SECTION EDITOR: HASSAN M. FATHALLAH-SHAYKH, MD

The Neurobiology of Dopamine Signaling

Jean-Antoine Girault, MD, PhD; Paul Greengard, PhD

The brain contains 2 major groups of dopamine neurons. One is located in the arcuate nucleus of the hypothalamic median eminence and is involved in neuroendocrine regulation. The other, which is the subject of this article, is located in the ventral mesencephalon and projects to the forebrain. Although dopamine neurons are few (<1/100 000 brain neurons), they play an important role in regulating several aspects of basic brain function. They are necessary for the normal tasks of the regions they innervate, including motor behavior, motivation, and working memory. Dopamine neurons are also a central element in the brain reward system that controls the learning of many behaviors. Disappearance of nigrostriatal neurons results in Parkinson disease, whereas blockade of dopamine receptors has therapeutic effects in psychosis. Finally, artificial increase in dopamine transmission is the common mechanism of action of drugs of abuse that leads to addiction. Understanding how dopamine works is a major goal of neurobiology. Much progress has been accomplished in identifying the intracellular signaling pathways that underlie the immediate actions of dopamine and account for its long-term effects on brain properties. Recent findings allow us to identify molecules that may represent future therapeutic targets in neurology and psychiatry. *Arch Neurol. 2004;61:641-644*

Dopamine was identified as a potential neurotransmitter in the brain in the late 1950s by Carlsson.[1] Soon thereafter, it was found that Parkinson disease was accompanied by a dramatic decrease in the dopamine content of the putamen and caudate nucleus. This led to the use of levodopa, the metabolic precursor of dopamine, as an efficient replacement therapy for alleviating the symptoms of Parkinson disease. The first dopamine receptors were identified by their ability to stimulate cyclic adenosine monophosphate (cAMP) production and were shown to be the targets of neuroleptic agents.[2] This discovery defined a class of *slow-acting neurotransmitters* that exert their effects through cascades of biochemical reactions, as opposed to *fast synaptic neurotransmitters* like glutamate or γ-aminobutyric acid that open ligand-gated ion channels.[3] Although fast neurotransmission accounts for most of the quasi-instantaneous functioning of the brain, slow neurotransmission is essential for neuromodulation and long-term regulation. Neuroleptic agents were found to block a second type of dopamine receptor.[4] This defined 2 classes of dopamine receptors, D1 and D2, which respectively stimulate and inhibit adenylyl cyclase. Later, cloning of these receptors demonstrated 2 D1-like receptors (D1 and D5) and 3 D2-like receptors (D2, D3, and D4). D1 and D2 receptors are abundant in the neostriatum, which comprises the caudate nucleus, putamen, and nucleus accumbens. These receptors are enriched in distinct populations of striatal neurons, although there is a degree of coexpression. While D1 and D2 receptors have opposite effects at the molecular level, they often have a synergistic action when more complex outputs are considered. Although D3 receptors seem to play a minor role in normal circumstances, they appear to be up-regulated in pathologic condi-

From the Institut National de la Santé et de la Recherche Médicale and Université Pierre et Marie Curie Unit 536, Institut du Fer à Moulin, Paris, France (Dr Girault); and the Laboratory of Molecular and Cellular Neuroscience, The Rockefeller University, New York, NY (Dr Greengard).

tions, making them prime targets for drug development.[5]

The cell bodies of dopamine neurons innervating the caudate nucleus and the putamen are located in the substantia nigra, while those of neurons innervating the ventral striatum and the prefrontal cortex have a more medial location in the ventral tegmental area. Dopamine is not a simple excitatory or inhibitory neurotransmitter. It is a neuromodulator that alters the responses of target neurons to other neurotransmitters in a manner that depends on the functional state of these neurons. Because of this complex modulatory role, trying to elucidate the physiological role of dopamine has been frustrating for many years. Recent progress in many areas of neuroscience, ranging from cellular electrophysiology to in vivo recordings and behavioral studies, allows us to better understand the function of dopamine neurons. One important finding was the identification of their role in reward systems.[6] In monkeys, unpredicted rewards increase the firing of dopamine neurons, whereas the absence of an expected reward has an inhibitory effect. This led to the proposal that dopamine neurons function as detectors of reward prediction errors. Although complex, this has profound implications for the role of dopamine in learning.[6]

