BIOPHYSICS AND BIOCHEMISTRY

Possible Role of the Phosphoinositide Pathway for Signal Transduction in Changes in the Sensitivity of δ-Opiate Receptors during Diabetes Mellitus

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We studied the effects of selective δ-opiate receptor agonists and antagonists on the phosphoinositide pathway in lymphocytes from healthy donors and patients with diabetes mellitus. The test compounds probably play a role in changes in the sensitivity to pharmacological substances binding to δ-opiate receptors during diabetes mellitus.

Key Words: *diabetes; phosphoinositides; opiate receptor agonists and antagonists; lymphocytes; nociception*

Recent studies revealed considerable variation in opioidergic neurotransmission during diabetes mellitus [8,10,12]. Dysfunction of the endogenous opioid system is accompanied by various changes in nociception of pharmacological substances mediated by different types of opiate receptors. During diabetes the antinociceptive effect of μ-opiate receptor agonists morphine and [d-Ala^2,N-MePhe^4,GLy-ol^5]enkephalin is less pronounced than under normal conditions. On the other hand, diabetes is accompanied by hypersensitivity to δ_1-receptor-mediated nociception [9,10]. For evaluation of biological mechanisms of these changes we studied rapid modifications of lipids in lymphocyte membranes under the effect of selective δ-opiate receptor agonist (deltorphin), δ_1-opiate receptor antagonist (7-benzylidene naltrexone), and δ_2-opiate receptor antagonist (naltriben). Published data show that these rapid modifications (5 sec) are associated with initiation of the phosphoinositide signaling pathway. They occur in various types of cells in response to the ligand-receptor interaction under the influence of physiologically active compounds (*e.g.*, agonists and antagonists of opiate receptors).

MATERIALS AND METHODS

We examined 35 patients with diabetes mellitus. Lymphocytes were isolated in a Ficoll-Verografin density gradient [7] and incubated with [1-^{14}C]arachidonic acid (Amersham, specific activity 58.3 mCi/mmol) in 5 ml Eagle medium containing 50 μmol $MgCl_2$, 12.5 μmol ATP, 1 μmol coenzyme A, and 1 μmol dithiothreitol. Labeled lymphocytes were resuspended in the same medium (5×10^6 cells/ml). Deltorphin II, 7-benzylidene naltrexone, and naltriben (Tocris) in a final concentration of 10^{-6} M were added to 0.2 ml cell suspension. Incubation was stopped after 5 sec by adding 2 ml cold chloroform-methanol mixture (1:2).

The baseline radioactivity incorporated into lipid fractions differed in healthy donors and patients with diabetes mellitus (Table 1).

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TABLE 1. Baseline Incorporation of Radioactivity in Healthy Donors and Patients with Diabetes Mellitus (cpm)

Compound	Healthy donors	Diabetes mellitus
Monoacylglycerol	151.2	230
1,2-Diacylglycerol	267	324-850
Arachidonic acid	640-853	113.6

Extraction of lipids and thin-layer chromatography were performed as described elsewhere [5]. The distribution of radioactivity between lipid fractions identified in iodine vapors was determined by thin-layer chromatography of plates on a Berthold radioscanning device. Radioactivity was quantified on a SL-4242 scintillation spectrophotometer (Roshe Bioelectronique).

RESULTS

The amount of 1,2-diacylglycerol markedly increased after 5-sec incubation with δ-opiate receptor agonist deltorphin. These results show that the phosphoinositide signaling pathway plays a role in the early receptor-mediated stage of translocation of this signal (Fig. 1, *a*). This assumption was confirmed by increased yield of arachidonyl glycerol, a product of diglyceride lipase activated after induction of phosphoinositide-specific phospholipase C. The early deltorphin-induced changes in lymphocytes from patients with diabetes mellitus included an opposite and more pronounced shift in the formation of 1,2-diacylglycerol. These results suggest that deltorphin produces different effects on the phosphoinositide signaling pathway under normal and pathological conditions. Therefore, this pathway can be considered as a membrane target for the ligand-receptor interaction.

