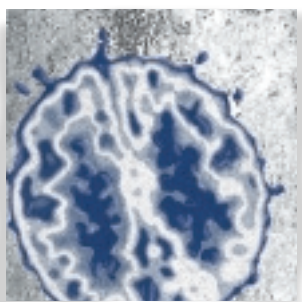# Basic research

Dopaminergic intracellular signal integrating proteins: relevance to schizophrenia

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Changes in dopaminergic function can be regulated by receptor-receptor interaction, or interaction with other proteins with dopamine receptors, and/or elements of the downstream signaling cascades. The complexity of dopaminergic signaling is far from being completely elucidated. It could, however, hold the key to the comprehension of the pathophysiology of neurological and psychiatric disorders, as well as to the identification of putative new targets for, and development of, more efficacious and selective drugs. Here, we review some of the current evidence and new ideas that are being proposed as a result, as well as future perspectives that are now being recognized.

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Dopamine receptors

Dopamine receptors are divided into two families, D_1-like and D_2-like receptors. Both families are of G-protein coupled receptors (GPCRs). Didactically, D_1-like (D_1 and D_5) are coupled to Gs and D_2-like (D_2, D_3 and D_4) are coupled to Gi. However, this is a simplified way of looking at the functions of the dopamine receptors. Recent evidence shows that these receptors do not work only via Gi or Gs, nor is their activation via ligand binding the same in every cell.[1]

Receptor-receptor interaction

G-protein coupled receptors are able to form dimers within arrays of oligomers.[2] Both homo- and hetero-oligomers were found for almost all kinds of GPCRs studied. Most interesting was the finding that the heteromeric receptors shown functions different from the individual and homo-oligomers, and dopamine receptors are no exception. It has been reported that D_1 and D_2 receptors associate within neurons, and that association results in a phospholipase C (PLC)-mediated increase in intracellular calcium, which is a pathway that is not activated by either receptor alone.[3-5] These findings gain relevance considering that abnormal calcium signaling seems to have a potentially important role in schizophrenia[6,7] and that the efficacy of antipsychotic drugs is closely related to their effect on dopamine receptors. D_2 and D_3 dopamine receptors also form heteromeric complexes with other neurotrans-

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Selected abbreviations and acronyms

AKT-1 *V-akt murine thymoma viral oncogene homolog-1*
DARPP-32 *dopamine- and cyclic AMP-regulated phosphoprotein-32*
GSK *glycogen synthase kinase*
NCS-1 *neuronal calcium sensor-1*
PAR-4 *prostate apoptosis response-4*
PK *protein kinase*
RGS *Regulator of G protein signaling*

mitter receptors, such as adenosine A_{2A},[8-10] which will encourage the development of adenosine and dopamine antagonist/agonist compounds with selective effect on heteromers as potential drugs in treatment of schizophrenia and other neuropsychiatric disorders.

DARPP-32

Recently, Albert et al[11] reported that DARPP-32 (dopamine- and cyclic AMP-regulated phosphoprotein, of relative molecular mass 32 000) was significantly reduced in the dorsolateral prefrontal cortex in more schizophrenic subjects when compared with paired controls, while the levels of other synaptic phosphoproteins, synapsin I and the calcium/calmodulin-dependent protein kinase II, did not differ between schizophrenics and controls. This was not due to medication, since subjects with Alzheimer's disease, treated or untreated with neuroleptic agents, did not show a decrease in DARPP-32 levels. Their finding was consistent with a selective reduction in DARPP-32 levels in schizophrenic subjects, which may be involved in the known prefrontal dysfunction associated with the disease. DARPP-32's inhibitory effect on protein phosphatase 1 (PP1) is activated when it is phosphorylated by protein kinase A (PKA) on threonine 34, and is terminated by the action of protein phosphatase 2B (PP-2B), which is in turn activated by calcium from intracellular stores, which can be mobilized by activation of D_2 receptors. Hernandez-Lopez et al[12] reported that D_2 suppresses transmembrane calcium (Ca^{2+}) currents through L-type Ca^{2+} channels mediated by $G\beta\gamma$ activation of phospholipase $C\beta1$, mobilization of intracellular Ca^{2+} stores, and activation of the calcium-dependent phosphatase calcineurin.

