1). In this context, it is interesting to remember that many peptide receptors are glycoproteins; for example the NPY/PYY receptor subtypes are structurally distinct glycoproteins (Sheikh and Williams, 1990). Also, monoamine receptors such as α_1 (Sawutz et al., 1987), α_2 (Convents et al., 1988), β_2 (Benovic et al., 1987b), and D2 (Clagett-Dame and McKelvy, 1989) receptors have been characterized as glycoproteins. In several instances deglycosylation has no major effects on receptor binding characteristics. Instead, it has been supposed to be important for the appropriate posttranslational routing of the receptor to the cell surface (Clagett-Dame and McKelvy, 1989). A further functional meaning of receptor glycosylation might be to favor the interactions among receptors via the formation of hydrogen bonds. If so, these interactions should also, be modulated by glycosidase activity. In agreement with this proposal is the detection of sialidase activity in brain synaptic membranes (Miyagi et al., 1990).

MECHANISM TYPE C

A second class of molecular mechanisms that may underlie interactions between receptors in the membrane is dependent on membrane-associated G proteins (Fig. 5).

The G proteins are heterotrimeric (consisting of α-, β-, and γ-subunits) guanine nucleotide binding proteins bound to the internal surface of the plasma membrane. They relay the signal from some types of receptors to the various membrane

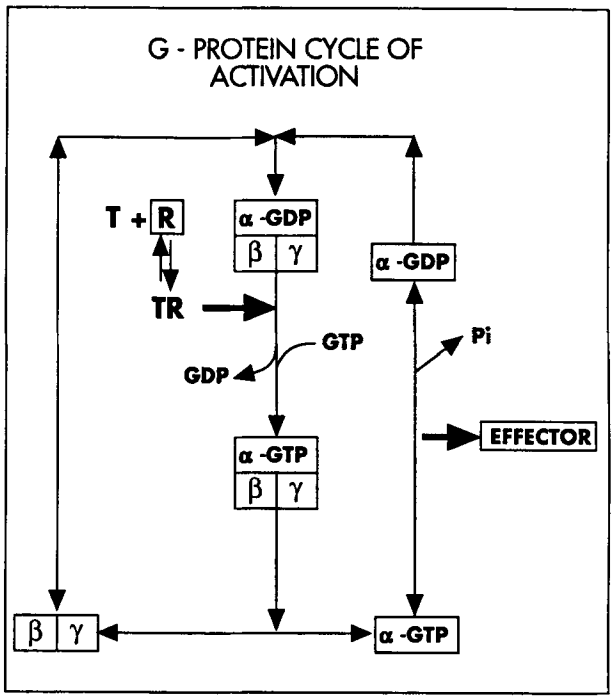

Fig. 5. Schematic drawing showing the activation of G proteins after the formation of the transmitter–receptor complex.

bound effectors (Gilman, 1987; Linder and Gilman, 1992). Evidence exists for a high diversity of G proteins and especially of the α-subunits. In fact, at least 20 distinct forms of α-subunits, five γ-subunits, and at least 10 β-subunits have been shown to exist, giving a strong structural basis for the formation of G protein networks within the nerve cell membrane (Simon et al., 1991).

G proteins appear to be anchored to the internal surface of the cellular membrane (Spiegel, 1992). Covalent interactions with lipids are responsible for this phenomenon. The βγ heterodimer is anchored through an isoprenoid molecule covalently bound to the carboxyl-terminal of the γ-subunit. Mutations that block isoprenylation prevent association to the membrane (Simonds et al., 1991). The mechanism for membrane association of the α-subunits is less well understood. Some classes of α-subunits (G_i and G_o) require a myristoylation of the amino-terminal portion to bind to the membrane (Spiegel et

al., 1991). However, there is also evidence that in some situations α-subunits can be released from the membrane.

In the resting state, GDP is bound to the α-subunit that forms a heterotrimer with the β- and γ-subunits. When an agonist activates the receptor, the receptor changes its conformational state and binds to the G protein (Fig. 5; Ross, 1989). This coupling facilitates the exchange of GDP for GTP, leading to a disassembly of the G protein. As a consequence, the α-subunit, which contains the GTP-binding site, becomes free to move along the internal surface of the plasma membrane (Fig. 5) and activates membrane-bound effectors, such as adenylate cyclase and calcium channels. In this way the G protein can transduce the signal from the receptor across the nerve cell membrane. The duration of activation depends on the rate of hydrolysis of GTP, which depends on the GTPase activity of the α-subunit. Usually, the activated α-subunits again become associated with the β- and γ-subunits within a few seconds as a result of the GTPase action.

Also, β- and γ-subunits seem to have a role in the activation of membrane effectors. The most frequently indicated action is the ability of β- and γ-subunits to counteract the actions of GTP activated α-subunits by binding to this subunit (Fig. 6) ("subunit exchange" phenomenon; Gilman, 1987; Axelrod et al., 1987; Simon et al., 1991; Linder and Gilman, 1992). In this way the GTP activated α-subunit can no longer activate its effector (*see*, e.g., the G_i mediated inhibition of adenylate cyclase activity via β- and γ-subunit binding to activated $G\alpha_i$, Ross, 1989). β- and γ-subunits can also, directly effect various effectors *inter alia* leading to alterations of adenylate cyclase activity (Federman et al., 1992). Thus, it seems possible that the β- and γ-subunits can act as coordinators of activity in parallel transduction lines over the nerve cell membranes by interacting with activated α-subunits and possibly by directly regulating membrane bound effectors.

Four major classes of G proteins exist (G_s, G_i, G_q, and G_{12}) based on the degree of sequence identities between the α-subunits (Simon et al., 1991). It must be emphasized that the same α-subunit can activate a number of different effectors and the same effector can be activated by several α-subunits. For instance, Gs can activate adenylate cyclase as well as calcium channels, whereas $G\alpha_{i1-3}$ and $G\alpha_o$-subunits all inhibit calcium channels and activate potassium channels (Fredholm, 1991). On the other hand, other subunits appear to be quite specific in switching on one type of effector in response to activation of a specific receptor subtype (Simon et al., 1991). As already mentioned, there also exists a diversity of the β- and γ-subunits. The β4 seems to be highly expressed in brain together with γ3. In addition, when discussing the G protein networks in the membrane, we must consider not only the diversity of receptors and of G proteins but also the fact that cloning studies demonstrate the existence of different types of effectors, such as adenylate cyclase, phospholipase C, and phospholipase A_2. In conclusion, each cell likely has a specific network of G-protein coupled receptors, G proteins, and membrane effectors. In this network, at every level of the cascade a certain degree of convergence and divergence between the different transmission lines will occur, allowing a wide range of crossactivations and signal integrations.

In light of the previous discussion, several mechanisms can be proposed for an involvement of G proteins in receptor–receptor interactions. A first mechanism can be the direct influence of α-subunit and/or βγ-subunits on a receptor molecule. Therefore, the target receptor molecule would be analogous to other G protein-dependent membrane effectors. This type of phenomenon has already been shown for some integral membrane proteins (ionic channels and transporters) other than classical enzyme effectors (Hille, 1992; Sternweis and Pang, 1990). Several types of ionic channels seem to be controlled by transmitter receptors through G proteins without involvement of a cytoplasmic second messenger system (Brown and Birnbaumer, 1990; Hille, 1992). This type of interaction has been called "membrane delimited" modulation (Brown and Birnbaumer, 1990). Thus, a direct

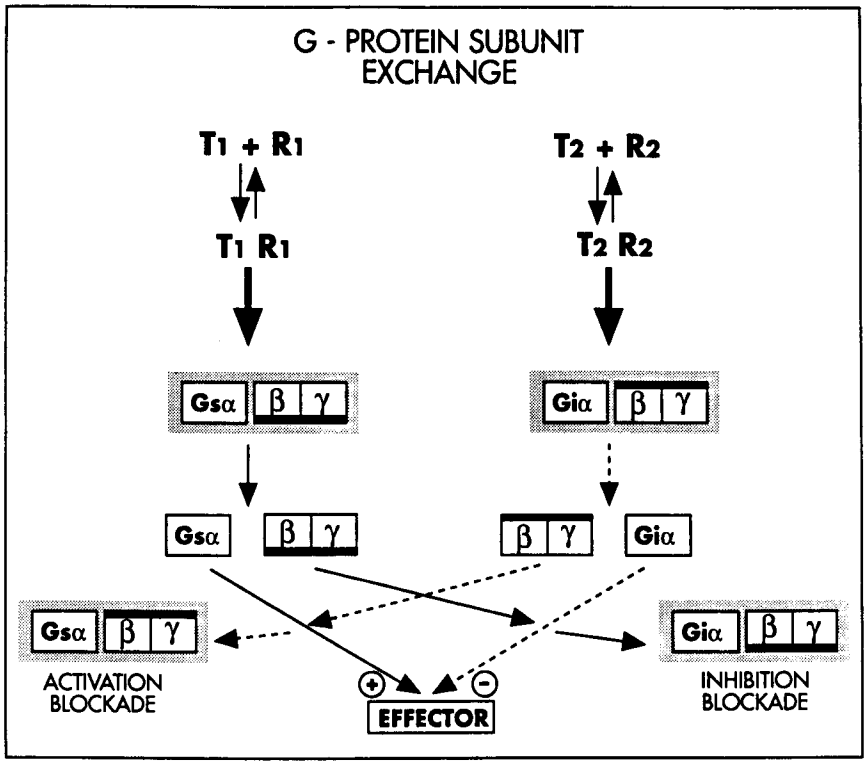

Fig. 6. Schematic drawing showing a possible mechanism for type C receptor–receptor interaction (G protein subunit exchange).

action of a G protein activated by one receptor on the functional state of another receptor may be the basis of receptor crosstalk in the membrane.

