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Norepinephrine induces rapid and long-lasting phosphorylation and redistribution of connexin 43 in cortical astrocytes

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ABSTRACT

Norepinephrine (NE) modulates brain functions depending on both the internal and external environment. While the neuromodulatory actions of NE have been well characterized, the response and involvement of cortical astrocytes to physiological noradrenergic systems remain largely unknown, especially at the molecular level. In this study, we biochemically characterize the action of NE on astrocytes of the murine neocortex. NE stimulation of acute brain slices rapidly increase phosphorylation of connexin 43 (Cx43) at Serine (Ser) 368, in slices from both juvenile and adolescent animals. The phosphorylation is mediated by the protein kinase C (PKC) pathway under the α1-adrenergic receptor and remains elevated for tens of minutes following brief exposure to NE, well after the intracellular calcium level returns to normal level, suggesting the plastic nature of this phosphorylation event. Importantly, this phosphorylation event persists in the absence of neuronal transmissions, suggesting that the effect of NE on Cx43 phosphorylation is induced directly on astrocytes. Furthermore, these NE-induced phosphorylations are associated with biochemical dissociation of Cx43 from gap-junctional plaques to non-junctional compartments. Finally, we show that pharmacological manipulation of the noradrenergic system using psychoactive drugs modulates phosphorylation of Cx43 in the cerebral cortex *in vivo*. These data suggest that NE acts directly on astrocytes in parallel with neurons and modulates functionally critical connexin channel proteins in a plastic manner. Thus, plasticity of astrocytes induced by the "gliomodulatory" actions of NE may play important roles in their physiological as well as pharmacological actions in the brain.

1. Introduction

Astrocytes play pivotal roles across broad aspects of brain function [1]. Astrocytes exist in a tiled fashion, each occupying a non-overlapping area but covering practically the entire cerebral cortex as a whole [2]. Using this extensive coverage, astrocytes appear to monitor and maintain the metabolic state of neurons [3]. In addition, accumulating evidence suggests that astrocytes regulate extracellular ionic conditions and thereby modulate neuronal activities [4,5]. Besides the various transporters and ion channels expressed on astrocytes, gap junctions are critical to their function in extracellular environment regulation. Gap junctions allow neighboring cells to communicate with each other in an analogue manner by transmitting small bioactive molecules with the size of less than ~1000 Da, based on the concentration gradient. Since astrocytes in the cerebral cortex are connected to neighboring cells via gap junctions, each occupying non-overlapping areas, this gap junctional network of astrocytes, called astrocytic syncytium, creates a long-range analogue cellular network [6]. Given these unique features, astrocyte gap junctions are critically involved in their physiological and pathophysiological functions [1,7–9].

At the molecular level, connexin 43 (Cx43) is the predominant constituent of functional gap junctions in astrocytes, especially in the juvenile animals [10]. Phosphorylation of Cx43 in cortical