Neuronal calcium sensor-1

Koh et al[13] demonstrated, also in the dorsolateral prefrontal cortex, that samples from schizophrenic and bipolar subjects display significantly elevated levels of neuronal calcium sensor-1 (NCS-1), which were not influenced by age, gender, hemisphere, cause of death, postmortem period, alcohol consumption, or use of psychotropic medication. These data were reproduced and expanded with the finding that another calcium sensor, calcyon, was also upregulated in the brains of schizophrenic patients compared with controls.[14,15] In striatum, NCS-1 and the D_2 dopamine receptor (DRD$_2$) were found to colocalize within sites of synaptic transmission and in close proximity to intracellular calcium stores.[16] Those authors proposed that NCS-1-D_2 receptor interaction may serve to couple dopamine and calcium signaling pathways, thus providing a component in the regulation of dopaminergic signaling which might be involved in brain diseases.

It has been a long-standing pursuit of biological psychiatry to define dopaminergic dysfunction in psychiatric patients, and the search for mechanisms downstream of membrane receptors has begun. In this particular case it is interesting to notice that both proteins are involved in dopaminergic signaling. DARPP-32 is phosphorylated by PKA activated via D_1 dopamine receptors which are coupled to Gs.[17] On the other hand, NCS-1 was shown to be able to mediate desensitization of D_2 dopamine receptors, attenuating agonist-induced receptor internalization via a mechanism that involves a reduction in D_2 receptor phosphorylation, which was accompanied by an increase in D_2 receptor-mediated cyclic adenosine monophosphate (cAMP) inhibition after dopamine stimulation.[16] This was recently confirmed by ultrastructural microscopic techniques.[18] Thus, DARPP-32 and NCS-1 seem to be participants in two opposing dopaminergic pathways, one linked to the D1-Gs-coupled receptors and the other to D_2-Gi-coupled receptors.

PC12 cells are commonly used as a neuronal model, given that they exhibit properties such as excitability, secretion, and expression of metabotropic and ionotropic receptors of different types, including dopamine receptors. In addition, PC12 cultures can be differentiated to develop extensive neurites resembling neuronal dendritic trees by using nerve growth factor (NGF). Thus, in order to further investigate the possible interrelations between NCS-1 and DARPP-32 pathways we used PC12 cells of wild type (WT) and a lineage (clone 2) stably overexpressing NCS-1. Our data showed that the amount of DARPP-32 in c2 cells was at least 3 times smaller than in the WT PC12 cells, which was in

agreement with the above described results obtained from prefrontal cortex of schizophrenic patients (Souza et al, unpublished data).

PAR-4 and calmodulin

Prostate apoptosis response 4 (PAR-4) is a leucine zipper containing protein that plays a role in apoptosis. Recently, it was reported that PAR-4 binds to the third intracellular loop of the D_2 receptor.[19] The PAR-4 binding site to D_2 is in the same region where calmodulin (CaM)binds to the receptor. In increased Ca^{2+} concentrations, calmodulin competes with PAR-4, decreasing its binding.

CaM is a small acidic protein that functions as a primary decoder of Ca^{2+} information in the cell. CaM acts as a switch when the Ca^{2+} concentration rises from resting.[20] CaM regulates several enzymes that are of interest for synaptic plasticity including adenylyl cyclases, protein kinases and phosphatases, nitric oxide synthase, and Ca^{2+} channels.[21] Among them is calcineurin, also known as PP2B.

Calmodulin is also a modulator of G protein signaling-4 (RGS4), a protein codified by a gene that was shown to have modest but significant association between polymorphisms and haplotypes in RGS4 and schizophrenia[22-25]; however, there have also been negative reports.[26,27] Using microarray technology Mirnics et al[28] found a decrease in RGS4 messenger ribonucleic acid (mRNA) in the prefrontal cortex of schizophrenic patients. The RGS4 gene is located at chromosome 1q23.3 within a known susceptibility locus for schizophrenia.[29] During the resting state RGS binds to phosphatidylinositol 3,4,5-trisphosphate (PIP3) inhibiting its action. During depolarization, Ca^{2+} enters the cells and binds to CaM, forming a complex that is able to recover RGS function by removing PIP3 inhibition.[30-34] It was found that there was a reciprocal control of RGS4 by PIP3 and CaM, and that CaM and PIP3 share the same binding site intercating competitively to controls RGS4 function.[35] Knockout mice for RGS4 did not present a decrease in prepulse inhibition (PPI) or any other behavioral alteration except for a subtle and complex sensorimotor deficit.[36]

