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Research paper

Reactive oxygen species mediate insulin signal transduction in mouse hypothalamus

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HIGHLIGHTS

- Insulin increased intracellular ROS in mouse hypothalamic explants.
- H_2O_2 by itself significantly increased p-IRβ and p-Akt levels.
- Insulin-induced p-IRβ and p-Akt increases were attenuated by NADPH oxidase inhibitor.
- NADPH oxidase inhibitor also attenuated insulin-induced intracellular ROS.
- Insulin-induced p-IRβ and p-Akt were mediated via ROS produced by NADPH oxidase.

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ABSTRACT

In the hypothalamus, several reports have implied that ROS mediate physiological effects of insulin. In this study, we investigated the mechanisms of insulin-induced ROS production and the effect of ROS on insulin signal transduction in mouse hypothalamic organotypic cultures. Insulin increased intracellular ROS, which were suppressed by NADPH oxidase inhibitor. H_2O_2 increased phospho-insulin receptor β (p-IRβ) and phospho-Akt (p-Akt) levels. Insulin-induced increases in p-IRβ and p-Akt levels were attenuated by ROS scavenger or NADPH oxidase inhibitor. Our data suggest that insulin-induced phosphorylation of IRβ and Akt is mediated via ROS which are predominantly produced by NADPH oxidase in mouse hypothalamus.

1. Introduction

Hydrogen peroxide (H_2O_2) and superoxide anion radicals, known as reactive oxygen species (ROS), are regarded as toxic byproducts of aerobic metabolism. The accumulation of ROS resulting from nutrient overload and mitochondrial exhaustion are suggested to play a causal role in insulin resistance in peripheral tissues [1]. Contrary to this, a growing body of evidence suggests that ROS also function as intracellular second messengers that promote signaling by hormones, including insulin [2–4].

Insulin plays a pivotal physiological role not only in peripheral tissues, but also in the central nervous system [5]. In the hypothalamus, insulin regulates energy balance by reduction of food intake [6,7], activation of sympathetic nerve outflow to the brown adipose tissue [8], or suppression of hepatic endogenous glucose production [9,10]. Recently, it was reported that insulin-stimulated mitochondrial H_2O_2 production triggers autophosphorylation of insulin receptors in cultured cerebellar granule neurons [11]. It was also reported that the increase of ROS levels within the hypothalamus decreases food intake [12]. Furthermore, centrally administered insulin reportedly increases hypothalamic ROS levels, and pharmacological suppression of ROS production by intracerebroventricular injection of NADPH oxidase inhibitor abolished the anorexigenic effect of insulin [13]. These evidences suggest that ROS are generated in response to insulin stimulation and enhance the physiological effects of insulin in the hypothalamus. However, the molecular mechanisms by which insulin produces ROS and whether or not ROS by themselves have an effect on the molecules involved in insulin signal transduction in the hypothalamus is not clear so far.

In this study, we investigated the precise molecular mechanisms of insulin-induced ROS production and the effect of ROS on insulin signal transduction in mouse hypothalamic organotypic cultures, which maintain the intrinsic properties [14,15].