Another mechanism of receptor modulation may depend on the changes in the affinity for the agonist induced by the binding of the G protein to the receptor. It is known that the affinity for the agonist of a G-protein coupled receptor is decreased by GTP (switch from the high- to the low-affinity state). This phenomenon depends on the fact that the coupling of the G protein to the receptor increases its affinity for the agonist and GTP causes the release of the G protein from the receptor (Ross, 1989). Therefore, the proportion of receptors bound to a G protein determines the apparent affinity of the overall receptor population. This may represent a negative feedback mechanism to turn off the activation of the receptor molecule at the same time as the effectors are switched on by the released GTP-activated α-subunit.

As several receptors converge on the same G proteins, there will be a competition among the receptor populations for the pool of G proteins. The activation of one receptor population will change the equilibria in the network and consequently the affinity for the agonist of some other receptor populations.

A correlated mechanism would be dependent on the subunit exchange phenomenon. As already discussed, the release of β- and γ-subunits on activation of the G-protein coupled receptor can bind to other GTP- activated α-subunits and block their action (Fig. 6).

Thus, βγ-subunits activated by one receptor population can sequester the α-subunits activatable by a second receptor population. In this way, the number of G proteins available for the second receptor population will be changed and thus its affinity for the agonist.

In conclusion, the presence of convergence and divergence in the receptor/G protein/effector

network can be the basis of receptor–receptor interactions within the membrane.

Type D Interactions—Interactions Between Nonadjacent Receptor Molecules in the Membrane via Intracytoplasmic Loops

Receptor–receptor type D interactions are based on long loops involving intracytoplasmic and also nuclear mechanisms. These loops have not only several steps, where crosstalks can take place, but they also consist of multiple feedback controls that allow a continuous tuning of the entire transmission line, thus controlling the progressive internalization of the signals. Details on some type D interactions have been provided by Huganir and Greengard (1987, 1990).

Shortly after the introduction of the concept of receptor–receptor interactions and the indication of the existence of intramembrane receptor–receptor interactions (Agnati et al., 1980, 1982; Fuxe et al., 1981a,b) evidence was obtained that protein phosphorylation may be an important mediator of receptor–receptor interactions (Huganir and Greengard 1983, 1986, 1987, 1990). The major function of receptor phosphorylation appears to be the regulation of the rate of desensitization of the neurotransmitter receptors. Protein phosphorylation takes place both at the ligand-gated ion channel receptors and at G-protein coupled receptors by modification of the hydroxyl groups of tyrosine, serine, and threonine residues.

It was early demonstrated by Huganir and Greengard (1983) that cAMP-dependent protein kinase phosphorylates muscle-type nicotinic ACh receptors. The phosphorylation takes place on the γ- and ∂-subunits and leads to a marked increase in the rate of desensitization of the nicotinic receptor (Huganir et al., 1986). Further studies showed that CGRP, which coexists with ACh in the nerve terminals of the motor endplates, increases cAMP levels in the myotubes, and is the major regulator of phosphorylation of nicotinic ACh receptors in muscle. Thus, CGRP can mimic the actions of forskolin and phosphorylates the α-, γ-, and ∂-subunits of the nicotinic ACh receptors, leading to a desensitization of these receptors (Miles et al., 1989; Mulle et al., 1988). Thus, cotransmission via neuropeptides appears to regulate the desensitization of synaptic receptors.

In the case of the nicotinic ACh receptors on skeletal muscle, the protein kinase C appears mainly to be involved in the phosphorylation induced by ACh of its own receptor (*see* Huganir and Greengard, 1990). It has also been proposed by Ross et al. (1987, 1988) that the subunit assembly of the ACh receptor is under the regulation of protein phosphorylation processes. Thus, it seems possible that a cotransmitter, in this case CGRP, is involved in the regulation of the organization of the nicotinic ACh receptors.

Protein kinase C is also, involved in the phosphorylation of the neuronal ACh nicotinic receptors. In this case, however, the protein kinase C appears to be activated by SP released from adjacent nerve terminals (*see* Stallcup and Patrick, 1980; Role, 1984). Thus, the receptor–receptor interaction appears to involve an activation of SP receptors leading to an increase in phosphoinositide turnover, and a subsequent activation of protein kinase C. SP has also, been shown to modulate the single channel properties of the neuronal nicotinic ACh receptors (Simmons et al., 1990). It seems likely that muscarinic cholinergic receptors via receptor–receptor interactions involving activation of protein kinase C also regulate the desensitization processes of the neuronal ACh nicotinic receptors.

Evidence also exists that other ligand-gated ion channels, such as $GABA_A$ receptors and glycine receptors, are regulated by cAMP-dependent protein kinase, by protein tyrosine kinase, and by protein kinase C (Huganir and Greengard, 1990). Thus, one important level of interaction in the case of metabotropic and ionotropic receptors is the receptor–receptor interaction taking place via second messengers and activation of protein kinases leading to the phosphorylation of the receptor subunits of the ligand-gated ion channels.

Interactions between different types of metabotropic receptors (G-protein coupled receptors) may involve protein phosphorylation. Activation of cAMP-dependent protein kinase has been shown to desensitize β-adrenergic receptors. However, in this case a direct phosphorylation of the G proteins and of the adenylate cyclase may also be involved in the desensitization process (Benovic et al., 1987a, 1988). Also, receptors activating phospholipase C are involved in the regulation of β-adrenergic receptor desensitization by stimulation of protein kinase C. Thus, different types of metabotropic receptors may participate in the control of the β-adrenergic receptors. In addition, other types of G-protein coupled receptors, such as the α_1 and α_2 adrenergic receptors and the muscarinic receptors, can be regulated via these types of receptor–receptor interactions, involving phosphorylation by cAMP-dependent protein kinase or by protein kinase C (*see* Rosenbaum et al., 1987).

Taken together, receptor–receptor interactions involving the second messengers leading to a subsequent protein phosphorylation appear to be the major mode to produce desensitization of synaptic ligand-gated ion channels as well as of other metabotropic G-protein coupled receptors (*see* Huganir and Greengard, 1990).

Another mechanism based on protein phosphorylation may be the basis of type D receptor–receptor interactions. For instance, the same cytosolic phosphoprotein, such as dopamine and cAMP-regulated phosphoprotein-32 (DARPP-32), is regulated by protein kinases and protein phosphatases activated by different receptors. Such an interaction at the effector level involving protein phosphorylation and dephosphorylation has been discovered for the interaction between the D1 and NMDA receptors in the basal ganglia (Halpain et al., 1990). D1 receptor activation leads to the phosphorylation of DARPP-32, which becomes a phosphatase inhibitor. In contrast, activation of NMDA receptors increases intracellular calcium ions leading to the activation of a calcium–calmodulin dependent phosphatase. This phosphatase will antagonize the action of D1 by favoring a dephosphorylation of DARPP-32 into an inactive form. Activation or inactivation of phosphatases can in turn regulate the phosphorylation state of receptors.

Type D interactions between receptors can also take place through regulation of receptor biosynthesis. It has been shown that the activation of cAMP-dependent protein kinase and of protein kinase C as well as increases of intracellular calcium levels can control the transcriptional activity of genes for receptors (for review *see* Laufer and Changeux, 1989). It has, for example, been shown that calcium ions and protein kinase C reduce the synthesis of nicotinic ACh receptor in the muscle. As discussed by Laufer and Changeux (1989), transcriptional regulation may only take place when a number of DNA-binding proteins (transacting factors) combine to the specific DNA sequences so that coordination may take place in their transcriptional regulation of the structural genes for neurotransmitter receptors, which may involve also anchoring proteins (*see* Laufer and Changeux, 1989). At the gene level also protein phosphorylation appears to play an important role in receptor–receptor interactions by their strong participation in the regulation of gene expression. As an example it may be mentioned that the CGRP can increase the number of ACh receptors in primary cultures, and appears to be involved in the regulation of ACh receptor α-subunit mRNA levels (Fontaine et al., 1986, 1987).

An Example of Receptor–Receptor Interactions: Dopamine D2 Receptors and Their Modulators in the Basal Ganglia

In the following we will analyze in more detail the case of interactions between DA receptor isotypes with other receptors in the basal ganglia.