Gene expression changes of RGS4 were observed with specific dopamine receptor agents, such as an increase in RGS4 by D_2 receptor stimulation or by D_1 receptor blockade.[37,38] It was found that spinophilin also binds to RGS4.[39] Using yeast two hybrid assays, Jeanneteau et al[40,41] identified an interaction between G alpha-interacting protein (GAIP-interacting protein), C terminus (GIPC) and D_2 and D_3, but not with D_4. They showed that when GIPC was recruited by dopamine receptors, their signaling function was reduced by increased sequestration into vesicles; however, at the same time they were protected from degradation. GIPC interacts specifically with another RGS protein, GAIP, which exerts GTPase function through direct interactions on activated (GTP-bound) form of G proteins to limit their lifetime and terminate signaling.

Park et al[19] have shown a correlation of PAR-4 with depressive symptoms in animal models. Although no data was reported in relation to schizophrenia, the regulation of D_2 activity by PAR-4 might show relevance in near future.

Actin-binding proteins

Spinophilin

Spinophilin was first described in 1997 as a novel F-actin and protein phosphatase-1 binding protein localized to dendritic spines.[42] It possesses a single PDZ domain, and was identified as a protein that specifically associates with the third cytoplasmic loop of the D_2 receptors.[43] The binding site with D_2 is distinct from that for PP1, meaning that spinophilin can bind both at the same time.

It was also recently reported that spinophilin antagonizes arrestin-stabilized receptor phosphorylation through blocking G-protein receptor kinase 2 (GRK2) association with receptor-G β–γ complexes, reducing receptor endocytosis.[39] This effect is similar to that reported for NCS-1.[16] Spinophilin was implicated in schizophrenia by a study showing that its expression levels were reduced in hippocampus of schizophrenic patients; however, the changes were not specific for schizophrenia, being similar to those found in mood disorder patients.[44] However, Clinton et al[45] showed contradictory data where spinophilin transcripts was increased in brains of schizophrenic patients, along with confirmation of increased levels of calcyon.

ABP-280; filamin A

Another actin-binding protein-280 (ABP-280) or filamin A is a abundant cytoplasmic protein that has an actin-binding domain at its N terminus. It was shown that ABP-280 can interact with several GPCRs, including with the 3i loop of the D_2 short and long isoforms of the

D2 and with the D_3. However, it does not interact with the D_4 or D_1 3i loops. In cells lacking ABP-280 the ability of the D_2 to inhibit forskolin-stimulated cAMP accumulation is significantly reduced, although the receptor affinities for agonists and antagonists were not altered.[46,47] It is interesting to notice that ABP-280 and spinophilin bind to the same region (third intracellular loop of D_2 receptors). However, no differences in ABP-280 expression were found in cortex from schizophrenic patients compared with controls.[14]

AKT/GSK3 pathway and dopaminergic signal

The human V-akt murine thymoma viral oncogene homolog *AKT1* and *AKT2* genes are mammalian proto-oncogenes of a viral oncogene known as V-AKT, related to leukemia in mice.[48] Latter studies have found that the proteins codified by these genes were related to protein kinases A and C (PKA and PKC).[49-51] Named as PKB, three family members with more than 80% homology have been so far identified (AKT1/PKBα, AKT2/PKBβ and AKT3/PKBγ). Disruption of the the serine/threonine kinase (PKB/AKT) has been implicated in several human cancers, and the enzyme seems to play an important role in their outcome.[52] In 1995, it was demonstrated that AKT/PKB was a direct effector of phosphatidylinositol-3-OH kinase (PI-3 kinase).[53,54] Later in the same year, Cross et al[55] showed that AKT/PKB phosphorylates glycogen synthase kinase-3 (GSK3), being a key factor in that signaling cascade, linking PI-3K to to basic metabolic functions, such as protein and lipid synthesis, carbohydrate metabolism, and transcription.[52]

AKT/PKB became interesting for psychiatry when it was found that both isoforms of GSK-3 (α and β) are inhibited by lithium (Li^+).[56,57] This inhibition seems to be due to competition for magnesium (Mg^{2+}) binding at a site distinct from the ATP binding site[58,59] and occurs at expected therapeutic concentrations of the drug used as a mood stabilizer. The neuroprotectant effect of Li^+ seems to be due to inhibition of GSK-3β that results in accumulation of the antiapoptotic factor β-catenin,[60] which is also affected by other mood stabilizers such as lamotrigine and valproate.[61] The first report of a possible involvement of GSK-3 in schizophrenia was by Yang et al,[62] where they showed that both cellular activities and protein levels of kinase FA/GSK-3α in the lymphocytes of schizophrenic patients were greatly impaired compared with normal controls.