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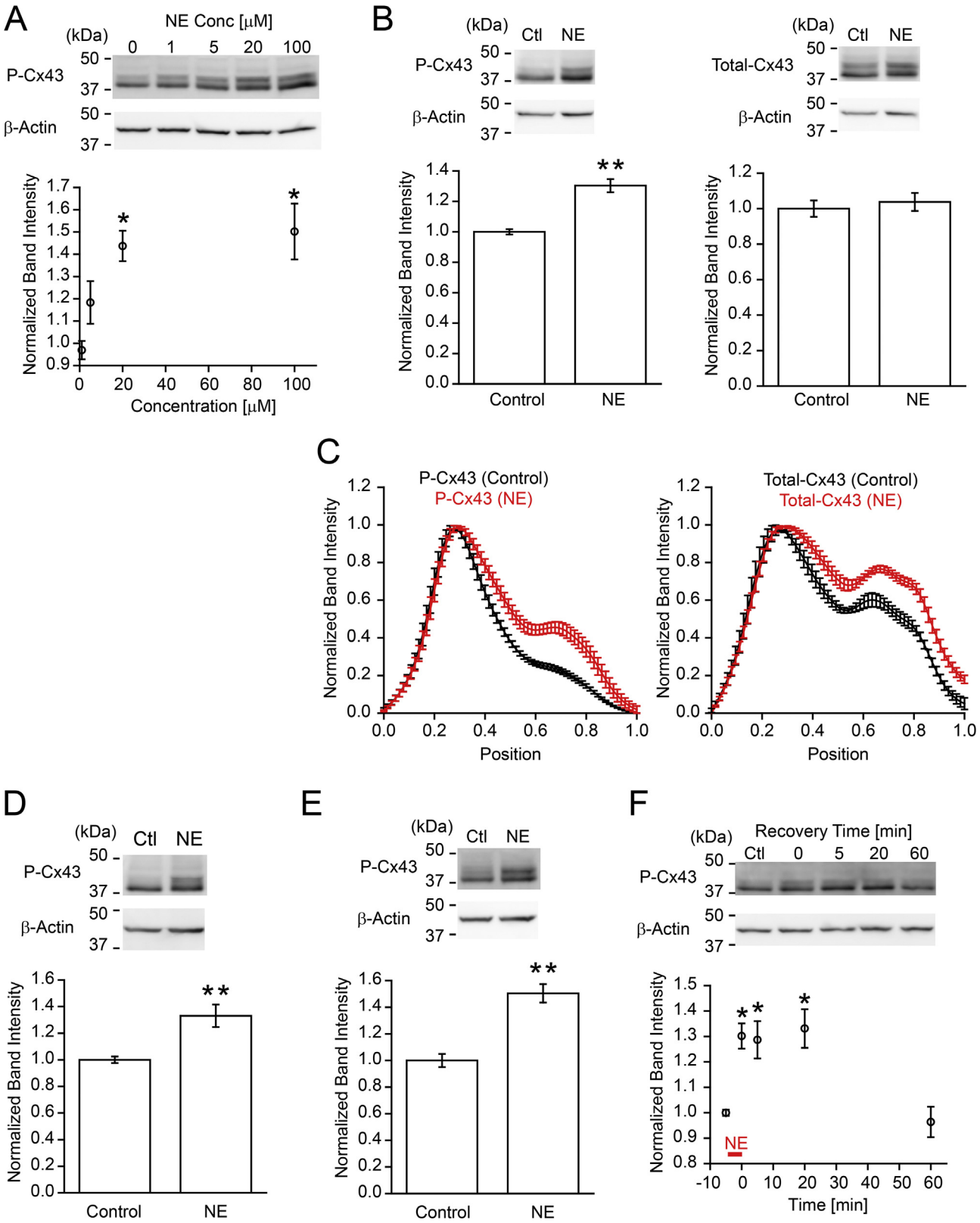

Fig. 1. NE induces phosphorylation of Cx43 at Ser368 in cortical brain slices. **(A). Dose dependency of phosphorylation**. Acutely prepared murine cortical slices were incubated with 1, 5, 20 and 100 μM of NE at 37 °C for 5 min and the brain lysates probed for Cx43 phosphorylated on Ser368. The band intensities were normalized to those of β-actin. **(B) NE induces phosphorylation of Cx43 at Ser368 without changes in total protein level**. Brain slices were treated with 20 μM NE for 5 min at 37 °C and probed for phosphorylated Cx43 (left) and total Cx43 (right). The band intensities were normalized to those of β-actin. (**C**). **Plot profile of the phospho- and total-Cx43 band**. Intensity profiles of phospho-Cx43 (left) and total Cx43 (right) were measured and plotted along the migration axis. Those under control conditions and NE-treated groups are shown in black and red, respectively. **(D). Confirmation of the phosphorylation using another antibody**. NE-induced change of the Cx43 S368 phosphorylation was confirmed by using another phospho-specific antibody from a different source. The antibody used in this panel was raised against the phosphopeptide corresponding to amino acid residues surrounding the phospho-

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