In mesostriatal DA pathways, two main types of DA receptors have been detected; the D1 and the D2. In the dorsal, somatomotor part of the striatum, these receptors appear to be substantially segregated, D1 receptors being expressed mainly in striatal medium-sized spiny neurons

projecting to the substantia nigra and D2 receptors mainly in those projecting to the globus pallidus and in the cholinergic interneurons (Le Moine et al., 1990, 1991; Gerfen, 1992). However, some DAceptive neurons contain both types of receptors. In addition, D2, but not D1, receptors are also expressed by DA cells and are located in cell bodies at the nigral level and in nerve terminals in the striatum. A similar distribution of DA receptors is thought to occur in the ventral, limbic part of the striatum, the nucleus accumbens, and the tuberculum olfactorium, although the cellular localization has not yet been clearly defined. In some DAergic neurons of the lateral substantia nigra and in some DAceptive neurons of the ventral striatum, D3 receptors have also been detected, although at a relatively lower level compared with D1 and D2 receptors (Bouthenet et al., 1992). D3 receptors have a pharmacology similar to that of D2 receptors and thus several studies on D2 binding and D2-controlled behaviors, especially in limbic areas, may partly reflect effects on D3 receptors.

The striatum is a useful model system for receptor–receptor interactions since alterations of striatal circuitry result in movement disorders and make it possible to investigate the behavioral outcome of modulations of striatal transmitters. Many transmitters and modulators have been identified in striatal circuits (*see*, e.g., Graybiel, 1990). We will describe the interaction between DA receptors and some modulators of DA transmission, i.e., CCK, NT, and adenosine. Cholecystokinin is costored with DA in selected groups of mesolimbic neurons and also appears to be weakly expressed in a few striatal DAceptive neurons. Neurotensin is present in some striatal DA target neurons and adenosine is released by neuronal and glial cell populations, especially following nervous tissue insults.

Reciprocal D1/D2 Receptor Interactions

All known DA receptors belong to the superfamily of G-protein coupled receptors. The general features of the G-protein-coupled receptors have recently been described (*see* Lefkowitz and Caron, 1988; O'Dowd et al., 1989). These proteins are integral membrane proteins with seven transmembrane spanning regions or domains, connected via hydrophilic loops, three of them facing the ECF and three of them facing the intracellular space. The *N*-terminal part is located in the extracellular space and the C-terminal part in the intracellular space (Lefkowitz and Caron, 1988; Lomasney et al., 1991). These extracellular loops are exposed to a ligand-rich environment, where hydrogen bonding interactions alter the ligand-binding site as well as the G-protein coupling (*see* Findlay and Eliopoulos, 1990). The transmembrane parts of the receptors span the membrane as α-helices. The ligand-binding site appears to be located within the intramembrane region among the seven transmembrane bundles, where a putative binding cavity or pocket may be formed (*see* Findlay and Eliopoulos, 1990). Instead, parts of the intracellular receptor surface appear to be involved in the coupling to the G protein.

Five subtypes (D1 to D5) (Fig. 2) of DA receptors (Bunzow et al., 1988; Van Tol et al., 1991) have been cloned in mammalian species. D1 receptors are linked to a G_s protein-activating adenylate cyclase and phospholipase C. The D2 receptors are known for their multiple transduction pathways mediated by the activation of a G_i protein, involving an inhibition of adenylate cyclase and of phospholipase C, opening of potassium channels, and increases of arachidonic acid release (*see* Vallar and Meldolesi, 1989; Strange, 1990). D2 receptors may also reduce the permeability of some calcium channels (*see* Vallar and Meldolesi, 1989).

As already mentioned, both D1 and D2 receptors are highly enriched in the striatum. Since the availability of selective ligands, a wealth of biochemical, electrophysiological, and behavioral studies have shown that DA effects in the striatum are often mediated by a positive or negative cooperation between D1 and D2 receptors (Clarke and White, 1987). For instance, a synergism between D1 and D2 receptors have been demonstrated for the protein phosphorylation-induced inhibition of sodium/potassium ATPase (Bertorello et al., 1990), and in the regulation of

electrical activity of neurons as well as of DA-induced behaviors (Clarke and White, 1987). This cooperation may, in part, be mediated by interactions between D1 and D2 receptors. Seeman et al. (1989) showed that in homogenates of striatal tissue from several mammalian species, including humans, DA was a noncompetitive antagonist for D2 binding, an effect that was prevented by D1 antagonist pretreatment. DA was also a noncompetitive antagonist for D1 binding, an effect that was blocked by pretreatment with a selective D2 antagonist. These results suggest that striatal D1 and D2 receptors can reciprocally modulate the binding characteristics of one another. Interestingly, the effect of D1 antagonists on D2 binding could be mimicked by a stable guanine nucleotide analog, which indicates that the interaction is mediated by G proteins.

Reciprocal Neurotensin/ D2 Receptor Interactions in the Basal Ganglia

NT/DA Coexistence and Codistribution of NT and D2 Receptors

Evidence exists that NT is costored in some nerve cell bodies and nerve terminals of the mesolimbocortical DA neurons originating in the ventromedial tegmental area of the midbrain (Hökfelt et al., 1984; Bean et al., 1989; Fuxe et al., 1990, 1992b,c). However, in the mesostriatal DA neurons projecting to the neostriatum, the nucleus accumbens and the olfactory tubercle NT is not a cotransmitter. Current evidence indicates that striatal NT content mainly, if not exclusively, derives from striatal intrinsic neurons. A few NT immunoreactive neurons and moderately dense plexa of NT immunoreactive nerve terminals have been detected in both the ventral and dorsal striatum. Treatments with D2 antagonists or striatal DA depleting agents markedly increased NT levels revealing a rich population of positive neurons in striatum (Merchant et al., 1992). Colocalization studies carried out in animals treated with a D2 antagonist showed that NT immunoreactivity is almost selectively contained in striatopallidal GABA/enkephalin (ENK) neurons (*see* Fuxe et al., 1992b).

Within the dorsal and ventral striatum the ^{3}H-NT binding sites (K_d value in the order of 2–5 n*M*) are codistributed with the very high densities of D2 and D1 receptors (Fig. 7) (*see* Fuxe et al., 1990). In addition, a high density of very high-affinity ^{125}I-NT receptors (K_d values from 0.1–0.2 n*M*) were also found within the DA cell body region of the substantia nigra where they are directly located on the DA cell bodies. These receptors are thought to function as autoreceptors regulating electrical activity (Shi and Bunney, 1992) and DA release (Tanganelli et al., 1989).

As previously discussed, the D2 receptors are coupled to adenylate cyclase, to phospholipase C, and to potassium channels, whereas the NT receptors, which are also G-protein coupled, can stimulate phospholipid hydrolysis in rat brain slices and increase intracellular calcium levels (Goedert et al., 1984a; Turner et al., 1990).

NT Modulation of DA Receptors

It is well known that centrally administered NT produces neuroleptic-like actions, probably as a result of an interaction with the DA pathways (*see* Nemeroff et al., 1983a,b, 1986). For instance, NT injected into the nucleus accumbens markedly counteracts the DA-induced locomotion and reward-related behaviors as well as the activation produced by amphetamine and cocaine (Agnati et al., 1986; Kalivas et al., 1984; Jolicoeur et al., 1985; Nemeroff et al., 1983a,b; Fuxe et al., 1992a).

Several lines of evidence indicate that NT-DA D2 receptor interactions are involved in NT-DA interactions in the basal ganglia. In vitro studies have demonstrated that NT in the lower nanomolar range (peak action at 3 n*M*) increases the K_d value (by 20–50%) without a modulation of the B_{max} value of the ^{3}H-*N*-propyl-norapomorphine (^{3}H-NPA) binding (Titeler and Seeman, 1979) in membrane preparations of both the dorsal and ventral striatum (Agnati et al., 1983c; von Euler and Fuxe, 1987; von Euler 1991; Fuxe et al., 1990; von Euler et al., 1990a, 1991). Kinetic analysis showed that this change is because of an increase of D2 agonist dissociation rate (von

