What dopamine does in the brain

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In the early 1970s, receptors for neurotransmitters acting via second messengers had not been identified biochemically nor were there definitive links to such messengers. The discovery by John W. Kebabian and Paul Greengard of a dopamine-sensitive adenyl cyclase, accordingly, was a giant step forward. The investigators first characterized the enzyme in sympathetic ganglia wherein dopamine-producing cells link pre- and post-synaptic neurons. Then, in the corpus striatum, the brain area enriched in dopamine, they delineated the enzyme's properties and showed that it was inhibited by antipsychotic drugs, leading to a large body of research on dopamine as a mediator of antipsychotic drug action and putative roles for this transmitter in the pathophysiology of schizophrenia.

In the early 1970s, a reasonable amount was known about the biochemistry of neurotransmitters. A few chemical transmitters were accepted—acetylcholine, norepinephrine, serotonin, and GABA. There were hints that certain common amino acids such as glutamate and glycine, besides their roles in general metabolism and protein synthesis, might also be neurotransmitters. However, this hint was still a controversial area. In 1970, Chang and Leeman (1) had isolated and sequenced Substance P in the brain as a peptide that stimulates salivary secretion, but there was little else to argue for peptides as transmitters. The explosion of peptide transmitters that commenced with the isolation of enkephalins was several years in the offing (2). Gases such as nitric oxide, carbon monoxide, and hydrogen sulfide as endogenous biologically active substances were not even fantasized.

The biosynthesis of acetylcholine, serotonin, norepinephrine, and GABA had been elucidated, and their degradative pathways had been established. Their release by exocytosis was generally accepted. Synaptic inactivation by an enzyme in the case of acetylcholine and for amines and amino acids, by reuptake into the nerve endings that had released them seemed on solid ground. However, molecular characterization of the most important element of synaptic transmission, transmitter receptors, was still unattained, at least in the brain.

It was generally assumed that neurotransmitters bound to receptor proteins on postsynaptic membranes. Several investigators had identified the receptor protein for the actions of acetylcholine in the electric organ of the electric eel through the binding of radiolabeled α-bungarotoxin (3). However, the receptor in these electric organs comprises 20% of membrane protein, about 1 million times as dense as what would be expected in the brain. Thus, few investigators anticipated ever understanding neurotransmitter receptors in the brain at a biochemical level, and the identification by ligand binding of neurotransmitter and drug receptors in the mid-1970s came as a surprise (4).

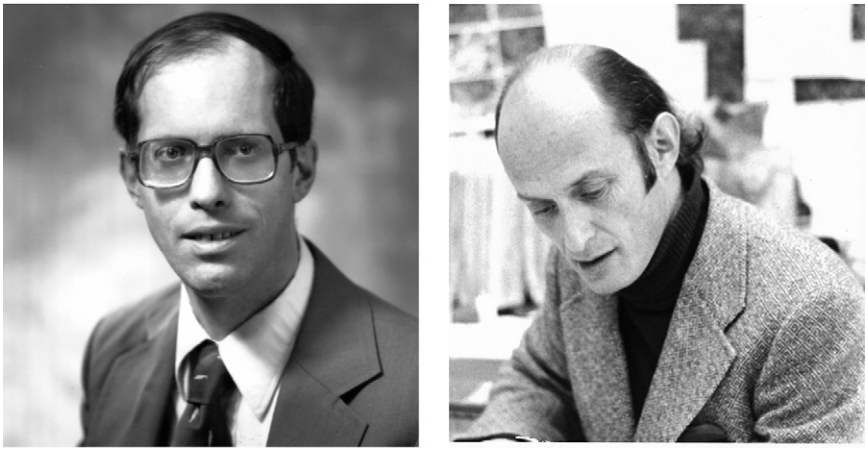

Fig. 1. Photographs of John W. Kebabian (*Left*) and Paul Greengard (*Right*) in the 1970s at about the time of their pioneering work on dopamine-activating adenylate cyclase.

For neurotransmitters that act by opening ion channels, such as acetylcholine at the neuromuscular junction, transmitter recognition was presumed to be transformed into an opening or closing of ion channels. For the biogenic amines, such as norepinephrine and serotonin, the picture was murkier. Hence, the work of Kebabian and Greengard (5) and Kebabian et al. (6) (Fig. 1) implying that dopamine in the superior cervical ganglion and the brain's caudate nucleus acts through a receptor coupled to a cAMP-forming enzyme—adenylate cyclase—was a giant step forward.

How did Kebabian and Greengard (5) and Kebabian et al. (6) come to explore adenylate cyclase as a target for the synaptic effects of a neurotransmitter? The deep background for this work comes from the pioneering efforts of Sutherland and Rall (7) that discovered cAMP as a second messenger molecule for a number of hormones. Sutherland and Rall (7) then identified adenylate cyclase as a cAMP-forming enzyme, which could be activated by hormones, each with its own distinctive receptor coupled somehow to a unitary adenylate cyclase.

