AMAZING PAPERS IN NEUROSCIENCE
Reviewing the Diverse Effects of Protein Phosphorylation in Neural Signaling Transduction

Abby K. Scurfield
School of Psychology and Neuroscience, St. Andrews, UK, KY16 9JP.

Signaling transduction pathways are now known to be foundational mechanisms for a wide variety of biological function. Nobel laureate Paul Greengard dedicated his early career to the exploration of how these molecular cascades are triggered by neurotransmitters, hence applying a general phenomenon to the nervous system. A review by Hemmings, Nairn, McGuinness, Huganir, and Greengard published in the FASEB journal in 1989 identifies three different effects of protein phosphorylation, namely vesicle release, modulation of receptor sensitivity, and initiation of positive or negative feedback systems. The work's focus on three specific examples, rather than the exhaustive approach taken by many other reviews, provides students with an accessible framework within which to learn fundamental concepts in molecular neuroscience. The review could be incorporated as assigned reading for introductory neuroscience or even upper level molecular neurobiology, as it holds very versatile teaching potential.

Key words: signaling pathways; neural communication; neurotransmitters; pathway regulation and modulation

Signaling transduction, the process by which extracellular signals reach target cells, bind to a receptor, and trigger a cascade of intracellular protein responses, is crucial to neural communication. Since it is largely driven by changes in protein phosphorylation states – presence or absence of adenosine triphosphate (ATP) – protein phosphorylation is critical for the maintenance of a wide variety of neural functions. The review article by Hemmings et al. (1989) highlights the importance of signaling transduction and protein phosphorylation by placing them in the context of neural communication. The paper is organized into three separate discussions on the physiological effects of phosphorylation of Synapsin I, nicotinic acetylcholine receptor (NAChR), and dopamine-and-cAMP-regulated-phosphoprotein (DARPP-32). The authors first discuss the two response mechanisms to extracellular signals and assign each phosphoprotein to one of these mechanisms. They also provide evidence of widespread synaptic localization of phosphoproteins and cite studies showing that addition of protein kinases is sufficient to initiate intracellular signaling, suggesting their importance in neurotransmission.

Hemmings and colleagues first introduce Synapsin I, which shows diffuse localization to all mammalian presynaptic nerve terminals and is specifically expressed on the cytoplasmic surface of neurotransmitter-carrying vesicles. Past studies have shown Synapsin I phosphorylation after injections of depolarizing agents, suggesting a role in neurotransmitter release. Phosphorylation of its two domains (tail and head) by cAMP and Ca^{2+}/Calmodulin (CaM)-dependent kinase I and II is discussed along with the physiological results of this action. The authors draw attention to the experiments of Llinas et al. (1985), which showed that injections of dephosphorylated Synapsin I decreased the amplitude and rise rate of the postsynaptic potential and injections of Ca2+/CaM Kinase II increased the rise rate and amplitude of the postsynaptic potential (Figure 4, Hemmings et al., 1989, originally Figures 1C, 2A in Llinas et al., 1985). This figure nicely sums up the effect of Synapsin I phosphorylation on synaptic transmission and even provides a teaching tool to introduce electrophysiological techniques such as voltage clamp. Examination of this empirical study led authors to postulate a comprehensive model of Synapsin I's role in synaptic vesicle release: in resting state, dephosphorylated Synapsin binds vesicles to prevent their movement while, in the active state, increased Ca^{2+} activates Ca^{2+}/CaM kinase II which phosphorylates the tail domain and releases Synapsin from vesicles. This allows for fusion with the plasma membrane. Using a thorough examination of past empirical studies and figures showing molecular cascades and subsequent intracellular events, the authors describe an important role of Synapsin I phosphorylation in increasing the availability of vesicles for membrane fusion and neurotransmitter release.

Next, the authors discuss protein phosphorylation in the context of NAChR regulation. NAChR is a membrane receptor/ion channel complex which conducts excitatory inward current upon binding of acetylcholine (ACh). While ionotropic in nature, the receptor has three intracellular phosphorylation sites which bind cyclic AMP (cAMP), protein kinase C (PKC), and a tyrosine kinase for regulatory purposes. Since all three phosphorylation sites are near one another, the authors discuss the idea that their phosphorylation must regulate a common property of the receptor. Empirical studies have shown that addition of cAMP and tyrosine kinases increases NAChR desensitization to ACh in the synaptic cleft. This is shown with an original figure (Figure 7, Hemmings et al., 1989, originally Figure 2 in Huganir et al., 1986) displaying NAChR activity after application of ACh to either phosphorylated or dephosphorylated receptors (Huganir et al., 1986). While no direct role for PKC had been

determined at this point, the authors postulate a similar desensitizing effect. The authors then discuss calcitonin gene-related peptide (CGRP), found with ACh in presynaptic terminals and released in the neuromuscular junction, as a potential protein kinase activator. This neuropeptide has been observed to increase phosphorylation and desensitization of the receptor in a regulatory manner through activation of the postsynaptic cAMP pathway. As for PKC and tyrosine kinase, authors acknowledge that, at this point (1989), identification of the remaining two neurotransmitters/activators is an area of active research, pointing toward an avenue for students to revisit today. As a concluding remark, the authors emphasize that NAChR regulation represents a situation in which three separate neurotransmitters are regulating cell sensitivity to a fourth (ACh) through receptor phosphorylation. They point this out with a comprehensive and straightforward schematic diagram (Figure 8, Hemmings et al., 1989, adapted from Huganir and Greengard, 1987) that students can use to ensure comprehension of the various pathways involved.