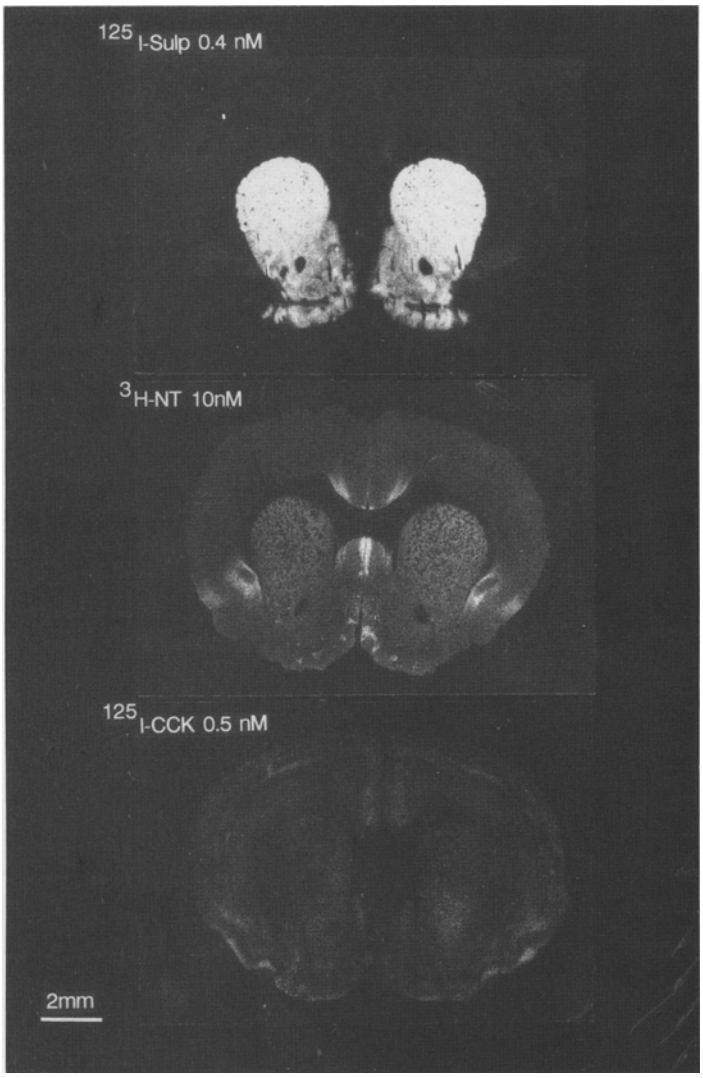

Fig. 7. Representative autoradiograms of the rat forebrain (Bregma 1.0–1.5 mm) showing the distribution of dopamine D2 receptors labeled with [^{125}I]sulpiride (0.5 n*M*, top panel), [^{3}H]neurotensin (NT) binding sites (10 n*M*, middle panel), and [^{125}I]cholecystokinin-8 binding sites (0.5 n*M*, bottom panel). Bar = 2 mm.

Euler, 1991). In contrast, NT was found not to influence the D2 antagonist binding and the D1 antagonist and agonist binding (*see* Fuxe et al., 1984a, 1992b; von Euler, 1991). A decrease of D2 agonist binding in basal ganglia was also found on NT (0.3–3 n*M*) intraventricular injections, demonstrating that NT-D2 receptor interactions are also present in vivo (von Euler et al., 1990b).

In competition experiments with DA vs ^{3}H-raclopride binding, NT (10 n*M*) produced marked increases in the dissociation constant of both high- and low- affinity binding sites (K_H and K_L) of D2 receptors without influencing the proportion of high vs low affinity binding sites (RH value) (von Euler, 1991; Li et al., 1993a). Since K_H values may in part reflect changes at high-affinity receptors located outside the synapses, whereas the K_L values may in part reflect changes at synaptic D2 receptors, D2 receptors for both synaptic and volume transmission may be influenced by NT receptors. The K_H and K_L state of the D2 receptors probably also reflects receptors

with a high and low G-protein binding, respectively, suggesting that D2 receptors with different coupling to G proteins are both affected by NT receptor activation.

It must be emphasized that the reduction of affinity induced in the D2 agonist-binding site is probably also associated with a marked reduction in the signal transduction, since the GTP-induced reduction in the proportion of high affinity binding sites in relation to total number of binding sites can only be demonstrated in the absence of NT (*see* von Euler, 1991). In the presence of GTP the modulation of affinity of the K_H and K_L values of DA also become more pronounced. Thus, NT receptor activation appears to interfere with the GTP/GDP exchange at the α_i-subunit of the D2 receptor, leading to an uncoupling to its biological effectors. In this way the K_d changes may be associated with powerful alterations in the D2 receptor transduction (Ariens et al., 1980).

More recently (Li et al., 1993a), NT modulation of D2 receptors has been confirmed by using the C-terminal NT 8–13 fragment. Already at 1 n*M*, the biologically active fragment NT 8–13 can produce a maximal increase of the K_H and K_L values in competition experiments with DA vs the D2 receptor antagonist ^{3}H-raclopride. The biologically inactive *N*-terminal fragment 1–7 had no action on the D2 receptors in the same concentration range. A higher potency of NT 8–13 vs NT 1–13 has also previously been noted in ^{125}I-NT binding studies on membranes derived from NT receptor cDNA transfected cells (Tanaka et al., 1990) as well as in membranes from rat brain (Goerdert et al., 1984b). Furthermore, neuromedin N, formed from the same precursor as NT (Dobner et al., 1987; Sato et al., 1991) and possessing NT agonist properties, can more potently than NT reduce the affinity of the DA D2 agonist- binding sites in the rat neostriatum (Li et al., 1993b). This rank order of potency indicates that NT receptors involved in the interaction with D2 receptors in basal ganglia are different from those cloned by Tanaka et al. (1990) and should be rather named neuromedin N-neurotensin receptors or type 2 NT receptors. These results also open up the possibility that biologically active C-terminal NT fragments and/or neuromedin N may be the major endogenous ligands for the striatal NT receptors (*see* Li et al., 1993a,b).

Mechanisms of NT-D2 Receptor Interactions

NT-D2 receptor interaction does not seem to involve G proteins, i.e., mechanism type C (von Euler et al., 1991). In fact, pretreatment with NEM or PTX, which block several types of G proteins, could not antagonize the ability of NT to reduce the affinity of the D2 agonist-binding sites. Since the experiments were carried out in washed membranes, mechanism type D can also be ruled out.

It has been suggested that the NT-induced reduction of the affinity of D2 agonist-binding sites is related to an ability of NT to directly bind DA and other DA agonists (Adachi et al., 1990). However, when calculating the amount of DA that can be complexed with NT at the doses used in behavioral experiments involving coadministration of NT and DA, it was found that NT can only bind about 2% of the administered DA (*see* Fuxe et al., 1992b). Furthermore, neuromedin N, formed from the same precursor as NT (Dobner et al., 1987) and possessing NT agonist properties *(see above)*, can more potently than NT reduce the affinity of the DA D2 agonist-binding sites in the rat neostriatum (Li et al., 1993b) in spite of the fact that neuromedin N cannot bind DA (Adachi et al., 1990).

Since NT even in very high doses cannot bind DA receptors (Nemeroff et al., 1983a), we are left with the possibility that intramembrane interactions between NT and D2 receptors consist of an interaction between adjacent receptors with or without the involvement of an interposed integral membrane molecule as discussed above for mechanism type B.

It has been shown that the effects of NT on D2 receptor sensitivity in nigral DA cells can be abolished by blocking protein kinase A (Shi and Bunney, 1992). Accordingly, cAMP-dependent phosphorylation can decrease agonist binding to the D2 receptor (Cain et al., 1988; Elazar and Fuchs, 1991). Even if phosphorylation reactions

cannot explain the effects observed in membrane homogenates, it seems quite likely that NT receptor activation in vivo can regulate the phosphorylation of the third intracytoplasmatic loop of the D2 receptor. Thus, as suggested by Shi and Bunney (1992), under in vivo conditions a type D interaction may also be involved in the NT/D2 interaction.

Possible Functional Relevance of the NT/D2 Receptor Interactions

Functional correlates of NT-D2 receptor interactions have been found in experiments on transmitter release and electrophysiology. In a series of experiments using intracerebral microdialysis in striatum, NT antagonism on D2 receptor-mediated effects has been demonstrated in both DAceptive GABA-containing neurons and DAergic nerve terminals. NT increases extracellular levels of GABA in the dorsal striatum of the halothane-anesthetized (O'Connor et al., 1992) as well as in the awake unrestrained male rat (Fuxe et al., 1992a). In the awake rat, local perfusion with NT, at a dose that is ineffective alone, counteracted the inhibitory effects of the D2 agonist pergolide on extracellular levels of GABA (Fuxe et al., 1992a). In addition, combined treatment with the D1 agonist SKF38393 and NT but not with the D1 agonist alone led to significant increases in the extracellular striatal levels of GABA. In the same animal model, NT could also antagonize the inhibitory effects of D2 agonists on extracellular levels of DA, DOPAC, and HVA (Tanganelli et al., 1989; Fuxe et al., 1992a). On the other hand it enhanced D1 receptor-mediated inhibition of DA release.

Electrophysiological studies demonstrated that NT is able to increase nigral DA cell firing (Kalivas, 1993). This effect is, at least, partly dependent on a desensitization of D2 autoreceptors. In fact, NT has been found to counteract the D2 agonist-induced inhibition of nigral DA cell firing (Shi and Bunney, 1991).

Overall, microdialysis and electrophysiological studies suggest that, in agreement with binding studies, NT receptors inhibit D2 receptor-mediated functions in DAceptive neurons and DAergic terminals and cell bodies. The interpretation of the NT effects on D1 receptor-mediated effects is less straightforward. Since D1 receptors are exclusively located in DAceptive cells, D1 agonist effects on DA release are thought to be mediated by the activation of a striatonigral feedback loop on DA nigral cells. Thus, NT may influence D1 receptor-mediated GABA and DA release through an indirect effect, although not via receptor crosstalk at the membrane level *(see above)* on D1 transmission and/or through inhibition of D2 transmission. Whatever mechanism is involved, both binding and functional evidence indicates that a major effect of NT on DA transmission in basal ganglia is a switch from D2 receptor-mediated to D1 receptor-mediated transmission, and thus from striopallidal to strionigral output pathways. This effect would be further potentiated by the NT-induced increase in DA release.