Why did Kebabian and Greengard (5) and Kebabian et al. (6) choose to explore dopamine rather than a better characterized transmitter such as norepinephrine or serotonin? Dopamine is the metabolic precursor of norepinephrine, differing only in the absence of a hydroxyl at the β-position, which is added by the enzyme dopamine β- hydroxylase (Fig. 2). Speculation that dopamine might be a biologically active molecule in its own right came in the late 1950s when Carlsson et al. (8) used the recently developed spectrophotofluorometric techniques for measuring biogenic amines to uncover extremely high concentrations of dopamine in the caudate nucleus of the brain, vastly greater than norepinephrine levels in this region. Dopamine became clinically relevant when Hornykiewicz (9) discovered its depletion in the caudate nucleus of

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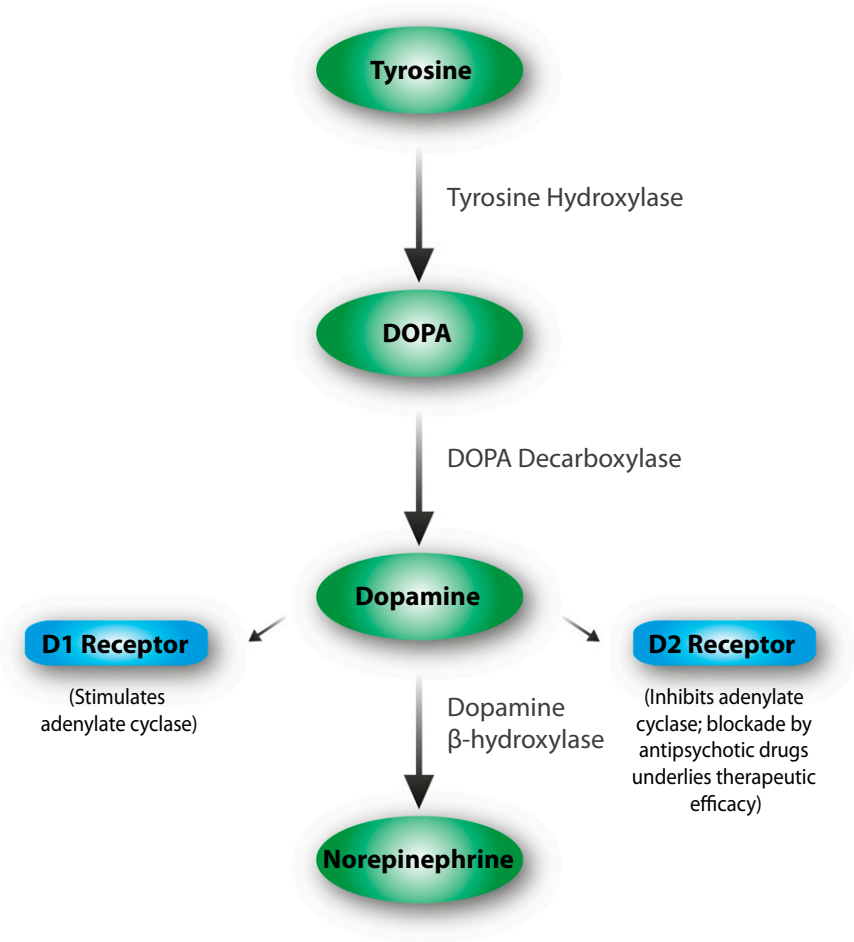

Fig. 2. Biosynthesis and receptor actions of dopamine.

patients with Parkinson's disease, whereas i.v. administration of L-dihydroxyphenylalanine, the amino acid precursor of dopamine, dramatically and rapidly alleviated Parkinsonian symptoms.

The path of Kebabian and Greengard (5) and Kebabian et al. (6) to characterizing synaptic actions of dopamine began in a peripheral system, the superior cervical ganglion, in which the presynaptic neuron uses acetylcholine as its transmitter, whereas the postsynaptic neuron employs norepinephrine. Besides rapid excitatory transmission from presynaptic to postsynaptic neuron in this ganglion, neurophysiologists had identified a slow hyperpolarizing inhibitory component that seemed to involve an interneuron. Histochemical studies revealed dopamine in these interneurons, suggesting that it might have a transmitter function. The interest of Kebabian and Greengard (5) and Kebabian et al. (6) in cAMP followed the discovery that the cAMP-dependent protein kinase, discovered by Krebs (10) and associated as a mediator of hormone effects (10), also was prominent in the brain (11).