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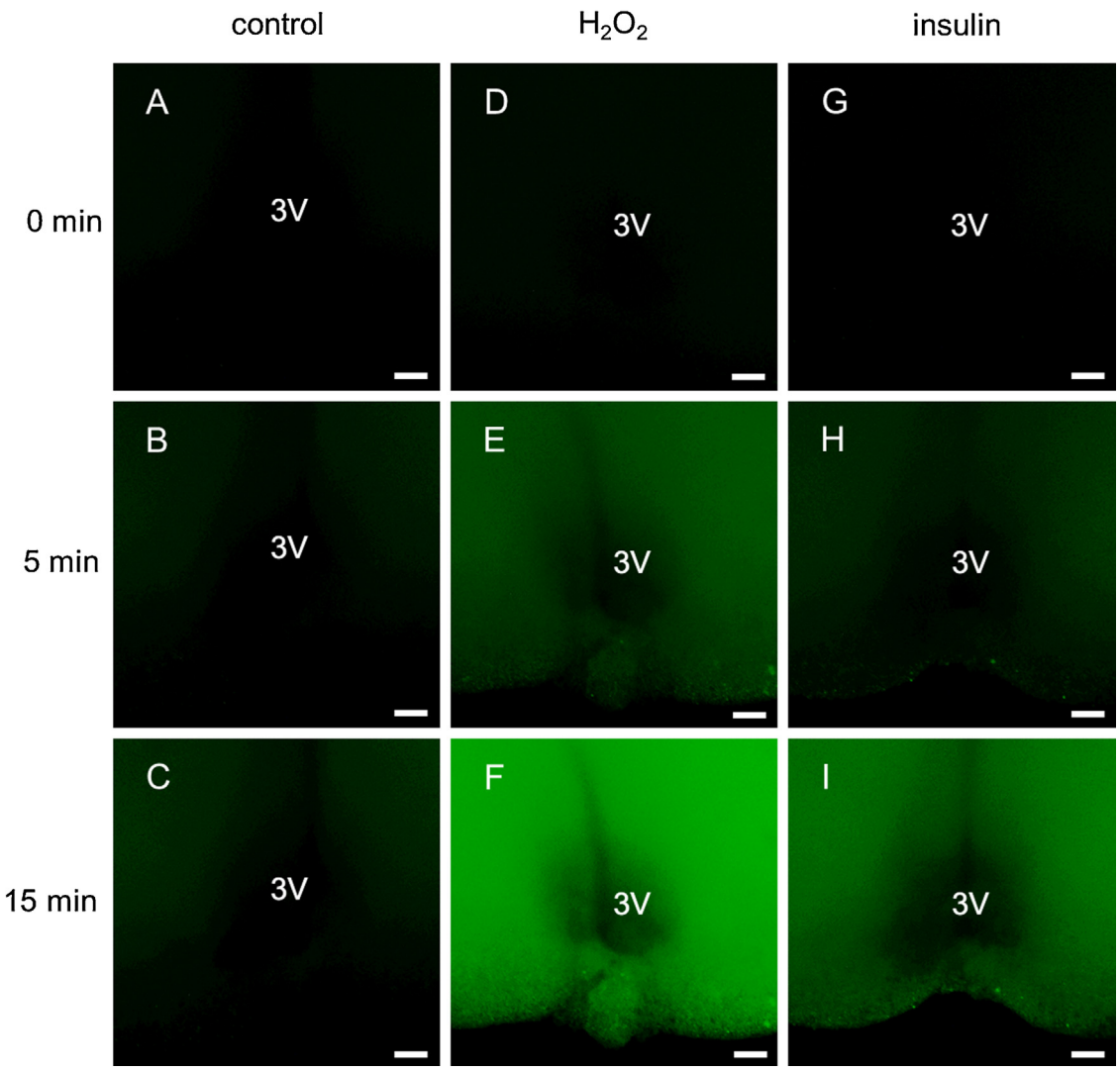

Fig. 1. Insulin-induced intracellular ROS production in hypothalamic organotypic cultures. Intracellular ROS production was assessed by fluorescence of CM-H_2DCF-DA with fluorescence microscopy. Fluorescence change in control (A–C), 10^{-4} M H_2O_2 (D–F), and 10^{-7} M insulin (G–I). 3 V, third ventricle. Scale bar 100 μm.

2. Materials and methods

2.1. Animals

All procedures were approved by the Animal Care and Use Committee of Nagoya University Graduate School of Medicine, and performed in accordance with the institutional guidelines which conform with the National Institutes of Health animal care guidelines.

2.2. Slice-explant culture procedure

Sixteen-day-old C57BL6/J mice (Chubu Science Materials, Nagoya, Japan) were sacrificed by decapitation, and hypothalamic tissue slice cultures were performed at 37 °C in 5% CO_2 enriched air under stationary conditions, as described previously [16].

2.3. Dispersed cell culture procedure

To assess intracellular ROS production, dispersed cell cultures were performed. Five-day-old C57BL6/J mice were sacrificed by decapitation, and hypothalamic cells were dissociated using a SUMITOMO Nerve-Cell culture system kit (MB-X0802, SUMITOMO Bakelite, Tokyo, Japan) according to the manufacturer's instructions. Dissociated cells were cultivated for 5 days at 37 °C in 5% CO_2 enriched air.

2.4. Intracellular ROS measurement of hypothalamic explants

Intracellular ROS production of hypothalamic explants was assessed with fluorescent probe 5,6-chloromethyl-2′,7′-dichlorodihydrofluorescein diacetate (CM-H_2DCF-DA, Life Technologies, MD, USA) as described previously [17]. Hypothalamic explants were serum-starved overnight, incubated in the dark with CM-H_2DCF-DA (10^{-5} M) for 60 min at 37 °C, and treated with insulin (10^{-7} M), H_2O_2 (10^{-4} M, Wako, Osaka, Japan) or control medium for 15 min. Fluorescence of CM-H_2DCF-DA was assessed with fluorescence microscopy (BX-51, Olympus, Tokyo, Japan) at an excitation wavelength of 488 nm and emission at 515–540 nm.

2.5. Intracellular ROS measurement of dispersed hypothalamic cells

Intracellular ROS production of the dispersed hypothalamic cells was assessed with fluorescent probe CellROX orange (Life Technologies, MD, USA) according to the manufacturer's