Evidence for DA/NT Receptor Interactions

Some evidence also points to the existence of a modulation of NT receptors by DA receptors in basal ganglia. DA was able to reduce the affinity and increase the number of ^{3}H-NT binding sites in the rat neostriatal membrane preparations, possibly involving an action via D1 receptors since D1 but not D2 agonists modulate the ^{3}H-NT binding (Agnati et al., 1985a,b). This interaction was found to be enhanced by the development of DA receptor supersensitivity induced by a 6-OHDA-induced lesion of the nigrostriatal DA pathway (Fuxe et al., 1986, 1990). These results probably reflect an intramembrane DA/NT receptor interaction. In this case the increase of the B_{max} value is substantial and may reflect an unmasking of the ^{3}H-NT binding sites in their interaction with the DA receptors, possibly of the D1 type. The relevance of this interaction for the control by NT receptors of D2 receptor transduction remains to be determined but it has been suggested that it may represent part of an inhibitory feedback loop controlling D2 transmission (*see* Fuxe et al., 1992b).

When discussing the role of NT receptor mechanisms in the control of D2 receptor transduction it must, however, also be mentioned that

the D2 receptors appear to exert a restraining influence on gene expression of NT and that D2 blockade results in increased NT synthesis in the GABA/ENK neurons of the neostriatum, probably associated with an enhancement of NT release. Such a continuous release may then result in a desensitization of the NT receptors restraining the D2 receptor sensitivity. Such a mechanism may be involved in producing the D2 receptor supersensitivity phenomenon after D2 receptor antagonist treatment (*see* Fuxe et al., 1990, 1992).

CCK-8/DA Receptor Interactions

CCK/DA Coexistence and Codistribution of CCK and D2 Receptors

The CCK innervation of the striatum is heterogeneous (Hökfelt et al., 1980; Gilles et al., 1983). In the rat, only a minor CCK-positive input derives from mesencephalic DA cells, most of which costore CCK (Hökfelt et al., 1980; Gilles et al., 1983). In addition, some cells containing CCK mRNA can be visualized in the striatum (Ding and Mocchetti, 1992). In the CNS, CCK binds to two types of G protein-linked receptors, the CCK-A and CCK-B receptors, with specific spectra of ligand selectivity (Moran et al., 1986). The former is present only in a few areas, which possibly include the ventral striatum and the midbrain DA cells (Hill et al., 1987), whereas the latter is largely distributed all over the brain (Moran et al., 1986).

CCK Modulation of DA Receptors

Neuroleptic-like actions have been demonstrated following central CCK-8 administration. Thus, antagonism of stereotypes induced by DAergic drugs, catalepsy, and ptosis are demonstrated as well as a reduction of self-stimulation behaviors and inhibition of conditioned avoidance responses (Zetler, 1985). Available evidence indicates that such actions of CCK-8 may be related to antagonistic CCK-D2 receptor interactions in the striatum.

Studies in striatal membranes from both the ventral and dorsal parts (Agnati and Fuxe 1983; Agnati et al., 1983a,b, 1985b; Li et al., 1993c) showed that CCK-8 (at concentrations ranging from 0.1–1 n*M*) selectively reduces the affinity without any change in the B_{max} value of the D2 agonist-binding site, the change being in the order of 20–40% depending on the concentration and the area studied. In a kinetic analysis, it was found that CCK-8 increased the K_d value by reducing the association of ^{3}H-NPA to binding sites in rat neostriatal membranes. Instead, NT induced an increase in the K_d value *(see above)* by increasing the dissociation rate constant (von Euler, 1991). CCK-D2 receptor interaction seems to be mediated by CCK-B receptors (Li et al., 1993c), since the increase of the K_d value of ^{3}H-NPA binding sites in rat striatal membranes can be counteracted by the selective CCK-B antagonist PD34308 (Hughes et al., 1990) but not by the selective CCK-A antagonist L364718 (Freidinger et al., 1990). These results are also in line with previous studies showing that CCK-4, a selective CCK-B agonist, can reduce the affinity of the D2 agonist-binding sites in striatal membranes (Agnati et al., 1983a,b).

It has been shown (Dumbrill-Ross and Seeman, 1984; Agnati et al., 1987) that, after 24 h intraventricular infusion, CCK-8 induces a long-term (up to 14 d) increase of ^{3}H-D2 receptor antagonist binding in dorsal and ventral striatum. These observations are apparently at variance with the results obtained in in vitro studies. However, a recent paper (Li et al., 1993c) gives some hints to reconcile these apparently contrasting findings. This study showed that, in competition experiments with DA vs ^{3}H-raclopride binding, CCK-8 increases D2 binding site affinity unless a D1 receptor antagonist is added. In this latter case CCK-8 causes a decrease in the affinity of DA for D2 binding sites. Thus, D1 receptor activation, which is likely to be present in vivo, appears to shift the CCK-8 action from a decrease to an increase of D2 receptor binding. An alternative hypothesis maintains that activation of CCK receptors in the nucleus accumbens can lead to an increase of D2 receptor biosynthesis. It

is likely that several CCK-8-induced alterations of D2 receptor transmission occur in vivo.

Mechanisms of CCK-D2 Receptor Interactions

Up until now, no mechanistic study has been carried out on CCK-D2 receptor interactions. In vivo studies suggest that CCK-8 may increase D2 receptor biosynthesis (mechanism type D). The presence of the modulation in membrane preparations implies that intramembrane receptor–receptor interactions take place (mechanism types B and C). It is not yet known if this interaction involves a G protein or not. Recent studies show that CCK-8-induced and NT-induced decreases of D2 receptor affinity in striatal membranes are additive at low, but not high, concentrations, which suggests that the CCK and NT operate via the same mechanism, i.e., without an intervention of G proteins *(see above).*

Possible Functional Relevance of the CCK/D2 Receptor Interactions

The presence of an antagonistic interaction between CCK and D2 receptors in striatum can be correlated with several electrophysiological and behavioral data. A functional antagonism between DA and CCK-8 has been found in single unit recordings from the *n. accumbens* (Wang and Hu, 1984). In intracerebral microdialysis experiments (*see* Tanganelli et al., 1990), it was shown that CCK-8 locally perfused in the neostriatum is able, in a concentration-related way, to counteract the inhibition of DA release caused by systemically given apomorphine acting on D2 autoreceptors. Finally, it is well known that CCK-8 has an antipsychotic agent profile, i.e., it mimics the actions of D2 receptor blockers (Van Ree, 1983). On the other hand, some behavioral (Crawley et al., 1989) and electrophysiological findings on nigral DA neurons (Kalivas, 1993) show that CCK-8 is able to increase DA transmission in some experimental paradigms, probably owing to coactivation of D1 and D2 receptors in these experiments *(see above).* It must also be considered that activation of D2 receptors increase CCK mRNA content in striatum (Ding and Mocchetti, 1992) and that D1 and D2 receptors increase the affinity of CCK-8 receptors (von Euler et al., 1992). It is then likely that CCK peptides in vivo have multiple influences on the DA mesostriatal system according to the subtype of CCK receptor activated and to the predominance of D2 or D1 transmission in the DA pathways.

A2/D2 Receptor Interactions in the Basal Ganglia

Codistribution of Adenosine A2 and DA D2 Receptors in the Brain

Adenosine has been shown to be a neuromodulator in the CNS (Fredholm and Hedqvist, 1979; Dunwiddie, 1985; Snyder, 1985). Its actions are mediated by receptors that have been subdivided into A1 and A2 according to agonist and antagonist potencies (Sattin and Rall, 1970; Daly et al., 1983; Bruns et al., 1988) and to their action on adenylate cyclase (Premont et al., 1979; Van Calker et al., 1979). Based on agonist potencies, the A2 receptors have been subdivided into high-affinity (A2a) and low-affinity (A2b) receptors (Bruns et al., 1986). Direct proof for the validity of this receptor classification lies on molecular cloning experiments, because A1 receptors and two forms of A2 receptors have recently been cloned (Maenhaut et al., 1991; Libert et al., 1991).