After treatment with δ_1-opiate receptor antagonist functional activity of the phosphoinositide pathway decreased in lymphocytes from healthy donors, but slightly increased in patients with diabetes mellitus. It was confirmed by increased release of 1,2-diacylglycerol (Fig. 1, *b*). The δ_2-opiate receptor antagonist markedly increased the content of 1,2-diacylglycerol in healthy donors, but decreased it in patients with diabetes mellitus. (Fig. 1, *c*). It should be emphasized that each of these three compounds decreased the release of arachidonic acid. However, the degree of these changes was different. It was probably related to rapid utilization of arachidonic acid due to the formation of eicosanoids. Some eicosanoids are involved in modulation of pain and nociception. Initiation of the phosphoinositide signaling pathway results in the release of not only 1,2-diacylglycerol activating protein kinases, but also inositol-1,4,5-triphosphate. This process is followed by mobilization of intracellular Ca^{2+}. Our results are consistent with published data that modification of nociception during diabetes is associated with changes in intracellular Ca^{2+} concentration.

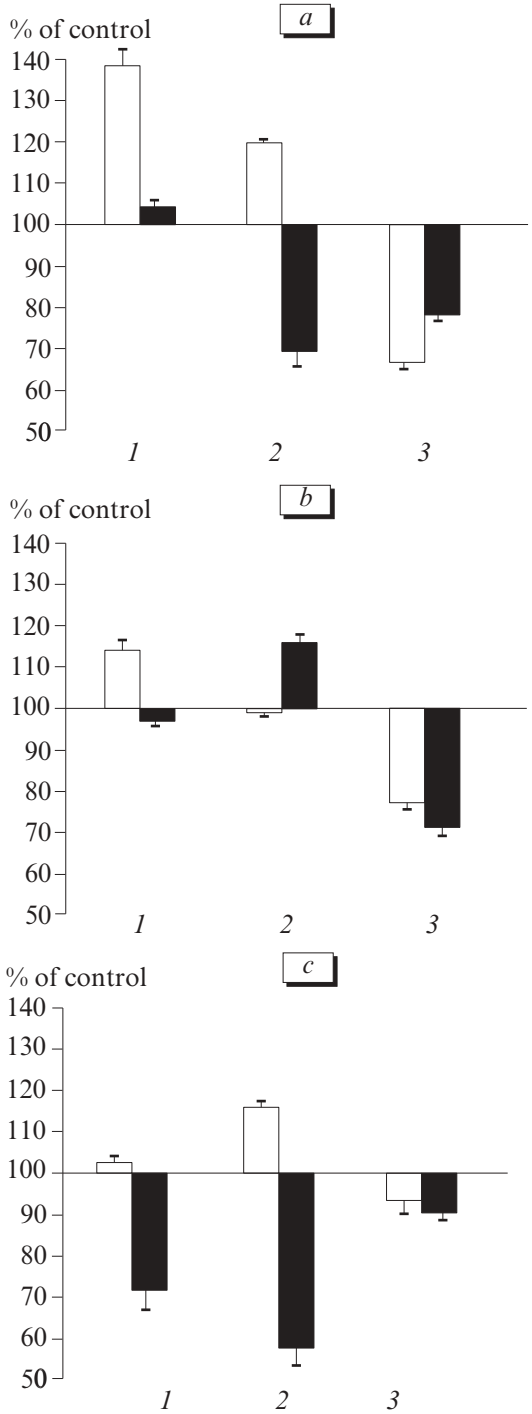

Fig. 1. Modifications of lipids in peripheral blood lymphocytes from healthy donors (light bars) and patients with diabetes mellitus (dark bars) 5 sec after treatment with deltorphin (*a*), 7-benzylidene naltrexone (*b*), and naltriben (*c*): Monoacylglycerol (*1*), diacylglycerol (*2*), and arachidonic acid (*3*). Ordinate: lipid fractions.

Our findings suggest that the phosphoinositide signaling pathway is involved in biological mechanisms underlying the effect of the test compounds. In patients with diabetes mellitus the phosphoinositide pathway was suppressed during the early stage of receptor-mediated signal translocation from selective agonists and antagonists. Therefore, this pathway plays a role in modification of nociception mediated by the δ-opiate receptor system in patients with diabetes mellitus.

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