GSM Phones and Central Nervous System in Rats: Quantification and Localisation of Neurotransmitters with Immunohistochemistry and Image Analysis

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Mobile phones could affect the brain by changing neurotransmitter metabolism and/or concentration. Measurement of changes in neurotransmitter (NT) concentrations in precisely-defined brain areas has been a challenge for many years. To achieve this goal, we developed and improved a protocol of image analysis coupled with immunodetection. This method allows to semi-quantify variations of NT quantities and can give useful information about cellular localisation of NT concentration changes. We applied this method to the GABAergic system. Gamma-Vinyl-GABA (GVG), an inhibitor of GABA-Transaminase, which is known to increase GABA concentration in central nervous system (CNS), was injected in rats (1, 100 and 1200 mg/kg). Rats were anaesthetised and perfused with a fixative solution. Cerebella were dissected, cut in 50 micrometers sagittal slices and processed for anti-GABA-immunohistochemistry (IHC), using the diaminobenzidine system. Parameters directly linked to the GABA concentration were quantified : Optical Density (O.D.), area, and number of immunoreactive stained cells in the slice. Image analysis was improved to perform differential analysis of defined cell layers (molecular, Purkinje and granular) and to measure the percentage of cellular-staining areas in each layer. As a positive control, we showed that GVG at 1200 mg/kg provoked a raise in number, O.D. and area of stained cells (up to 150% above control values), mainly in molecular and granular cell layers. It is therefore possible to detect spatially localised changes in NT concentrations thanks to IHC coupled with image analysis. We used this approach to study the effects of microwaves emitted by mobile telephones on GABA concentration in the cerebellum. Rats were exposed to 2 different 900 MHz radiofrequencies (RF) emitted either on a continuous mode (CW) inducing a specific absorption rate (SAR) of 32 W/kg, or with 576µs impulses at a repetition rate of 217 Hz (PW) inducing a SAR of 4 W/kg. After exposure to PW (SAR 4 W/kg) for 2 hours, a slight 16% decrease of the area of processes in the Purkinje cell layer was observed ($p=0.008$). Rats were then exposed to higher SAR (32 W/kg, CW) and, as they did not show any trouble, the exposure was also performed for 2hours. We observed a significant decrease of O.D. (about 30%) in the 3 cerebellar layers ($p<0.001$), together with a decrease of the area of stained processes in the Purkinje cell layer similar to the one observed after exposure to RF 4 W/kg.

CONCLUSION

Immunohistochemistry seems to be suitable to investigate the effects of various factors on NT content in the CNS with a good cellular resolution. Indeed, we were able to show that RF exposure induced a decrease of the cerebellar GABA content. This effect clearly depended upon the type and the power of RF emitted. Further investigations with complementary techniques are needed to elucidate the mechanisms of RF interaction with NT and their receptors.

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