

PRELIMINARY COMMUNICATION

INHIBITION OF ARACHIDONIC ACID CASCADE ATTENUATES THE INDUCTION OF c-FOS PROTEINS BY DOI, 5-HT_{2A/2C} RECEPTOR AGONIST, IN THE RAT CORTEX

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Inhibition of arachidonic acid cascade attenuates the induction of c-Fos proteins by DOI, 5-HT_{2A/2C} receptor agonist, in the rat cortex. M. MAĆKOWIAK, A. CZYRAK, K. WĘDZONY. *Pol. J. Pharmacol.*, 2002, 54, 73–76.

Previous immunohistochemical studies have shown that c-Fos proteins induced by DOI, a 5-HT_{2A/2C} agonist, are present in the population of cortical neurons, which are devoid of 5-HT_{2A} receptors. A mechanism of the induction of c-Fos proteins expression by DOI is still unclear. However, the involvement of the 5-HT_{2A} and AMPA, but not 5-HT_{2C} receptors in this process has been reported. In the present study, we investigated whether arachidonic acid, a retrograde messenger, is involved in the above mechanism of c-Fos induction. Phospholipase A₂ pathway, which leads to the subsequent generation of arachidonic acid and its metabolites, is known to be coupled to 5-HT_{2A} receptor activation. The inhibition of arachidonic acid cascade both at the level of phospholipase A₂ (by dexamethasone, 1.5 mg/kg) or at the level of cyclooxygenases that catalyze arachidonic acid biotransformation (by indomethacin, 3 mg/kg), decreased the number of c-Fos immunopositive cells after induction by DOI (8 mg/kg). Our results suggest that arachidonic acid cascade may be involved in the induction of c-Fos proteins by DOI in the rat parietal cortex.

Key words: DOI, c-Fos proteins, phospholipase A₂, arachidonic acid, dexamethasone, indomethacin

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The hallucinogen 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI), a 5-HT_{2A/2C} agonist, induces the expression of c-Fos proteins in the cerebral cortex. Immunohistochemical studies have shown that c-Fos proteins induced by DOI, in the parietal [6] or somatosensory cortex [8], are not present in 5-HT_{2A}-immunopositive neurons. 5-HT_{2A} receptor protein has been observed in cell bodies and apical dendrites of pyramidal neurons in layer V, whereas c-Fos positive neurons were seen mainly in cortical layer IV and upper part of layer V [6, 8]. The study examining the mechanism through which DOI activates cortical neurons, as reflected by c-Fos proteins induction, showed that DOI acts through 5-HT_{2A} but not 5-HT_{2C} receptor [8]. The available data also suggest that DOI activates 5-HT_{2A} receptors on thalamocortical glutamatergic neurons, thereby increasing glutamate release, which in consequence induces c-Fos expression in cortical neurons *via* AMPA receptors activation [8]. However, electrophysiological studies suggested also the possibility (at least theoretical) of asynchronous glutamate release in the cortex *via* a retrograde messenger released in response to 5-HT_{2A} receptor activation [7]. One of such retrograde messengers, arachidonic acid, is known to be coupled to 5-HT_{2A} receptors activation *via* the phospholipase A₂ (PLA₂) pathway [2]. Moreover, arachidonic acid cascade is known to be involved in the modulation of glutamate release, and can also activate the expression of c-Fos gene [3]. Since the mechanism of c-Fos proteins induction by DOI is still unclear, we decided to investigate whether the retrograde messenger, such as arachidonic acid is involved in the above mechanism. PLA₂ is the enzyme that catalyzes phospholipid degradation and formation of arachidonic acid. Arachidonic acid itself is transformed to biologically active metabolites by multiple enzymes, including cyclooxygenases (COX-1, COX-2) and lipoxygenases [9]. We used pharmacological tools, such as dexamethasone (PLA₂ inhibitor) [9] or indomethacin (COX-1, COX-2 inhibitor) [9] to block arachidonic acid cascade, and examined the level of DOI-induced expression of c-Fos proteins in the rat parietal cortex, using immunohistochemical method. DOI-induced expression of c-Fos proteins has been used as a marker of 5-HT_{2A} activation.