Receptors mediate the action of neurotransmitters on target neurons by altering the permeability of ion channels and thereby changing the membrane potential, or by activating signaling pathways that include different biochemical reactions. These should not be considered independent mechanisms because calcium influx through ion channels activates signaling pathways, while signaling pathways modify the properties of several ion channels. Dopamine receptors comprise 7-transmembrane domain receptors and are associated with guanosine triphosphate–binding proteins (or G proteins) that mediate their effects. One major effect of D1 receptors is to raise cAMP levels and thereby activate a cAMP-dependent protein kinase (PKA). This enzyme transfers a phosphate group from adenosine triphosphate to several specific protein substrates, modifying their properties in many ways. D2 receptors are coupled to different types of G proteins that decrease cAMP levels and alter the permeability of potassium channels. Because regulation of cAMP levels is a major effect of dopamine receptors and because PKA is the major target of cAMP, understanding the action of dopamine at the molecular level required the identification and characterization of PKA substrates in neurons innervated by dopamine.

EXPERIMENTAL METHODS

Most of the initial experiments done to understand the action of dopamine at a molecular level have involved the striatum, which contains high levels of dopamine receptors. Several approaches have been used to identify the relevant PKA substrates in striatal neurons, including the search for proteins that are phosphorylated by this enzyme in vitro.[7] These proteins were then purified, their genes cloned, and their properties studied. Another different strategy started from well-characterized proteins such as ion channels or neurotransmitter receptors. Neurobiologists examined whether these proteins were phosphorylated and how this phosphorylation altered their properties. In all cases, it was crucial to determine how the phosphorylation of relevant proteins is regulated. To do so, a practical method is to use antibodies that recognize a given protein only when it is phosphorylated at a precise location on a specific amino acid residue.[8] Such antibodies are usually obtained by immunizing rabbits or mice against a short phosphorylated peptide that corresponds to the site of interest. The specificity of the antibodies can be improved by selecting those that react with the phosphorylated antigen but not with its unphosphorylated form, using a method called *affinity purification*. A similar strategy can be used to generate antibodies reacting only with the unphosphorylated form of a protein. Phosphorylation state–specific antibodies can be used in 2 types of experiments (**Figure 1**). First, the antibodies allow the identification of phosphorylated proteins by *immunoblot* (also termed *Western blot*). Within limits, this allows a quantitative measurement of specific proteins, phosphorylated or not, depending on the selectivity of the antibodies used. Another useful application of such antibodies is to reveal the presence of phosphorylated proteins in fixed cells or tissues using immunofluorescence. Only phosphorylation state–specific antibodies that have no cross-reactivity with any other antigen can be used for immunofluorescence studies because there is no prior separation of proteins.

RELEVANCE TO THE STUDY OF NEUROSCIENCE

Using the methods herein and other molecular and cellular techniques, many proteins and phosphorylation reactions involved in the action of dopamine have been identified. Some phosphorylated proteins are directly responsible for dopamine's effects, eg, dopamine controls the activity of glutamate receptors that mediate the corticostriatal neurotransmission. It does this through phosphorylation by PKA of 2 major subtypes of glutamate receptors, so-called AMPA (α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid) and NMDA (*N*-methyl-D-aspartate) receptors, according to their selective synthetic agonist. Dopamine also regulates the activity of voltage-gated ion channels, including sodium and calcium channels, by modulating the phosphorylation state of these channels or of associated proteins. Another class of proteins is critical for dopamine's long-term effects comprises transcription factors that regulate the expression of specific genes. Through complicated phosphorylation cascades, dopamine increases the phosphorylation of specific transcription factors, leading to their increased activity and the expression of immediate early genes. In turn, immediate early genes activate the expression of late genes that are thought to be essential for the long-lasting modifications of synaptic transmission controlled by dopamine.