The authors finish with a discussion of DARPP-32 which they implicate in dopaminergic signaling. This cytosolic phosphoprotein is found in high concentrations in the basal ganglia, specifically in cells expressing D1 dopamine receptors (Hemmings and Greengard, 1986). Studies have shown that both dopamine and cAMP increase DARPP-32 phosphorylation, leading the authors to suggest it may have a role in modulation of dopaminergic signaling. They consider previous findings that have shown phosphorylation by cAMP, cGMP-dependent protein kinase, and casein kinase, and next report that phosphorylated-DARPP-32 is a noncompetitive inhibitor of protein phosphatase-1. The authors point out that, because protein phosphatase-1 dephosphorylates many dopaminergic-signaling effector proteins, the phosphorylation of DARPP-32 could trigger a positive feedback system to increase dopamine effects. Alternatively, by inhibition of this phosphatase, DARPP-32 may modulate other signaling pathways by allowing buildup of phosphoproteins, downregulating the cell response through a negative feedback system. The discussion of both contrasting regulatory mechanisms is complemented by another detailed schematic (Figure 10, Hemmings et al., 1989, adapted from Hemmings et al., 1987) which concisely illustrates complicated phosphorylation cycles that may otherwise be difficult for students with limited cell biology background to understand. This section provides an interesting window into the multifaceted effects of DARPP-32 phosphorylation, from vesicle release to receptor desensitization and feedback pathways, all offering very relevant insights into the importance of protein phosphorylation.

VALUE

This review quickly covers several important findings in neural signaling transduction, distinguishing its potential from that of primary articles. Through exploration of past studies (many generated by the same lab), the authors take the reader through the diverse effects of protein phosphorylation with three examples showing vesicle release, receptor desensitization, and modulatory activity. All three topics are central ideas in neuroscience, and thus crucial classroom material. Rather than learning about these ideas separately, students can easily grasp concepts from material presented in this combined format. While signaling pathways and the importance of phosphorylation events had by this time been well established in other types of cells, Paul Greengard and his team were some of the first to implicate these findings in nervous system function, and students should be aware of this step in history. This paper also offers teaching platforms for experimental design, basics of signaling pathways, and imaging techniques such as immunocytochemistry and electron and light microscopy.

Even though this review is quite aged and some signaling mechanisms have since been further elucidated, the foundational ideas presented here have stood the test of time, further establishing this review as one of unique importance. This work continues to be cited and influential in the field (Fernandez et al., 2006; Bykhovskaia, 2011; Stokes et al., 2015). This characteristic of the work strengthens its teaching potential, as the limitations and ideas for subsequent inquiries encourage students to follow this story through to the present day. While there are two other more widely cited reviews, namely Greengard et al., 1993 and Greengard, 1978; they do not offer material as well suited to undergraduate education or neuroscience. Greengard et al. (1993) is an extensive review of Synapsin I and the intracellular events upon its phosphorylation (at which students can look for further detail), while Greengard (1978) provides a more general review of signaling pathways throughout the entire body. More recent reviews (Cohen, 2000; Pawson and Scott, 2005) provide thorough reports on protein phosphorylation in the neural context but are too detailed and biochemistry-heavy for incorporation in most undergraduate courses. For the most recent updates on these three examples of protein phosphorylation, students should look to Kuroiwa et al. (2012), Lee et al. (2015), and Marsh et al. (2017). Marsh et al. (2017) is an empirical study which demonstrates that AB42 oligomers induce persistent phosphorylation of Synapsin I at a pathological level implicated in Alzheimer's Disease, highlighting the importance of maintaining the normal phosphorylation cycle of this protein. Lee et al. (2015) reveals the role of PKC phosphorylation of the NAChR (which Hemmings and colleagues had not yet identified). Instead of increasing receptor desensitization, as Hemmings and colleagues hypothesized, PKC phosphorylation of the alpha4 NAChR subunit triggers recovery from desensitization. This is an important turn of events that can easily be highlighted in undergraduate curriculum. Lastly, the Kuroiwa et al. (2012) paper provides insight into the interactions between muscarinic receptors and DARPP-32 signaling, implicating roles for M5 and M1 type receptors in modulating DARPP-32 dopamine responses in D1 and D2 neurons. The ability of the Hemming et al. (1989) review, however, to concisely report on specific neural examples of protein phosphorylation confirms its value for educators and

students alike. It is potentially a 'hidden gem' for easy and efficient implementation in undergraduate neuroscience education.