All the experiments were carried out on male Wistar rats (200–250 g). The experimental protocols were approved by the Committee for Labora-

tory Animal Welfare and Ethics of the Institute of Pharmacology, Polish Academy of Sciences in Kraków, and met the requirements of the European Council Guide for the Care and Use of Laboratory Animals (86/609/EEC). The rats were injected with dexamethasone (Sigma, USA; 1.5 mg/kg *sc*), indomethacin (Sigma, USA; 3 mg/kg *ip*) or respective vehicles 30 min before DOI (Sigma/RBI, USA; 8 mg/kg *ip*) or saline. Dexamethasone was suspended in 1% Tween 80 by Polytron homogenization. Indomethacin was dissolved in a minimum volume of 0.2 M Na₂CO₃ buffered with 0.1 M HCl, and then diluted with saline. DOI was dissolved in saline. Control (vehicle-treated animals) and all drug-treated animals were deeply anesthetized (3 h after DOI or saline treatment) with sodium pentobarbital (100 mg/kg) and were transcardially perfused with 0.9% NaCl followed by 4% paraformaldehyde in 0.1 M phosphate-buffered saline (PBS). Immunocytochemical visualization of c-Fos proteins has been described previously [6]. Briefly, free floating, 50 µm thick vibratome sections were incubated for 10 min in PBS containing 0.3% hydrogen peroxide, rinsed and incubated for 1 h in a blocking buffer (5% normal rabbit serum and 0.1% Triton X-100 in 0.01 M PBS). Then the sections were incubated (48 h at 4°C) with primary polyclonal sheep antibody (Sigma-Genosys, UK) against residues 2–16 of the N-terminal region of c-Fos proteins (dilution of 1:2000 in 1% normal rabbit serum and 1% Triton X-100 in 0.01 M PBS). The reaction was visualized with biotinylated anti-sheep IgG antibody (Vector Lab, USA) and the avidin-biotin horseradish peroxidase complex (Vectastain Elite ABC Kit, Vector Lab, USA), which produced brown color. Digital images were captured using Spot II digital camera (Diagnostic Instruments, USA) attached to a Nikon Optiphot II microscope and Image Pro Plus programme (Media Cybernetics, USA).

It was observed that DOI (8 mg/kg) induced marked appearance of c-Fos proteins in the rat parietal cortex (Fig. 1 and 2). c-Fos-immunopositive cells were present throughout the cortex with main location in layers IV/V (Fig. 1). The observed distribution of c-Fos proteins in the rat cortex was similar to that noticed in our previous study [6] and observed by other authors [8]. Acute treatment of rats with dexamethasone (1.5 mg/kg) significantly decreased the number of DOI-induced c-Fos-immunopositive neurons (by about 50%) in the parietal

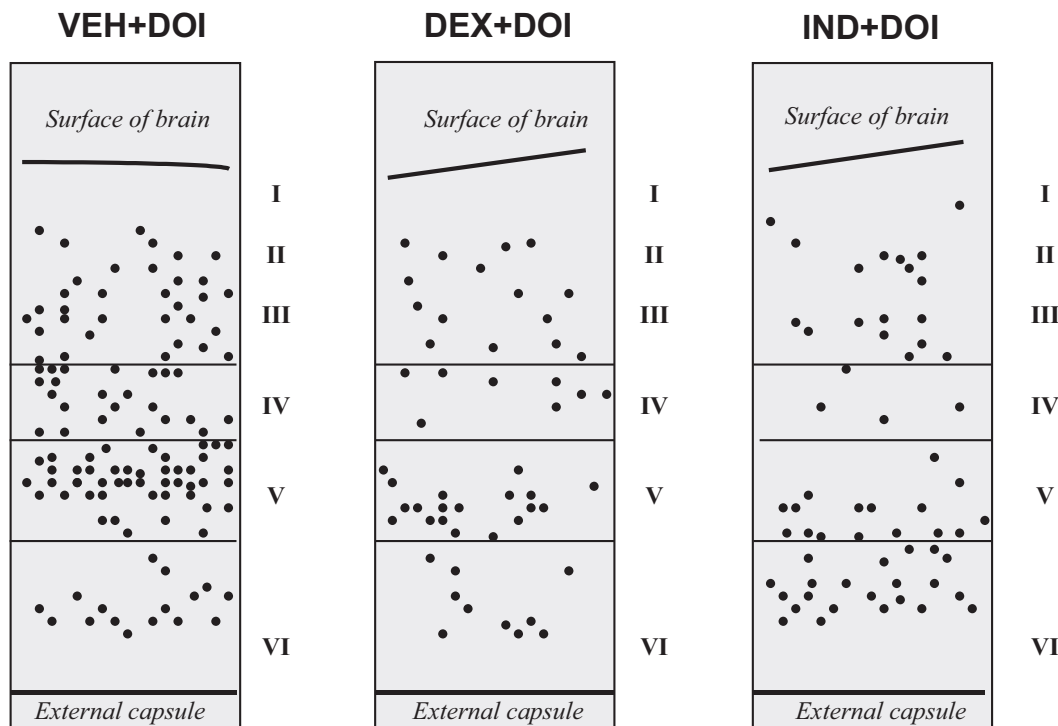


Fig. 1. Maps of c-Fos-positive nuclei (circles) in the parietal cortex cross-section taken from digital images. (VEH + DOI): the expression of c-Fos proteins induced by DOI (8 mg/kg). (DEX + DOI): the effect of dexamethasone (DEX) 1.5 mg/kg on DOI-induced expression of c-Fos proteins. (IND + DOI): the effect of indomethacin (IND) 3 mg/kg on DOI-induced expression of c-Fos proteins