With radioligand-binding techniques, using membrane preparations or autoradiography, it has been found, both in rodents and in humans, that high-affinity adenosine A2 receptors are highly enriched in the striatum (caudate nucleus and putamen in humans), nucleus accumbens and olfactory tubercle (Fig. 8) (Bruns et al., 1986; Alexander and Reddington, 1989; Jarvis et al., 1989; Jarvis and Williams, 1991; Parkinson and Fredholm, 1990; Martinez-Mir et al., 1991), areas with very high numbers of DA receptors (Boyson et al., 1986). Accordingly, A2 receptor mRNA is exclusively localized in the basal ganglia of the rat, dog, and human brain (Schiffmann et al., 1991a,b). Further studies at the cellular level showed that adenosine A2 receptor mRNA is

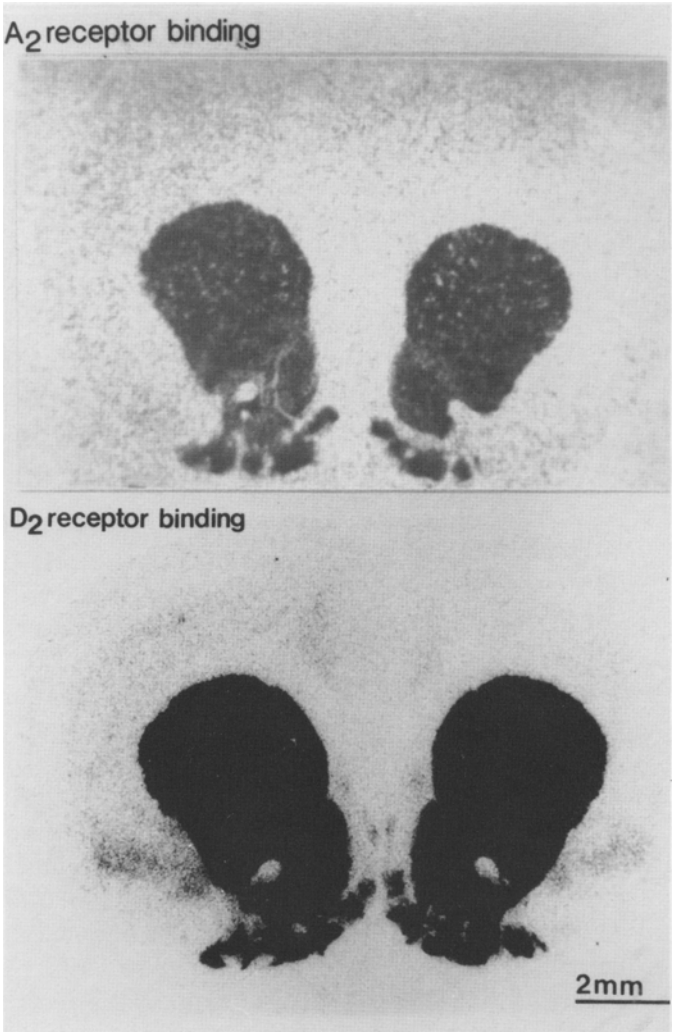

Fig. 8. Representative autoradiograms of the rat forebrain (Bregma 1.6–1.7 mm) showing the distribution of dopamine D2 receptors labeled with [^{125}I]sulpiride (top panel) and adenosine A2 receptors labeled with [^{3}H]CGS 21680 (bottom panel). Modified from Ferré et al. (1992). High densities of both D2 and A2 receptors codistribute in the ventral and dorsal striatum.

selectively expressed by GABAergic-ENK striatal neurons, which also contain D2 receptors (Schiffmann et al., 1991b; Fink et al., 1992).

Adenosine Modulation of D2 Binding

In rat striatal membrane preparations, the specific A2 adenosine agonist CGS 21680 induces a decrease in the affinity of DA D2 receptors for DA agonists, like DA and NPA, but not for DA antagonists, like raclopride (Ferré et al., 1991d). This CGS 21680 effect was inhibited by the adenosine antagonist 8-phenyltheophylline and was mimicked by L-PIA (Bruns et al., 1986), provided the concentration used was high enough to stimulate high-affinity A2 receptors in addition to A1 ones. Furthermore, CGS 21680 was ineffective in altering D1 binding (Ferré et al., 1991d).

In subsequent studies it was found that, in addition to the increase in both K_H and K_L values of DA D2 receptors, stimulation of adenosine A2 receptors is associated with an increase in the

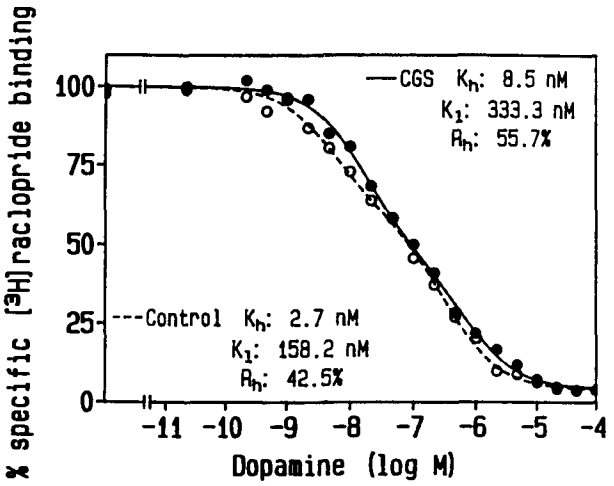

Fig. 9. Representative competition–inhibition curves illustrating the effect of the adenosine A2 agonist CGS 21680 (30 n*M*) on DA-induced inhibition of the binding of the DA D2 antagonist ^{3}H-raclopride. The $MgCl_2$ concentration was 1 m*M* (for further details on methods, *see* Ferré et al. 1991). The presence of CGS 21680 causes an increase in the dissociation constants of both high- and low-affinity states (K_H and K_L, respectively) and an increase in the proportion of the high-affinity state (R_H) of the D2 receptors. This suggests that adenosine A2 receptor stimulation causes a decrease of both the affinity of D2 receptors and the transduction of the signal from the D2 receptor to the G protein (for further details, *see text*).

proportion of high-affinity binding sites (R_H) (Fig. 9) (Ferré and Fuxe, 1992; Ferré et al., 1993). In view of the role of guanine nucleotides and divalent cations (like Mg^{2+}) to regulate in opposing ways the proportion of high-affinity D2 receptors (Sibley and Creese, 1983), interaction experiments with CGS 21680, the GTP analog Gpp (NH)p, and Mg^{2+} were performed. It was found that these three factors play significant although independent roles on R_H values of the DA D2 receptors, CGS 21680, and Mg^{2+} increasing and Gpp(NH)p decreasing R_H (Ferré et al., 1993). Both the GTP analog and Mg^{2+} did not alter the effect of CGS 21680 on K_H and K_L values of the dopamine D2 receptor (Ferré et al., 1993).

GTP binding to the α-subunit causes its dissociation from the receptor, which changes to a low-affinity state, the GTP-α-subunit complex being responsible for the changes in adenylate cyclase activity, ion channel permeability, and so on (Gilman, 1987). Consequently, an increase in the proportion of high-affinity D2 binding sites is expected to be associated with an impairment in the transduction of the signal from the D2 receptor to the G protein.

Mechanisms of A2-D2 Receptor Interactions

Both adenosine A2 and DA D2 receptors belong to the family of G protein-coupled receptors (Bunzow et al., 1988; Libert et al., 1989). The A2 receptor is coupled to G_s proteins, which link A2 receptors to adenylate cyclase (Libert et al., 1989), and the D2 receptor is coupled to G_i proteins, which link D2 receptors to adenylate cyclase, ion channels, and phospholipase C (Vallar and Meldolesi, 1989). Consequently, A2 and D2 transmission lines are likely to interact at the signal transduction level, since activation of the adenosine A2 receptor stimulates and activation of the DA D2 receptor inhibits adenylate cyclase activity (Premont et al., 1979; Van Calker et al., 1979; Onali et al., 1985).

One possible mechanism underlying the A2-induced increase in the R_H values of D2 receptors could be an interaction between G_s and G_i proteins (Gilman, 1987). An increased formation of βγ-subunits from the A2-linked G_s, after A2 receptor activation, could bind to the α-subunits from the D2-linked G_i, leading to an inhibition of the G_i protein dissociation from the D2 receptor (mechanism type C). However, our results suggest that the A2-mediated increase in R_H values of the D2 receptors is independent of the effect of GTP. In fact, this A2 adenosine effect was not affected by the GTP antagonist GDP-βS (Ferré et al., 1993). Also, our biochemical data, in membrane preparations, rule out the involvement of adenylate cyclase in the A2-mediated modulation of D2 receptors, because adenylate cyclase activity depends on factors, such as ATP, that were not included in the incubating medium (mechanism type D). It is therefore likely that mechanism type B is involved in A2–D2 receptor interaction in the cell membrane. Nevertheless, the abovementioned mechanisms, the G_s–G_i

protein interaction and the interaction at the level of adenylate cyclase, are very probably additional mechanisms that amplify the intramembrane allosteric A2/D2 receptor–receptor interaction in vivo.

