Interactions of the neuropeptide Galanin with cortical spreading depolarization and cortical neuronal excitability

F. Gimeno-Ferrer[1], A. Eitner[2], R. Bauer[3], A. Lehmenkühler[4], H.-G. Schaible[1], F. Richter[1]

[1]Institute of Physiology I / Neurophysiology, University Hospital Jena, Germany; [2]Department of Trauma, Hand and Reconstructive Surgery, Experimental Trauma Surgery, University Hospital Jena, Germany; [3]Institute of Molecular Cell Biology, CMB-Center for Molecular Biomedicine, University Hospital Jena, Germany; [4]Pain Institute & Center for Medical Education, Düsseldorf, Germany

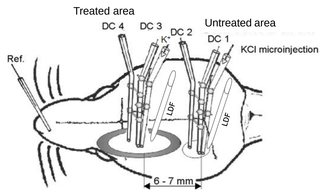
The neuropeptide Galanin has pleiotropic functions ranging from influencing the release of hormones in the hypothalamus, control of food intake, control of the release of neurotransmitters in the hippocampus. In the literature data are showing that Galanin thereby influences neuronal excitability. It is also discussed to have an impact on pain and to turn down epileptic activity in the brain. In the here presented study we investigated the influence of external applied Galanin on neuronal excitability in normal rat cerebral cortex and on the neuronal/glial mass depolarization called cortical spreading depolarization (CSD). For this, we recorded the electrocorticogram (ECoG), regional cerebral blood flow (rCBF) and parameters of CSD in a restricted cortical region treated with Galanin at different concentrations and in remote untreated brain area. We compared these parameters in both cortical areas before and during Galanin application and after washout of Galanin.

METHODS

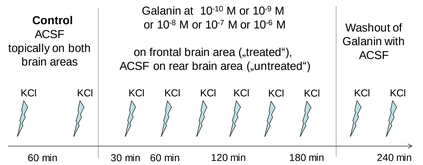
Experiments were performed on spontaneously breathing adult male Wistar rats (anesthetized with sodium thiopentone 100 mg/kg i.p.). Two trephinations were made over the left parieto-occipital cortex, the dura mater was opened, and the exposed brain areas were superfused with regular artificial cerebrospinal fluid (ACSF, warmed to 37 ° C, equilibrated with carbogen). DC potentials were recorded at two sites in the cerebral cortex with pairs of glass microelectrodes (tip diameter 5 µm) in cortical layers II and V. The frontal trephination hole was surrounded by a wall of dental acrylic, and there Galanin was applied topically to the cortical surface (see Figure). The electrocardiogram and the systemic blood pressure were continuously monitored.

Single CSDs were elicited at intervals of 30 min by microinjections of 1 M KCl solution (100 kPa, 100 – 500 ms) at the untreated recording site. CSDs were evaluated regarding failure in propagation to frontal electrodes, peak amplitudes at each electrode, and propagation times between the site of elicitation and electrodes in the treated area. In addition, in the treated area CSD-related changes in extracellular potassium concentration ($[K^+]_e$) were measured with a micropipette filled with Corning IE-190 ionic exchanger. In the remote area 100 µl of Galanin at concentrations from 10^{-10} M to 10^{-6} M (only one concentration per experiment) was applied topically and left there for three hours, followed by a rapid washout with ACSF. In both cortical areas rCBF was measured.

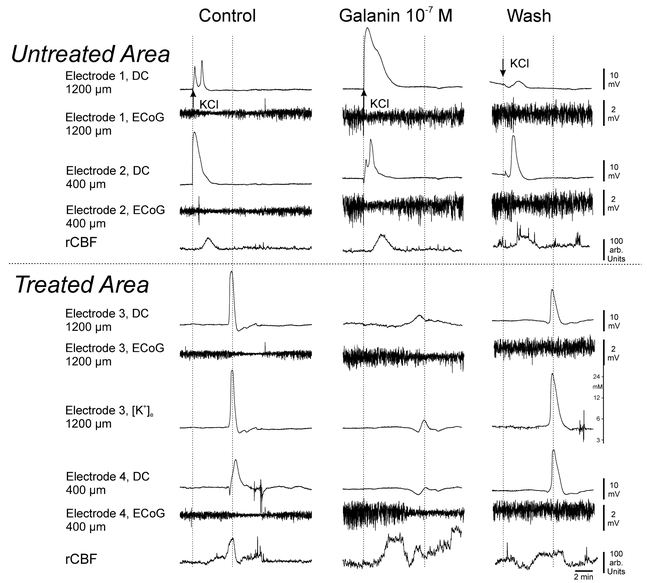
Rat cortices from native brains were prepared after intracardial perfusion. 10 µm thick cryosections were sliced and stained with Anti-GalR1 (Alomone Labs) and Anti-GalR2 (Raybiotech-Hölzel) antibodies to localize the Galanin receptor in the cortex. Alexa 488 goat anti-rabbit (Thermofisher) was used as secondary antibody. Images were recorded using the confocal laser scanning microscope TCS SP5 (Leica, Wetzlar, Germany).



Schematic drawing of the rat skull (not to scale) with the two trephinations and inserted electrodes and LDF probes in treated and untreated areas.