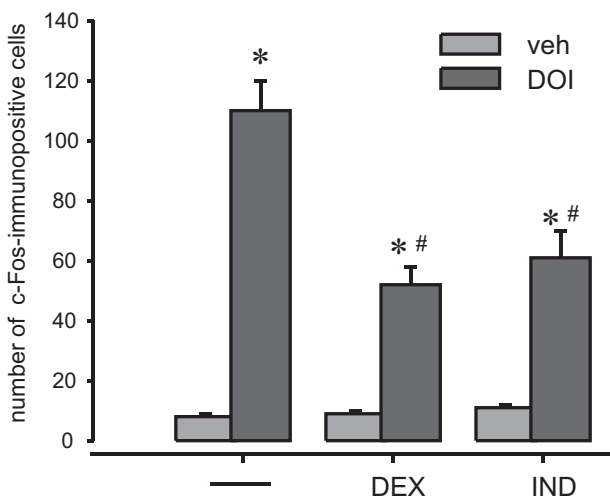


Fig. 2. The effects of dexamethasone (DEX) 1.5 mg/kg and indomethacin (IND) 3 mg/kg on the number of c-Fos-immunopositive cells induced by DOI (8 mg/kg) in the parietal cortex. The number of c-Fos positive nuclei has been counted in the equal cross-sections of the parietal cortex. * $p < 0.05$ vs. the control (vehicle-treated animals), # $p < 0.05$ vs. DOI-treated animals; one-way analysis of variance (ANOVA) followed by the Dunnett's test, $n = 5$

cortex (Fig. 1 and 2). The strongest effect of dexamethasone was observed in layers IV/V, where the induction of c-Fos was the highest. Similarly as in the case of dexamethasone, single injection of indomethacin (3 mg/kg) also attenuated DOI-induced c-Fos proteins expression (by approximately 50%) in the rat cortex (Fig. 1 and 2). Both dexamethasone and indomethacin given alone did not affect c-Fos proteins expression in the parietal cortex of the control (vehicle-treated animals) (Fig. 2).

The present study demonstrates that inhibition of the arachidonic acid cascade by dexamethasone and indomethacin attenuated the induction of c-Fos proteins by DOI. Dexamethasone, the synthetic glucocorticoid, blocks arachidonic acid cascade either by inducing synthesis of annexins PLA_2 inhibiting compounds, or by direct inhibition of PLA_2 synthesis [9]. It has also been found that glucocorticoids can inhibit COX-2 expression by binding with their receptors acting as transcription factors and affecting the binding activity of other crucial transcription factors, e.g. AP-1 [1]. Indomethacin inhibits activity of cyclooxygenases, both COX-1

and COX-2 [1, 9], i.e. the enzymes responsible for arachidonic acid metabolism. The ability of both PLA₂ and cyclooxygenase inhibitors to decrease DOI-induced c-Fos expression indicates that not only arachidonic acid, but also its active metabolites associated with cyclooxygenases, such as prostaglandins and thromboxanes, may be involved in the mechanism of DOI-induced c-Fos proteins appearance. This suggestion is in line with the observation that, at least in *in vitro* studies, cyclooxygenase metabolic pathway of arachidonic acid but not lipoxygenase pathway, is required for the activation of c-Fos transcription by NMDA receptors [3, 4]. However, the involvement of NMDA receptors in the process of DOI-induced c-Fos expression in the rat cortex is unclear. On the one hand, immunohistochemical study showed that the inhibition of AMPA/KA receptors blocked the expression of c-Fos induced by DOI treatment [8]. On the other hand, it has also been reported that the inhibitors of PLA₂ and cyclooxygenases abolished NMDA- but not kainic acid-induced increases in c-fos mRNA in the dentate gyrus cells [5]. Because of the lack of data demonstrating the regulation of AMPA receptors by arachidonic acid cascade in the rat cortex, it is difficult to speculate, if DOI-induced c-Fos expression can be linked with AMPA receptor activation by arachidonic acid.

In conclusion, arachidonic acid cascade is clearly engaged in the mechanism of DOI-induced c-Fos proteins expression in the rat parietal cortex. However, since both dexamethasone and indomethacin did not prevent DOI-induced c-Fos expression but only decreased the number of c-Fos-immunopositive neurons by approximately 50%, it may be hypothesized that more than one mechanism is involved in the DOI-induced c-Fos proteins appearance.

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