Possible Functional Relevance for Adenosine A2 Receptor-DA D2 Receptor Interaction

In the experimental animal, adenosine agonists produce psychomotor depressant effects (Snyder et al., 1981; Vapaatalo and McGuffin-Clineschmidt, 1981; Barraco et al., 1984; Durcan and Morgan, 1989a; Heffner et al., 1989) whereas adenosine antagonists, which include the methylxanthines caffeine and theophylline, not only inhibit the adenosine agonist-induced psychomotor effects, but, when administered alone, also exert psychomotor stimulant effects suggesting the existence of a basal adenosinergic tone in vivo (Boissier and Simon, 1965; Thithapandha et al., 1972; Snyder et al., 1981; Coffin and Carney, 1986; Spealman and Coffin, 1986). The potencies of different adenosine agonists and antagonists to produce their psychomotor effects correlate with their affinities for A2 but not for A1 adenosine receptors (Spealman and Coffin, 1986; Durcan and Morgan, 1989b; Nikodijevic et al., 1990). A large amount of experimental data suggest that central DAergic neurotransmission is involved in the mediation of the psychomotor effects of adenosine agonists and antagonists. In rats adenosine agonists inhibit and methylxanthines potentiate the locomotor activity induced by DA agonists (Fuxe and Ungerstedt, 1974; Fredholm et al, 1976, 1983; Green et al., 1982; Heffner et al., 1989; Brown et al., 1991; Ferré at al., 1991a,b). Also, methylxanthine-induced locomotor activity in rodents is antagonized by treatments that cause DA depletion or blockade of DA receptors (Waldeck, 1973; White et al., 1978; Erinoff and Snodgrass, 1986; Herrera-Marschitz et al., 1988; Heffner et al., 1989; Josselyn and Beninger, 1991; Ferré et al., 1991b).

Thus, a central adenosine-DA interaction exists by which activation of adenosine receptors leads to an inhibition of DA neurotransmission and methylxanthines, by blocking the effect of endogenous adenosine, produce an enhancement of this transmission (*see* Ferré et al., 1992).

A presynaptic adenosine-DA interaction has been shown, with adenosine agonists causing a decrease and methylxanthines an increase of striatal DA release in rodents (Harms et al., 1979; Michaelis et al., 1979; Fredholm, 1985; Morgan and Vestal, 1989; Wood et al., 1989; Lupica et al., 1990). However this interaction seems to involve adenosine A1 receptors (Lupica et al., 1990) and the evidence regarding the behavioral effects rather suggests that A2 receptors are the major subtype involved in the interaction with DA neurotransmission *(see above)*.

Recently, it has been shown that adenosine analogs inhibit and methylxanthines potentiate a DA D2-mediated behavior in short-term reserpinized mice (Ferré et al., 1991a,b). Consequently, a postsynaptic adenosine A2/DA D2 interaction was suggested as being the main mechanism of action mediating the behavioral effects of adenosine agonists and antagonists. In line with this hypothesis, it was found that stimulation of central adenosine A2 receptors by the icv administration of CGS 21680 induces catalepsy in the rat, which can be counteracted both by an adenosine antagonist and by a specific DA D2 agonist (Ferré et al., 1991d). These behavioral data have a neurochemical counterpart (Schiffman et al., 1993). In fact, A2 antagonists, like D2 agonists, decrease the expression of ENK mRNA in striatal neurons containing both A2 and D2 receptors.

Striatal DA denervation induces both a higher sensitivity to DA agonists and a higher sensitivity to methylxanthines. Thus, systemic or intrastriatal administration of DA agonists or methylxanthines have a stronger effect in the denervated striatum (Pycock, 1980; Herrera-Marschitz et al., 1985, 1988; Josselyn and Beninger, 1991). The increased effect of DA agonists is most probably owing to the development of a denervation-induced supersensitivity of DA receptors (Ungerstedt, 1971; Creese et al., 1977). The increased effect of methylxanthines after DA den-

ervation could be explained by an increased interaction between adenosine A2 and DA D2 receptors. In fact, it has been recently found that, in membrane preparations from the DA denervated neostriatum, the DA D2 receptors are more sensitive to the modulatory effect of CGS 21680 on D2 receptors (Ferré and Fuxe, 1992).

Overall View

The set of phenomena presented in this section clearly shows that a complex network of interactions between transmission lines takes place in striatal cells (Fig. 10). It must, however, be underlined that this picture is highly simplified, since several major modulators (SP, ENK, dynorphin, and so forth) and the transmitter of intrinsic striatal neurons (GABA) as well as that of the main striatal input (glutamate) have not been taken into account.

The interactions appear to be at the same time specific (e.g., in the case presented the modulations are selective for D2 receptors) but not exclusive (several modulators have the same effect on the same molecular target). In addition, they can take place at different levels of the informational cascade (receptor molecules, G proteins, second messenger systems, gene expression). It is likely that complex equilibria within this network of transmissions will take place according to the degree of activation of each receptor population. It must, therefore, be stressed that it is the outcome of multiple receptor activations that finally determine the sensitivity and transduction of each individual receptor population.

In the frame of a reductionistic approach, the next steps in the comprehension of the function of striatal network will be, *inter alia*, to analyze the coordination between multiple modulatory systems. An example of synergism between modulators is that of CCK and NT on D2 receptors. It has been shown *(see above)* that CCK-8 reduces the dissociation rate constant, whereas NT increases the association rate constant of the D2 agonist-binding sites. This neurochemical synergism has a functional counterpart. In fact, NT and CCK-8 have a synergistic inhibitory interaction on D2 autoreceptors provided very low concentrations of NT and CCK-8 are used (Fig. 11) (*see* Tanganelli et al., 1993). The interaction was not observed at higher concentrations, suggesting that the two neuropeptide receptors modify D2 receptor transduction via a common regulatory mechanism.

At the neuronal network level in the neostriatum, the synergism of the two peptides may have a major integrative function *inter alia* by allowing a switching from D2 receptor-mediated transmission (striopallidal GABA/ENK pathway) toward a D1 receptor-mediated transmission (strionigral GABA/SP pathway). Thus, CCK and NT would favor the restraining influence of the indirect pathway on movements, since movements become elicited via the direct pathway when the cortical–striatal glutamate pathway becomes activated. In this way discrete movements will be elicited.

Integrative Aspects of Receptor-Receptor Interactions

Experimental evidence has been presented for the presence in the brain of interactions among different transmission lines at the level of membrane receptors. In this last section, our goal will be to discuss, often on a speculative basis, what is the importance of receptor–receptor interactions for the integrative mechanisms in the CNS. Previously, we have underlined their pharmacological importance by representing a new site of action for drug development (Fuxe et al., 1989b). Thus, our efforts will be focused on showing the possible links between these molecular mechanisms and nerve cell and neural network functions.

Integration of Signals Through Receptor-Receptor Interactions

Receptor–receptor interactions belong to the broader class of the interactions between transmission lines. Therefore, we will first discuss some general properties of these latter phenomena and then some distinctive features of the crosstalk at receptor level.

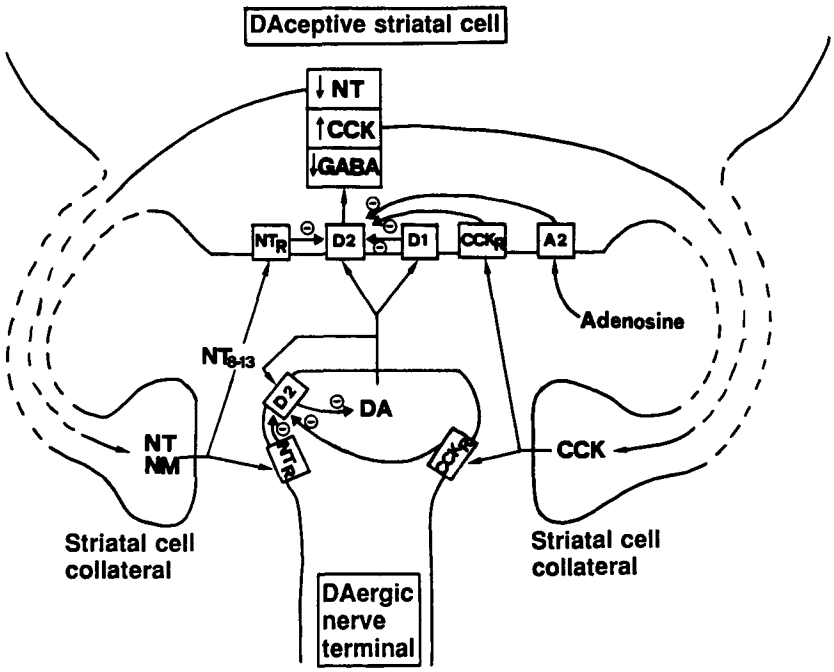

Fig. 10. Schematic representation of some receptor–receptor interactions, and their consequences on target cells, occurring in striatal membranes. Adenosine present in the extracellular space can derive from several cellular sources, including intrinsic striatal neurons and glial cells. For further details and discussion, *see text*. Abbreviations: CCK = cholecystokinin, CCK_R = cholecystokinin receptor, DA = dopamine, GABA = γ-aminobutyric acid, NM = neuromedin, NT = neurotensin, NT_{8-13} = neurotensin fragment 8–13, NT_R = neurotensin receptor.

