Samardžić *et al.: Intrinsic Activity*, 2015; **3**(Suppl. 2):A2.9
http://www.intrinsicactivity.org/2015/3/S2/A2.9
published online: 9 September 2015

21st Scientific Symposium of the Austrian Pharmacological Society: Joint Meeting with the British Pharmacological Society and the Pharmacological Societies of Croatia, Serbia and Slovenia

Graz, 16–18 September 2015

MEETING ABSTRACT

A2.9

In vitro effects of ethanol and gabapentin treatment

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Background: The anticonvulsant drug gabapentin, a structural analogue of GABA, showed beneficial effects in the treatment of alcoholism and its consequences. Although the mechanisms of action of gabapentin are not fully understood, studies suggested that gabapentin's neuroprotective effects could be achieved via $GABA_A$ receptors. The aim of this study was to investigate the potential protective action of gabapentin on the well-known neurotoxic effects of chronic alcohol consumption and withdrawal.

Methods: The dose–response relationship and the time course of ethanol and gabapentin treatment were established on human embryonic kidney (HEK) 293 cells, either non-transfected or stably expressing α1β2γ2S $GABA_A$ receptors. A trypan blue exclusion assay and MTT test were performed to assess cell viability. Membrane preparations of stably transfected HEK 293 cells treated with 100 mM ethanol in combination with 1 µM gabapentin for 96 h were used in [^{3}H]flunitrazepam and [^{3}H]TBOB binding studies to determine the number and affinity of benzodiazepine and convulsant binding sites, and their allosteric interactions with GABA binding sites. 100 µM bicuculline, a $GABA_A$ receptor antagonist, was used in other to counteract the effects of gabapentin. The levels of mRNA encoding the α1, β2 and γ2S receptor subunits in stably transfected HEK 293 cells following ethanol and gabapentin treatment were determined by semiquantitative RT-PCR analysis.

Results: Treatment with ethanol at concentrations higher than 100 mM for 96 h reduced the number of HEK 293 cells, non-transfected or transfected with α1β2γ2S $GABA_A$ receptors. The cytotoxic effect of ethanol was counteracted by co-administration of 1 µM gabapentin in transfected, but not in non-transfected cells. Exposure to 100 mM ethanol induced an increase in the number of binding sites for benzodiazepines and convulsants, while this effect was reversed by simultaneous exposure to 1 µM gabapentin, 100 µM bicuculline or their combination. Administration of ethanol, as well as bicuculline, induced functional allosteric uncoupling of the GABA and the benzodiazepine binding site, which was prevented by simultaneous gabapentin treatment. The mRNA expression levels of α1, β2 and γ2S $GABA_A$ receptor subunits were not influenced by the exposure to either ethanol or gabapentin, but only by their co-administration.

Discussion: Although our results support the hypothesis of an involvement of $GABA_A$ receptors in the actions of gabapentin, further research is needed to elucidate the mechanisms of gabapentin's protective effects against ethanol-induced cytotoxicity.

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