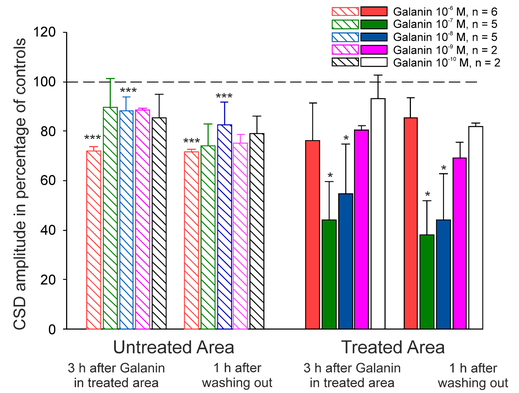
Schedule of an experiment with application of one concentration of Galanin followed by the washout. Flash symbols indicate microinjection of KCl to ignite a CSD.



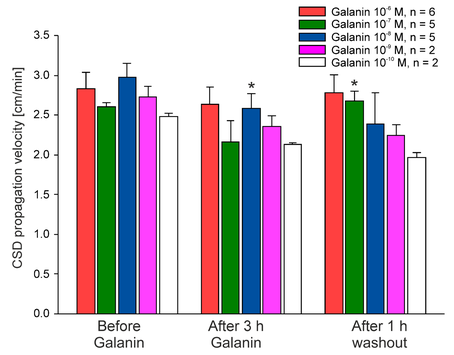
Representative CSD from treated and untreated brain areas before, 3 h after topical application of Galanin at 10^{-7} M, and after 1 h wash with ACSF. Arrows mark the microinjection of KCl to elicit CSD. Dotted lines accentuate CSD propagation times from site of elicitation to treated area that was slowed after Galanin. The panels show the DC-electrocorticogram (DC-ECoG) and the respective band-pass filtered electrocorticographic data (0.5 to 45 Hz) thus indicating that there is spreading depression of electroencephalographic activity. The threshold for CSD ignition increased due to Galanin, causing a larger amount of KCl injected and resulting in a lager DC amplitude (middle column, top).

Effects of Galanin treatment on CSD elicitability and CSD propagation

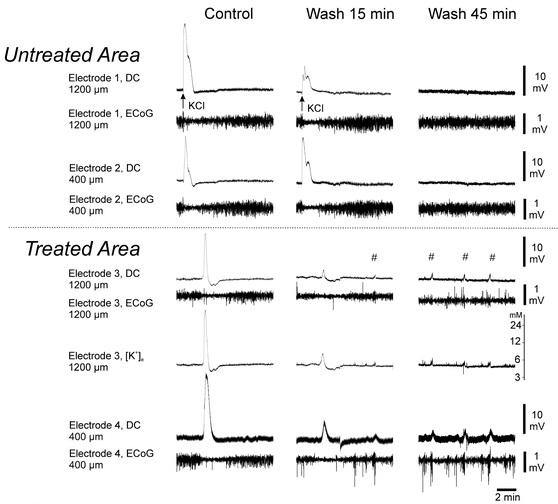
Concentration	Tested animals	% Experiments with increase of threshold	Mean increase in threshold (duration of injection)	Attempts of CSD by KCl injection	Propagating CSD by KCl injection	% Propagating CSD
Galanin 10^{-6}M	6	50%	283 ms	38	36	94,7%
Galanin 10^{-7}M	5	40%	430 ms	30	26	86,7%
Galanin 10^{-8}M	5	40%	500 ms	30	27	90%
Galanin 10^{-9}M	2	0%	0 ms	12	12	100%
Galanin 10^{-10}M	2	0%	0 ms	12	12	100%



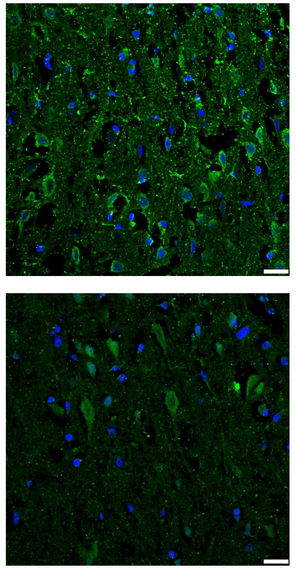
Comparison of percentage changes in CSD amplitudes 3 hours after topical application of different concentrations of Galanin and 1 hour after washout with ACSF. Bars shown mean values ± standard error. Control values prior to application are set to 100 % (dashed line), *** $p < 0.001$, * $p < 0.05$, t-test versus controls.



Application of Galanin at different concentrations resulted in some slowing of CSD propagation that was barely reversed by the washout with ACSF. The columns show mean values ± standard errors, * $p < 0.05$, t-test Galanin versus controls or washout versus Galanin.



CSD samples and electrocorticographic activity from an animal treated with Galanin at 10^{-7} M for 3 hours and then Galanin was washed out. Arrows mark the microinjection of KCl to elicit CSD. The reduction of CSD amplitudes was still existent in the washing phase. In addition after removal of Galanin short periods of ictal discharging activity appeared in treated area only first together with CSD and then alone (marked by #).



Immunohistology of rat cortical slices revealed the expression of the GalR1 (top) and the GalR2 (bottom) in a proportion of neurons that are stained in bright green. Scale bars are 25 µm.

SUMMARY AND CONCLUSION

- Galanin decreases CSD amplitudes and slows their propagation velocity. Too low or too high concentrations of Galanin evoked smaller or even no effects than intermediate doses (e.g. 10^{-7} or 10^{-8} M).
- Rapid removal of effective concentration of Galanin by washout barely restitutes CSD amplitudes, but induces in 25 % of the rats ictal discharging neuronal activity.
- Galanin exerts its effect via Galanin receptors that are expressed on cortical neurons. The specific network functions involved are under investigation.
- Galanin is an important neuropeptide for controlling neuronal excitability in cerebral cortex. This could be of interest for diseases connected with neuronal hyperactivity, e.g. migraine or epilepsy.

TOBeATPAIN

europa.eu *This project has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No 764860*