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Short Communication

Inhibition of cytochrome P450 by proadifen diminishes the excitability of brain serotonin neurons in rats

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Abstract. The aim of this study was to investigate the effect an inhibitor of cytochrome-P450, proadifen hydrochloride (SKF525), on the excitability of serotonin neurons. Adult male Wistar rats were administered SKF525 forty-eight, twenty-four, and one hour before electrophysiological assessments. Control animals were injected saline. Rats were anesthetized with chloral hydrate and glass electrodes were stereotaxically inserted into the dorsal raphe nucleus (DRN). Serotonin neurons were identified and their firing activity was recorded. It was found that the SKF525 inhibits the excitability of 5-HT neurons. We suggest that corticosterone might play a key role in the SKF525-induced inhibition of 5-HT neurons.

Key words: Serotonin — Dorsal raphe nucleus — *in vivo* electrophysiology — Proadifen hydrochloride — Cytochrome P450 — Corticosterone

Cytochrome-P450 (CYP) is a superfamily of microsomal and mitochondrial enzymes which catalyze oxidation of various endogenous and exogenous biological molecules, such as steroid hormones, arachidonic and fatty acids, catecholamines, lipid-soluble vitamins, various medications including antidepressant drugs, and carcinogens (Rendic 2002; Munro et al. 2018). CYP irreversibly metabolizes corticosterone into 6β-corticosterone in rodents and cortisol into 6β-cortisol in humans (Peng et al. 2011).

Brain serotonin (5-HT) system consists of 5-HT-secreting neurons, located in several brain nuclei, such as rostral, median, and dorsal raphe nucleus (DRN). The axons of these neurons innervate various areas of the central nervous system. The 5-HT neurons of the DRN densely innervate the limbic areas of the brain and play a key role in depression, anxiety, and in response to antidepressant drugs (Pavlovicova et al. 2015).

Brain 5-HT neurotransmission regulates hepatic CYP activity, and *vice versa*. The selective lesion of 5-HT neurons or inhibition of 5-HT synthesis led to a robust activation of the hepatic CYP (Kot and Daniel 2011). An injection of a 5-HT precursor 5-hydroxytryptophan into the lateral cerebral ventriculi increased brain 5-HT concentrations and diminished the activity of CYP in the liver (Rysz et al. 2016). It was found that rats with higher hepatic CYP activity had also higher brain monoamine oxidase A (MAO-A, an enzyme metabolizing the 5-HT) activity and reduced 5-HT levels in the plasma (Tseilikman et al. 2016). Finally, CYP inhibitor proadifen hydrochloride (SKF525) was reported to reduce MAO-A activity (Kozochkin et al. 2016). Since CYP is inhibited by literally all antidepressant drugs (Nassan et al. 2016; Ornoy and Koren 2018), and since brain 5-HT system is one of their primary targets of therapeutic action, interaction between CYP inhibition and excitability of brain 5-HT neuron is of special interest. The aim of the present study was to investigate the effect of the CYP inhibition by SKF525 on the excitability of 5-HT neurons of the DRN, using *in vivo* electrophysiology.

Adult male Wistar rats (200–250 g) were ordered from the Breading Facility of the Institute of Experimental Pharmacology and Toxicology, Centre for Experimental Medicine, Slovak Academy of Sciences (Dobrá voda, Slovakia) and housed in a temperature-controlled room (22–24°C) with a 12:12 hours light-dark cycle, and had *ad libitum* access to

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food and water. All experimental procedures were approved by the Animal Health and Animal Welfare Division of the State Veterinary and Food Administration of the Slovak Republic (Permit number Ro 3054/17-221/3) and conformed to the Directive 2010/63/EU of the European Parliament and of the Council on the Protection of Animals Used for Scientific Purposes. Rats were allowed to acclimatize for one week after their arrival in our animal facility. SKF525 was ordered from Abcam (Cambridge, UK) and dissolved in saline. To achieve the steady-state inhibition of the CYP, the rats received three intraperitoneal (i.p.) injections of SKF525 (25 mg/kg): forty-eight, twenty-four, and one hour before electrophysiological assessments. Control animals were injected saline using the same protocol.