AUDIENCE

While there is a wide range of courses into which this paper could be incorporated, it is best suited to an upper division molecular neuroscience or neurobiology course. Students can start with discussions on the material covered here and perhaps delve into primary articles in the same field, such as Fienberg et al. (1998), an article exploring dopaminergic signaling regulation by DARPP-32 in more depth. I could also see this paper incorporated into cell biology (nervous system unit), introductory neuroscience, or more specific courses such as neurotransmission. It can be easily applied to teaching at many levels, with a prerequisite of basic knowledge in signaling pathways. In sum, this review provides an ideal platform on which to further develop signal transduction knowledge within a neural context by highlighting specific examples easily understood by undergraduate students.

REFERENCES

Bykhovskaia M (2011) Synapsin regulation of vesicle organization and functional pools. Semin Cell Dev Biol 22:387-392.

Cohen P (2000) The regulation of protein function by multisite phosphorylation – a 25 year update. Trends Biochem Sci 25:596-601.

Fernandez E, Schiappa R, Girault J, Le Novère N (2006) DARPP-32 is a robust integrator of dopamine and glutamate signals. PLoS Comput Biol 2:e176.

Fienberg A, Hiroi N, Mermelstein P, Song W, Snyder G, Nishi A, Cheramy A, O'Callaghan JP, Miller DB, Cole DG, Corbett R, Haile CN, Cooper DC, Onn SP, Grace AA, Ouimet CC, White FJ, Hyman SE, Surmeier DJ, Girault J, Nestler EJ, Greengard P (1998) DARPP-32: regulator of the efficacy of dopaminergic neurotransmission. Science 281:838-842.

Greengard P (1978) Phosphorylated proteins as physiological effectors. Science 199:146-152.

Greengard P, Valtorta F, Czernik AJ, Benfenati F (1993) Synaptic vesicle phosphoproteins and regulation of synaptic function. Science 259:780-785.

Hemmings HC Jr, Greengard P (1986) DARPP-32, a dopamine- and adenosine 3':5'-monophosphate-regulated phosphoprotein: regional, tissue, and phylogenetic distribution. J Neurosci 6:1469-1481.

Hemmings HC, Walaas SI, Ouimet CC, Greengard P (1987) Dopaminergic regulation of protein phosphorylation in the striatum: DARPP-32. Trends Neurosci 10:377-383.

Hemmings HC Jr, Nairn AC, McGuinness TL, Huganir RL, Greengard P (1989) Role of protein phosphorylation in neuronal signal transduction. FASEB J 3:1583-1592.

Huganir RL, Greengard P (1987) Regulation of receptor function by protein phosphorylation. Trends Pharmacol Sci 8:472-477.

Huganir RL, Delcour AH, Greengard P, Hess GP (1986) Phosphorylation of the nicotinic acetylcholine receptor regulates its rate of desensitization. Nature 321:774-776.

Kuroiwa M, Hamada M, Hieda E, Shuto T, Sotogaku N, Flajolet M, Snyder GL, Hendrick JP, Fienberg A, Nishi A (2012) Muscarinic receptors acting at pre- and post-synaptic sites differentially regulate dopamine/DARPP-32 signaling in striatonigral and striatopallidal neurons. Neuropharmacology 63:1248-1257.

Lee AM, Wu DF, Dadgar J, Wang D, McMahon T, Messing RO (2015) PKCε phosphorylates a4β2 nicotinic ACh receptors and promotes recovery from desensitization. Br J Pharmacol 172:4430-4441.

Llinas R, McGuinness TL, Leonard CS, Sugimori M, Greengard P (1985) Intraterminal injection of synapsin I or of calcium/calmodulin-dependent protein kinase II alters neurotransmitter release at the squid giant synapse. Proc Natl Acad Sci U S A 82:3035-3039.

Marsh J, Bagol SH, Williams RB, Dickson G, Alifragis P (2017) Synapsin I phosphorylation is dysregulated by beta-amyloid oligomers and restored by valproic acid. Neurobiol Dis 106:63-75.

Pawson T, Scott JD (2005) Protein phosphorylation in signaling – 50 years and counting. Trends Biochem Sci 30:286-290.

Stokes C, Treinin M, Papke RL (2015) Looking below the surface of nicotinic acetylcholine receptors. Trends Pharmacol Sci 36:514-523.

The author thanks fellow MRes Neuroscience students and advisors, particularly Dr. Stefan Pulver, for their constructive feedback.

Address correspondence to: Abby Scurfield, School of Psychology and Neuroscience, St Mary's Quad, South Street, St. Andrews, Fife, KY16 9JP. Email: as382@st-andrews.ac.uk

Received March 09, 2018; revised May 23, 2018; accepted June 05, 2018.

This work was supported by The Association for the Development of Undergraduate Neuroscience Education (SRA & RLN), The Endowment for Science Education (EA), and The Synaptic State Faculty Research Foundation (EA).