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Effect of the Opioid Agonist Fenaridin and its Antagonist on the Phosphoinositide Catabolism in Brain Synaptosomes of White Rats *In Vitro*

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The release of 1,2-diacylglycerol and arachidonic acid increases considerably by the 10th sec of incubation of arachidonate-labeled synaptosomes with the opioid antagonist fenetham. The opioid agonist fenaridin elicits an opposite effect.

Key Words: *fenaridin; fenetham; phosphoinositides*

Initiation of the phosphoinositide turnover, a membrane-coupled cascade transducing external signal via the second messenger pathway, is a universal mechanism involved in cell response to hormones, transmitters, and physiologically active compounds [4,5].

Signal transfer to phosphoinositide-specific phospholipase C or phosphoinositide-phosphodiesterase is a key event in the cascade of molecular events initiated by the ligand-receptor interaction. These enzymes cleave phosphoinositide to form the second messengers inositol triphosphate (IP_3) and 1,2-diacylglycerol (1,2-DG) that induce the release of intracellular Ca^{2+} and activation of protein kinase C, respectively [14].

The role of phosphoinositide turnover in the interactions of opioid agonists and antagonists with specific receptors is poorly investigated [13]. Inositol triphosphate may be involved in the release of endogenous opioid peptides [15], while protein kinase C may inhibit the analgesic effect of morphine [1].

Fenaridin (a compound belonging to 4-aminopiperidines) is a potent narcotic analgesic [1]. Its activity is higher than that of morphine, promedol, and fentanyl [2]. Fenetham, a compound with a similar structure, is a "pure" opioid antagonist of the naloxone type [2].

MATERIALS AND METHODS

Experiments were carried out on random-bred male rats weighing 150-180 g. Synaptosomes were isolated as described elsewhere [9]. Incorporation of ^{14}C-arachidonic acid in phosphoinositides was assessed by measuring the radioactivity of the monophosphoinositide fraction [12]. Synaptosomes (6-8 mg protein) were incubated for 45 min at 37°C in a mixture containing 80 mM arachidonic acid, 1 μCi

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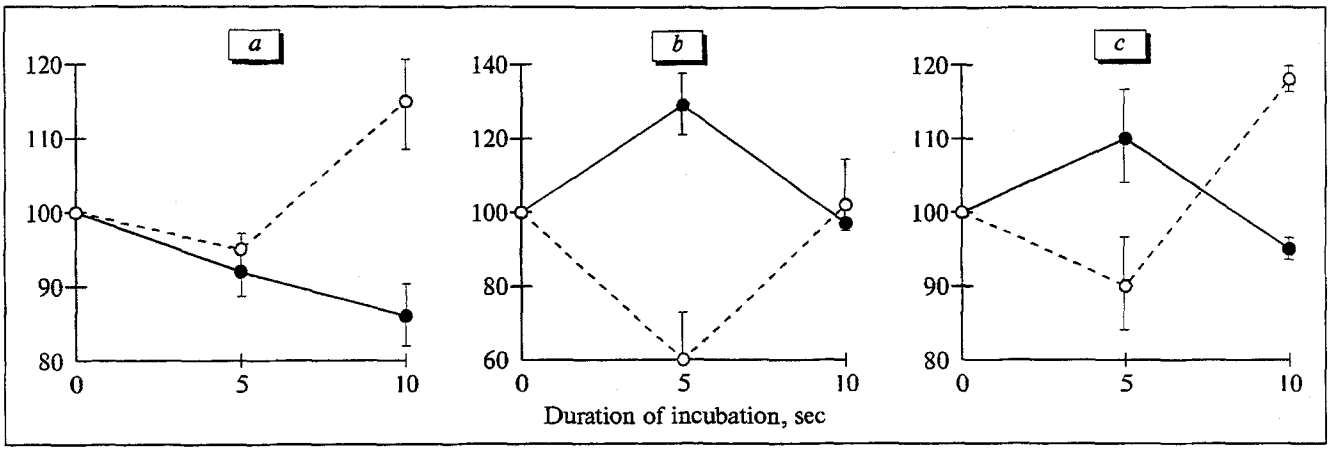

Fig. 1. Effect of fenaridin (solid line) and fenetham (broken line) on the release of 1,2-diacylglycerol (*a*), triglycerides (*b*), and arachidonic acid (*c*). Ordinate: changes in radioactivity, percent of the baseline value. The baseline value of incorporation of radiolabeled arachidonic acid in the studied lipid fractions is assumed as 100%.

^{14}C-arachidonic acid (56 μCi/mmol, Amersham), 30 nm lysophosphatidylinositol (Sigma), 50 μM $MgCl_2$, 12.5 μM ATP, 10 μM coenzyme A, and 1.0 μM dithiothreitol.

Phosphoinositide turnover was investigated in a mixture containing labeled synaptosomes (150-200 μg protein), 2.5 mM $CaCl_2$, 0.32 M sucrose in 50 mM Tris-HCl buffer, and 0.1 ml studied compounds (a final concentration of 10^{-5} M). Incubation was carried out at 37°C. Hydrolysis products were extracted after 5 and 10 min and separated by linear thin-layer chromatography on silica gel plates (Estonia) using a petroleum ether:diethyl ether:formic acid (30:20:2) system [3]. Radioactivity was measured in Bray's scintillation cocktail with a Roche-Bioelectronique SL-4221 scintillation spectrometer. Protein content was determined as described elsewhere [11]. The results were statistically processed.

RESULTS

Noticeable shifts in the release of 1,2-DG occurred after 5 sec of incubation with fenaridin and fenetham; by the 10th sec it was clear that these agents produce opposite effects (Fig. 1). Such rapid and pronounced changes in the release of 1,2-DG may result from a specific receptor-mediated effect of fenaridin and fenetham on phosphoinositide-phosphodiesterase [6].

Fenetham markedly enhances the release of 1,2-DG, which is consistent with the inhibitory effect of protein kinase C on opiate-mediated analgesia [10].

By the 10th sec of incubation, it was clear that fenaridin and fenetham produce different effects on the release of arachidonic acid. This may be due to opposite effects of these compounds on the phosphoinositide deacylation enzymes (and probably diglyceride lipase activity) and on the enzymes participating in the biosynthesis of eicosanoids, which are known to provoke hyperalgesia [7,8]. This assumption is consistent with pharmacological effects of fenaridin and fenetham.

Changes in the content of arachidonyl-triglycerides (intermediate "depots" of arachidonic acid) coincide with the dynamics of the deacylation reactions.

Our findings indicate that the opioid agonist fenaridin and the opioid antagonist fenetham modulate the receptor-mediated phosphoinositide turnover at the initial stage of signal transduction.

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