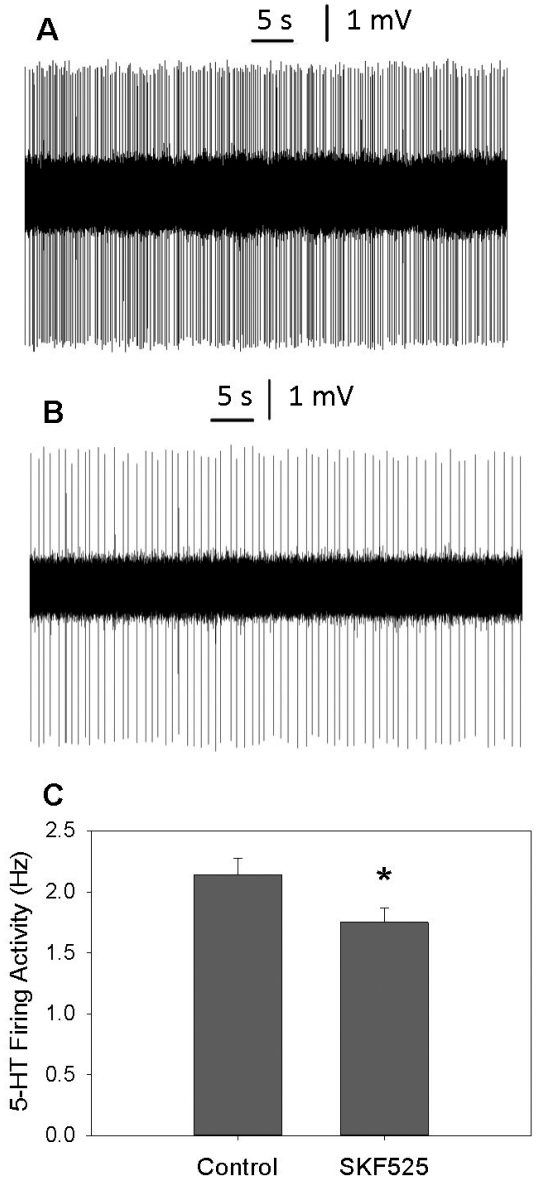

Figure 1. Effect of SKF525 on the excitability of 5-HT neurons. **A.** Representative recording from a 5-HT neuron from the DRN of a control rat. **B.** Representative recording from a 5-HT neuron from the DRN of an SKF525-adminstered animal. **C.** Summary effect calculated from 97 neurons from eight control rats and 119 neurons from seven SKF525-adminstered rats. * $p < 0.05$, two-tailed Student's *t*-test.

One hour after the last saline or SKF525 injection, rats were anesthetized with chloral hydrate (Sigma-Aldrich, 0.4 g/kg, i.p.) and mounted into the stereotaxic frame (David Kopf Instruments, Tujunga, CA). Rat body temperature was maintained at 37°C with a heating pad (Gaymor Instruments, Orchard Park, NY, USA). The scalp was opened and a 3 mm hole was drilled in the skull for insertion of electrodes. Glass-pipettes were pulled with a DMZ-Universal Puller (Zeitz-Instruments GmbH, Martinsried, Germany) to a fine tip approximately 1 μm in diameter and filled with 2 M NaCl solution. Electrode impedance ranged from 7 to 8 MΩ. The pipettes were lowered into the DRN, 7.8–8.3 mm posterior to bregma and 4.5–7.0 mm ventral to brain surface (Paxinos and Watson 2014), by a hydraulic micro-positioner (David Kopf Instruments, Tujunga, CA). Serotonin neurons were identified by their regular, low-frequency (less than 5 Hz) firing rate and positive bi- or tri-phasic action potential of the total duration of 2.0–5.0 ms and cumulative duration of depolarization and repolarization phases of 0.8–1.2 ms, as described in the previous studies (Aghajanian and Vandermaelen 1982; Dremencov et al. 2017) and recorded for at least two minutes using the Power Lab data acquisition system and Lab Chart software (AD Instruments, Dunedin, New Zealand).

We found a significant ($p = 0.03$, two-tailed Student's *t*-test) 18%-decrease in 5-HT neuronal firing activity in SKF525-administered rats (1.75 ± 0.12 Hz, 119 cells from 7 rats) in comparison to controls (2.14 ± 0.14 Hz, 97 neurons from 8 rats; Fig. 1). The mean number of the spontaneously active 5-HT neurons *per* electrode track was not statistically different between the groups (SKF525: 5.67 ± 0.95; control: 3.69 ± 0.57; $p = 0.08$, two-tailed Student's *t*-test).

As a potent CYP inhibitor, SKF525 was previously reported to increase the plasma levels of corticosterone in rats (Magus et al. 1968). On the other side, corticosterone inhibits the excitatory glutamatergic input to 5-HT neurons of the DRN (Wang et al. 2012). It is therefore possible that corticosterone mediates, at least in part, the inhibitory effect of SKF525 on brain 5-HT neurons.

It was previously reported that the suppression of 5-HT neurons by intra-DRN injection of γ-aminobutyric acid (GABA) induced depression-like behavior in mice (Xiao et al. 2017). It is possible that the partial inhibition of 5-HT neurons by SKF525 have a depressogenic effect as well. It was indeed reported that SKF525 reversed the antidepressant-like behavioral effect of imipramine and desipramine in rats (Maj et al. 1981).

Brain 5-HT system is a target of literally all antidepressant drugs and liver CYP is their major metabolizer. As CYP substrates, antidepressants inhibit CYP activity (Nassan et al. 2016; Ornoy and Koren 2018). Since CYP suppression attenuates 5-HT neurotransmission, the inhibition of this enzyme by antidepressants may interfere with their primary therapeutic effect.

The main limitations of this study are the using of a non-selective CYP inhibitor and non-distinguishing between brain and hepatic CYP inhibition. In the future studies, the effect of the selective inhibitors of the specific CYP subtypes, such as CYP3A1, CYP3A2, CYP3A4 and CYP3A5, which are fundamental in glucocorticoid metabolism (Peng et al. 2011), should be tested.

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