In addition to these proteins regulated by phosphorylation, which may be thought of as the effectors of dopamine signaling, another class of proteins plays a critical role in the

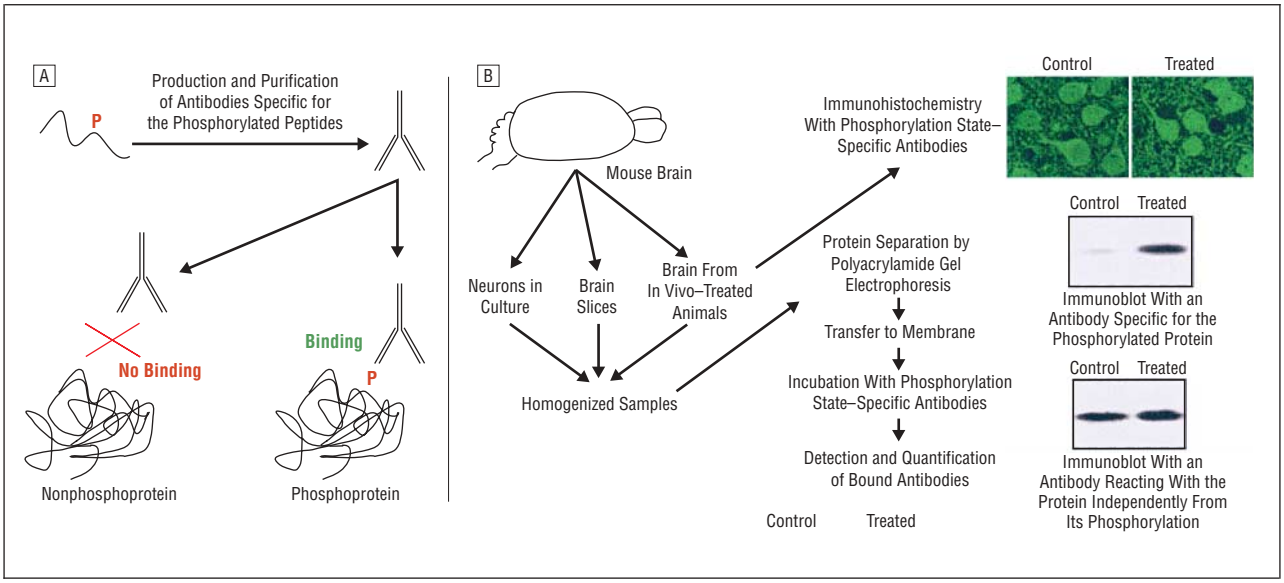

Figure 1. Phosphorylation state–specific antibodies. A, Antibodies are raised against a phosphorylated peptide corresponding to a specific phosphorylation site in a protein of interest. If necessary, they are purified by affinity chromatography to obtain antibodies that react only with the phosphorylated form of the protein. P indicates phosphate. B, Such antibodies can be used to detect phosphoproteins in cells or tissues by different techniques, including immunofluorescence (right upper panel) and immunoblot (right middle panel). In this example, antibodies against phosphothreonine-34–DARPP-32 (right upper and middle panels) or against DARPP-32 (right lower panel) were used. Mice were injected with 10 mg/kg of body weight of d-amphetamine (treated) or vehicle (control) and their striatum examined by immunofluorescence or immunoblot.