The protein kinase was at least two steps removed from the neurotransmitter. Hence, to link cAMP to neurotransmitters would require monitoring cAMP levels, which was a tedious procedure in small ganglia at that time. Because of his background as a neurophysiologist, Greengard focused on the hyperpolarizing influences of presumed interneurons in the superior cervical ganglion. First, he showed that preganglionic stimulation increases cAMP levels several fold. At this point, he had no reason to favor dopamine over norepinephrine as the transmitter, because ganglionic levels of the two catecholamines are about the same. In slices of the ganglion, dopamine increased cAMP levels with substantially greater potency than norepinephrine. In homogenates, dopamine and norepinephrine increased adenylate cyclase activity to a similar extent. In the ganglion, norepinephrine was well-established as the principal catecholamine in the postganglionic cells, but histochemical studies had revealed that dopamine, rather than norepinephrine, predominated in the interneurons. Based on these findings, Kebabian and Greengard (5) cautiously concluded that "the physiologic effects of dopamine in the ganglion, and possibly elsewhere in the nervous system, may be mediated by stimulating the synthesis of adenosine-3′,5′-monophosphate" (5).

The superior cervical ganglion is an ideal tissue for investigating synaptic transmission, with a single presynaptic and a single postsynaptic neuron along with small interneurons linking the pre- and postsynaptic elements. Greengard then proceeded to the more challenging caudate nucleus, the area of highest dopamine density in the brain (8). He used similar strategies as in the ganglion. In homogenates of the caudate, dopamine stimulated adenylate cyclase with a potency about threefold greater than norepinephrine, whereas at the known α- and β-receptors for norepinephrine, dopamine is much weaker. Moreover, the β-adrenergic receptor is most potently activated by isoproterenol, which failed to stimulate adenylate cyclase in the caudate, even at high concentrations. Apomorphine, known to mimic dopamine pharmacologically, was even more potent than dopamine in stimulating the cyclase.

In this study, Kebabian et al. (6) noted that two antipsychotic drugs, chlorpromazine and haloperidol, blocked dopamine's effects. These drugs, first introduced into psychiatry in 1952, had revolutionized the treatment of schizophrenia, and therefore, understanding their mechanism of action was of great importance. The first insights into how they acted came from the studies of Carlsson and Lindqvist (12) just a few years after they identified dopamine as a putative neurotransmitter/neuromodulator. Carlsson and Lindqvist (12) noted that the pharmacology of these drugs resembled reserpine, an antipsychotic drug that acts by depleting the brain of dopamine, serotonin, and norepinephrine. Chlorpromazine and haloperidol failed to influence levels of dopamine or norepinephrine but increased metabolic products of dopamine fairly selectively. Carlsson and Lindqvist (12) speculated that the antipsychotics were causing a functional dopamine depletion by blocking receptors, leading to a feedback augmentation of dopamine turnover. The increased turnover of dopamine was confirmed by numerous investigators, but no one had examined a receptor-linked event directly.

In a subsequent study, Clement-Cormier et al. (13) explored potencies of a series of antipsychotics of the phenothiazine and butyrophenone class and found substantial potency in inhibiting the dopamine-sensitive adenylate cyclase. Among the phenothiazines, clinical potency paralleled inhibition of the enzymes. Thus, the very potent antipsychotic fluphenazine inhibited the enzyme about 10 times more potently than chlorpromazine.

Promethazine, a phenothiazine antihistamine that is not antipsychotic, was about 25-fold weaker than chlorpromazine. This finding suggested that these drugs act by blocking dopamine receptors associated with the adenylate cyclase. However, there were exceptions to the rule. Thus, haloperidol and pimozide, which clinically, are one to two orders of magnitude more potent then chlorpromazine, were, respectively, 3 and 20 times weaker than chlorpromazine.

The resolution to this knotty problem came a few years later when it was possible to identify dopamine receptors by ligand binding (4). Two classes of dopamine receptors could be labeled with [^{3}H]haloperidol and [^{3}H]dopamine (14) corresponding, respectively, to dopamine-D2 and dopamine-D1 receptors, which were subsequently enunciated by Kebabian and Calne (15). At D1 receptors, dopamine activates adenylate cyclase, whereas at D2 receptors, the transmitter inhibits the enzyme (Fig. 2). Antipsychotic drug potencies are predicted by blockade of D2 receptors at which haloperidol and pimozide are much more potent than chlorpromazine. The dopamine-sensitive adenylate cyclase characterized by Kebabian et al. (6) corresponds to D1 receptors.

The studies of dopamine by Greengard, coupled with his epic contributions to neural regulation of protein kinases, have been landmarks in the biochemical elucidation of synaptic transmission in the brain, which was acknowledged by his coreceiving of the Nobel Prize in Physiology or Medicine in 2000. In regards to the question raised at the outset of this essay (how does one pinpoint a neurotransmitter receptor at a biochemical level), Greengard was cautious. The title of the publication by Kebabian et al. (6) that is the subject of this essay, "Dopamine-sensitive adenylate cyclase in caudate nucleus of rat brain, and its similarity to the 'dopamine receptor,'" was itself a hedge. Throughout the paper, Kebabian et al. (6) always adumbrated the term in quotation marks. With the use of ligand binding techniques just a few years after the publication by Kebabian et al. (6), it became easy to characterize neurotransmitter receptors in depth and investigate the linkage between the receptor, defined as the recognition site for the neurotransmitter, and coupling to G proteins followed by influences on adenylate cyclase and related molecules (4). Greengard's efforts lie at the base of this edifice.

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