More recently, low immunoreactivity and activity of GSK-3β was demonstrated in the postmortem frontal cortex of schizophrenic patients,[63-65] but at least one negative study was also reported for a different brain collection,[66] which made the authors stress the need to be cautious with interepretation of data from postmortem samples.

In an elegant study, Emamian et al[67] have shown that levels of AKT1, but not of AKT2 or AKT3, was reduced in lymphocytes and in frontal cortex of schizophrenic patients compared with controls. In contrast, the expression levels of GSK-3β was not altered in patient's lymphocytes and was slightly decreased in brain tissue. However, when the AKT1-dependent phosphorylation levels of GSK-3β at serine-9 was significantly lower in lymphocytes and frontal cortex of schizophrenic patients. In addition, they have shown that treatment of mice with haloperidol induced an increase in phosphorylation of GSK-3β Ser-9.

Evidence of the involvement of the AKT/GSK-3β pathway with dopamine-dependent behaviors and signaling was recently shown.[68] The authors demonstrated that increased dopaminergic neurotransmission in mice striatum, resulted in inactivation of AKT and concomitant activation of GSK-3α and GSK-3β, which was affected by activation of the cAMP pathway, as shown by the lack of phosphorylation changes in DARRP-32 Thr-34. However, these effects were effectively reversed either by inhibition of dopamine synthesis, dopamine D_2 receptor (D_2) blockade, or administration of lithium salts. In addition, it was also shown that pharmacological or genetic inhibition of GSK-3 significantly reduces dopamine-dependent locomotor behaviors.[68] Taken together, their findings suggest that D_2 is responsible for the regulation of AKT by dopamine.[67,68] In a further development, Beaulieu et al[69] were able to show that a β-arrestin 2-mediated kinase/phosphatase scaffolding of AKT and protein phosphatase-2A (PP2A) was responsible for the regulation of AKT by DA receptors.

Conclusion

There are many putative crossover points between the abovementioned proteins and their regulated pathways, and only an extensive investigation of many of these steps will allow better comprehension of cellular signaling mechanisms. These could turn out to be therapeutic targets in the treatment of serious mental illnesses such as schizophrenia. ❏

Las proteínas integradoras de la señal dopaminérgica intracelular y su relevancia para la esquizofrenia

Las modificaciones de la función dopaminérgica se pueden regular a través de interacciones entre los receptores o entre los receptores de dopamina y otras proteínas o con los elementos de las cascadas señalizadoras efectoras. La complejidad de la señalización dopaminérgica no se ha aclarado ni mucho menos. Sin embargo, podría aportar la clave para entender la fisiopatología de los trastornos neurológicos y psiquiátricos e identificar posibles objetivos nuevos que permitieran el desarrollo de fármacos más eficaces y selectivos. En este artículo se revisan algunas de las pruebas actuales y se proponen nuevas ideas, además de exponer las perspectivas futuras.

Application à la schizophrénie du signal intracellulaire dopaminergique intégrant les protéines

L'interaction récepteur-récepteur, l'interaction d'autres protéines avec des récepteurs à la dopamine et/ou des éléments de la cascade de signalisation en aval peuvent réguler les modifications de la fonction dopaminergique. La complexité de la signalisation dopaminergique est loin d'être complètement élucidée. Elle pourrait cependant être la clé de la compréhension de la physiopathologie des troubles neurologiques et psychiatriques, ainsi que de l'identification d'éventuelles nouvelles cibles et du développement de médicaments plus efficaces et sélectifs. Cet article fait la mise au point de quelques preuves actuelles et des nouvelles idées qui en découlent ainsi que des perspectives d'avenir maintenant bien reconnues.

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