physiology of dopamine-innervated brain regions. These are regulatory proteins that are essential components of the signaling cascades activated by dopamine. The best studied example is DARPP-32, a dopamine- and cAMP-regulated phosphoprotein with a molecular mass of 32 kDa.[9,10] This protein is enriched in striatal medium-sized spiny neurons and is expressed at lower levels in other cell populations of the nervous system and other tissues. DARPP-32 is a small protein that can be phosphorylated on several residues by different protein kinases. Depending on the state of phosphorylation of these various residues, DARPP-32 exerts different regulatory effects on signaling pathways. DARPP-32 is phosphorylated on threonine 34 by PKA in response to stimulation of D1 receptors. When phosphorylated, DARPP-32 is a potent inhibitor of a particular type of protein phosphatase, termed *protein phosphatase 1*. Protein phosphatases are key enzymes that dephosphorylate proteins and account for the reversibility of phosphorylation reactions within cells. The regulation of protein phosphatases is a finely tuned process and an important aspect of signaling pathways. When phosphorylated by PKA, DARPP-32 inhibits protein phosphatase 1 and amplifies the response to PKA and to other protein kinases by preventing dephosphorylation of residues that are substrates for protein phosphatase 1. The importance of this role of DARPP-32 has been demonstrated by the generation of mutant mice lacking DARPP-32.[11] Although these mice appear normal in standard laboratory housing conditions, they have altered responses to all challenges of the dopamine systems. DARPP-32 is also phosphorylated on a different residue, threonine 75, by a protein kinase called *cyclin-dependent kinase 5* (CDK5).[12] Although CDKs are generally known for their role in the regulation of cell cycle, CDK5 has important functions in the mature nervous system and during development. Phosphorylation of DARPP-32 by CDK5 turns it into an inhibitor of PKA. When phosphorylated on threonine 75, instead of amplifying PKA actions, DARPP-32 has an inhibitory role on this pathway. Therefore, DARPP-32 can be thought of as a switch that can be turned from an amplification regulator to an inhibitor of the cAMP pathway (**Figure 2**). This regulation of DARPP-32 by CDK5 appears to be important for adaptive responses of striatal neurons to repetitive stimulation of dopamine receptors by cocaine.[13] Finally, the role of DARPP-32 is not limited to the action of dopamine. Because of its multiple phosphorylation sites, DARPP-32 is at the crossroads between many signaling pathways activated by various neurotransmitters and plays a critical role in their action in striatal neurons and in other cell types.[9]

APPLICATIONS AND RELEVANCE TO THE PRACTICE OF NEUROLOGY AND PSYCHIATRY

Dopamine exerts important effects on its target neurons by modulating their responses and by altering synaptic plasticity. These functions of dopamine account for its role in different human conditions, including Parkinson disease, drug addiction, compulsive behavior, attention-deficit/hyperactivity disorder, and schizophrenia. Although diverse, these diseases correspond to, or include, alterations of the same basic neuronal processing mechanisms that are normally controlled by dopamine. Depending on the type of alterations and the predominant anatomical sites of dysfunction, the clinical manifestations are different and do not necessarily respect