The presence of interactions between transmission lines leads to the formation of molecular networks within and between different levels in the transduction pathways: membrane receptors, G proteins, second messenger systems, and so forth. Each informational molecule in the network has a set of specific interactions with other molecules. The more these events are studied the more is emerging a kind of molecular grammar: molecules that block or potentiate the action of other molecules, molecules with permissive roles, molecules that converge to the same target or diverge to a number of different targets, molecules that are necessary events for a certain function or that are only modulating it, and so on.

In most instances these molecular events reproduce the integrative phenomena known at the neuronal network level: The idea is therefore emerging that they constitute the basic computational units of the integration occurring in the cell. In comparison with neuronal circuits, these molecular circuits are characterized by an extraordinary miniaturization of computational elements (Agnati et al., 1984, 1988). It has been proposed (Agnati et al., 1990) that intracellular reactions elicited by neurotransmitters should be considered as a computation (microcomputation) in a series with respect to the computation taking place at the network level (macrocomputation). Actually, the information handling in the CNS should be considered the result of integrations at several successive networks, i.e., neural network, receptor network, G protein network, and intracellular chemical network.

The existence of networks implies that the reading out of the extracellular information carried out by the intracellular mechanisms is a holistic process. In this process it may be convenient to distinguish on the one hand fast electrical events, and on the other hand, slow electrical

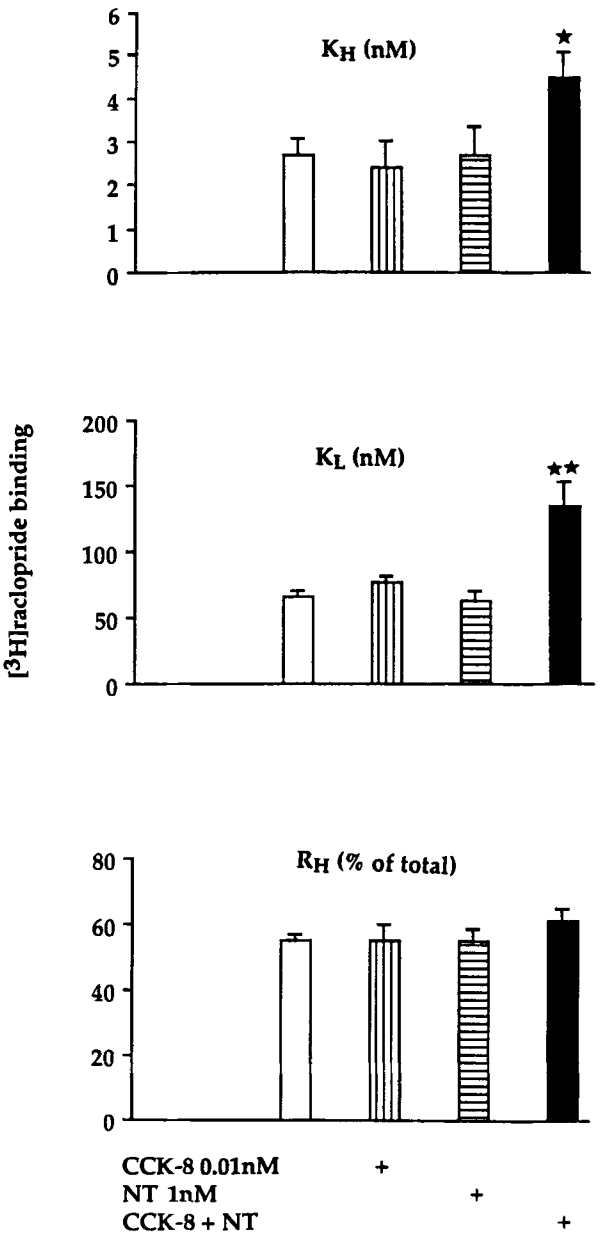

Fig. 11. Effects of NT plus CCK-8 on the K_H (upper panel), K_L (middle panel), and R_H (bottom panel) values of [^{3}H]racloprlde binding in rat neostriatal membranes. The K_H values were 2.65 ± 0.47, 2.38 ± 0.63, 2.65 ± 0.75, and 4.44 ± 0.65, the K_L values were 65 ± 7, 77 ± 8, 62 ± 8, and 135 ± 13 n*M*, and the R_H values were 55 ± 2, 55 ± 5, 55 ± 4, and 61 ± 4, for the control, 1 n*M* of NT, 0.01 n*M* of CCK-8, and NT ± CCK, respectively. Each treatment represents the mean ± SEM of nine separate experiments. Statistical analysis according to one-way ANOVA with Fischer's PLSD test, $^*p < 0.05$, $^{**}p < 0.01$.

events and metabolic events. The first set of phenomena poses fewer problems of interpretation, since the integration takes place at the membrane level according to chemicophysical rules that are largely understood. A more difficult task is the interpretation of the second set of phenomena, since no rules are known of how segregation and integration of the different signals, many times affecting the same intracellular effectors, take place. Segregation of transmission lines from a membrane receptor to the nucleus is certainly possible but seems rare.

The specific characteristic of receptor–receptor interactions is to intervene at the beginning of the transduction cascade. From a general standpoint, we can distinguish the interactions occurring at the membrane level (types A, B, and C) from those involving intracellular loops (type D).

The former ones can achieve a fast setting of activity in a transmission line in the light of ongoing activity in other transmission lines. In this way, physically distinct receptor molecules can behave as a "functional supramolecular complex" in the cell membrane (Agnati et al., 1982). The latter ones allow receptor heteroregulation at relatively long (phosphorylation) to very long (receptor synthesis) time scale.

Because of their location at the cell interface with the ECF, receptor crosstalk mechanisms constitute a first molecular network for the cellular integration. For this reason they seem particularly suited for a number of integrative tasks.

Filtration of Incoming Signals

Thanks to the existence of intramembrane receptor–receptor interactions it is possible to attain a predominance of some signals over others if they are contemporaneously presented to the cell. A signal will be potentiated, inhibited, or left unchanged in relation to the state of activation of the other receptors with which its receptor interacts. Inhibitory interactions make it possible to hinder incoming signals that are irrelevant or redundant in a particular cellular state. The advantage of this mechanism over blocking the signal at the source, i.e., blocking transmitter release via presynaptic inhibition, is

that the state of the target cell and the influence of the other incoming signals can also be taken into account.

Integration of Coincident Signals

A growing amount of evidence indicates the occurrence in the brain of detectors of coincident signals (Bourne and Nicoll, 1993). This type of integrative phenomenon consists of the generation of an output that is qualitatively or quantitatively different when two or more inputs are simultaneously present as compared to when they are presented alone. Coincidence detectors can be recognized in the brain at several levels of complexity, from network to molecules. Intramembrane receptor–receptor interactions are by definition molecular machines for integration of coincident signals. In fact, the alteration in the modulated receptor is functionally relevant only if the target receptor is active, i.e., when both transmitter and modulator are present at the same time.

Receptor–Receptor Interactions and Neuronal Plasticity

In the previous section we have discussed receptor–receptor interactions as elementary computational events in the cell. A second aspect of these molecular events is that they can be the basis for permanent modifications in cell characteristics.

Starting from the work of Hebb (1949), theories on neuronal plasticity have underlined the necessity of co-occurrence of several events to obtain a permanent change in neural tissue characteristics. At the network level, this principle implies that the simultaneous activity of several convergent inputs causes the discharge of a target cell. The study of plastic phenomena in mammalian brains, such as hippocampal and cortical long-term potentiation (LTP, Stevens, 1993; Bear and Kirkwood 1993) and cerebellar long-term depression (LTD, Ito, 1991), have substantiated this hypothesis. For instance, in order to obtain LTP in hippocampus, the activation of several synapses is necessary together with the discharge of the target cell (Gustafsson et al., 1987). The molecular basis of this phenomenon is partially understood (Colley and Routtenberg, 1993; Stevens, 1993). Again, the contemporaneous activation of several molecules is necessary for LTP, namely the activation of NMDA receptor by glutamate in the presence of depolarization of the membrane induced by the activation of other excitatory receptors. In general, convergence of simultaneous signals on a detector seems to play a key role in plastic phenomena (Bourne and Nicoll, 1993).

Since already discussed, membrane receptor–receptor interactions are coincidence detectors and are therefore likely molecular candidates for this type of plastic phenomena. A type A receptor–receptor interaction, i.e., the glycine–glutamate interaction in the activation of NMDA receptors, is thought to be involved in hippocampal LTP. It is in fact known that glycine is a coagonist at NMDA receptors *(see above)*. It is not known at this moment if the glycine site is already saturated in hippocampal NMDA receptors, or if glycine secretion is another event that must co-occur to obtain LTP. However, this example underlines the potentiality of receptor crosstalk for plastic phenomena in neuronal membranes.

Acknowledgments

The research on receptor–receptor interactions and the conceptual developments are mainly the results of a strict long-term collaboration between Agnati's group in Modena and Fuxe's group in Stockholm and also involve other collaborators (Agnati's group: F. Benfenati and E. Merlo-Pich; Fuxe's group: A. Härfstrand and G. von Euler). We fully appreciate the fruitful collaboration with B. Fredholm in this field.

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