Mechanism of Action of the Stimulatory Effect of Kaempferol-3-neohesperidoside on $^{14}$C-Glucose Uptake on skeletal muscle

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Introduction: kaempferol-3-neohesperidoside (K-3N) exhibits insulin-like properties in terms of glucose lowering. Objectives: We investigated the mechanism of action of K-3N effect on $^{14}$C-glucose uptake on skeletal muscle. Methods: For [U-$^{14}$C]-2-deoxi-D-glucose ($[^{14}$C]DG) uptake, rat muscles (Protocol CEUA/PP007) were pre-incubated (30min) with KRb, K-3N (1.0nM), insulin (10.0nM) or inhibitors: 100 microM HNMPA(AM), 10 microM SB239063, 1 microM colchicine. After, muscles were incubated (60min) with KRb, insulin or K-3N with/without inhibitors plus $[^{14}$C]DG (0.1 microCi/ml) (37°C, pH 7.4, gassed with O$_2$:CO$_2$ (95%:5%)). The samples were processed and results expressed as nmol glycosyl/microg of protein. For GLUT4 immunoblotting, muscles were incubated (60min) with KRb, K-3N (1.0nM) or insulin (10.0nM). To total homogenate (TH), muscles were homogenized in a cold lysis solution. To plasma membrane (PM) and post-plasma membrane (PPM) fractions, muscles were homogenized in Buffer A (50mM Tris–HCl, pH 8.0, 0.1% Triton X-100, protease inhibitors cocktail), centrifuged and supernatants collected, centrifuged (16,000g, 4°C) to obtain the PPM fraction. The pellet of the centrifugation was resuspended in Buffer A plus 1% Triton X-100 followed by 1h ice bath and centrifuged (16,000g, 4°C). The supernatant was collected as the PM fraction. Proteins from TH or each fraction were separated by SDS-PAGE. Proteins were transferred onto a nitrocellulose membrane, blocked, incubated overnight (4°C) with anti-GLUT-4 (1:500). Membranes were incubated (2h) with anti-rabbit IgG (1:1000) and immunoreactive bands visualized by Immobilon™ Western chemiluminescence HRP substrate. Autoradiograms were quantified by scanning the films and determining the optical densities. Results and conclusions: The stimulatory effect of K-3N on glucose uptake was blocked by the inhibitors pretreatments. K-3N stimulated GLUT4 translocation to the PM without changes in the protein immunoccontent. The presence of insulin inhibitors showed that K-3N triggers pathways involved in the GLUT4 translocation and activation in skeletal muscle, reinforcing that it represents a primary site at which K-3N promotes glucose homeostasis.

Keywords: glucose uptake, K-3N, flavonoids, mechanism of action.
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