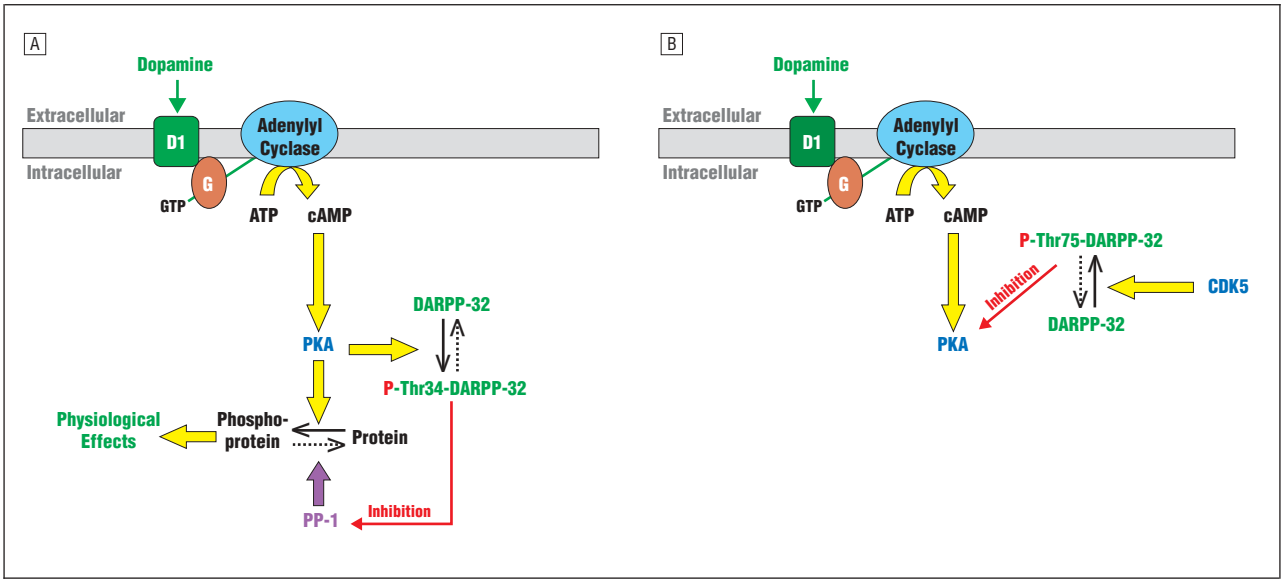

Figure 2. The role of DARPP-32 in the action of dopamine. A, In basal conditions, dopamine stimulates the phosphorylation of DARPP-32 on threonine 34 (P-Thr34–DARPP-32) through a signaling cascade that includes dopamine D1 receptors, a specific heterotrimeric G protein (G), adenylyl cyclase that raises cyclic adenosine monophosphate (cAMP) levels, and cAMP-dependent protein kinase (PKA). Because phosphorylated DARPP-32 is a potent inhibitor of protein phosphatase 1 (PP-1), it increases the phosphorylation of numerous substrates and plays a major role in dopamine actions. B, When cyclin-dependent kinase 5 (CDK5) is activated (eg, following treatment for cocaine abuse), it phosphorylates DARPP-32 on threonine 75, turning it into an inhibitor of PKA and switching off the regulations depicted in A. ATP indicates adenosine triphosphate; GTP, guanosine triphosphate.

the traditional boundaries between neurology and psychiatry. Until recently, virtually all the drugs available to neurologists or psychiatrists were acting at the level of the neuronal membranes, modifying neurotransmitters' metabolism or interactions with receptors. Understanding the intracellular signaling pathways underlying the action of neurotransmitters, especially dopamine, suggests a new class of potential therapeutic agents that could target a specific protein kinase or a phosphatase. In principle, one advantage of pharmacological manipulation of signaling pathways is that it would not alter basic function but only modify regulatory processes. For example, inhibitors of protein kinases modulating DARPP-32 could have applications in enhancing or decreasing specific aspects of dopamine actions. Knowledge of the signaling pathways activated by dopamine also allows us to look for changes in these pathways in pathologic conditions. At present, this approach is limited in humans to the search for mutations in the genes of the relevant proteins and for variation of their levels in postmortem brain samples. Therefore, it will be challenging to design novel methods allowing the study of intracellular signaling pathways in vivo by noninvasive approaches. The use of experimental animal models and the design of drugs capable of selectively altering these pathways should allow important progress to be made.

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Corresponding author: Jean-Antoine Girault, MD, PhD, Institut National de la Santé et de la Recherche Médicale and Université Pierre et Marie Curie Unit 536, Institut du Fer à Moulin, 17, rue du Fer à Moulin, 75005 Paris, France (e-mail: girault@fer-a-moulin.inserm.fr).

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Correction

Error in Figure Legend. In the article titled "The Neurobiology of Dopamine Signaling," published in the May issue of the ARCHIVES (2004;61:641-644), the legend to Figure 2 included an error. The description for part B should have read as follows: When cyclin-dependent kinase 5 (CDK5) is activated (eg, following long-term cocaine administration), it phosphorylates DARPP-32 on threonine 75, turning it into an inhibitor of PKA and switching off the regulations depicted in A.