Evidence for the in vivo analgesic effect of carboxamido steroids via lipid rafts

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INTRODUCTION

Transient Receptor Potential ion channels, such as TRP Vanilloid 1 and Ankyrin repeat domailn 1 (TRPV1, TRPA1), are expressed in nociceptive primary sensory neurons. TRPV1 can be activated by capsaicin (CAPS), resiniferatoxin (RTX), low pH, noxious heat, arachidonic acid or other fatty acid metabolites. Irritant moleculas (allyl-isothiocyanate (AITC), formaldehyde) activate TRPA1. Lipid rafts are defined as liquid ordered plasma membran microdomains rich in cholesterol, sphingomyelin and gangliosides. Our previous finding have revealed that cholesterol depletion by methyl-β-cyclodextrin inhibits the function of TRPV1 receptor. We described also that a carboxamido-steroid compoud (C1) had an inhibitory effect on TRP ion channel activation through lipid raft disruption. The aim of this study is to examine the *in vitro* actions, and the potential analgesic effect of C1 compoud in *in vivo* mouse models.

RESULTS

Our results show, that C1 (Fig.2) treatment diminished the percentage of responsive cells (Fig.3), and the magnitude of Ca^{2+} transients in TRG neurones (Fig.4), and decreased the ^{45}Ca-uptake on receptor-expressing CHO cells (Fig.5). C1 treatment significantly reduced the RTX-induced thermal, and mechanical hyperalgesia (Fig.6,7) the formaldehyde-evoked hyperalgesia (Fig.8) and the number of capsaicin-evoked eye-wiping movements (Fig.9) in *in vivo* models.

METHODS

The effect of C1 was analysed on isolated trigeminal (TRG) neurons by measuring agonist induced Ca^{2+}-transients with ratiometric technique (Fig. 1), and on TRPV1-, or TRPA1-expressing CHO cells by measuring ^{45}Ca-uptake. We investigated the mechanonociceptive and thermonociceptive threshold of the animals in RTX-induced thermal-, mechanical hyperalgesia, and formaldehyde-evoked hyperalgesia model. The analgesic effect of C1 was also measured in capsaicin-evoked acute nocifensive response („eye-wiping") test.

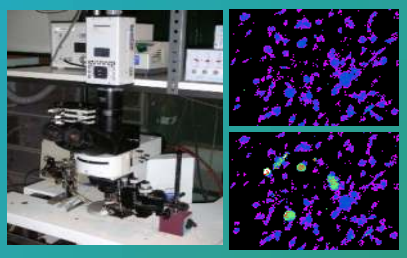

Fig.1: Fluorescent Ca-imaging setup, fura 2-AM-loaded cells before and after the Ca^{2+}-influx

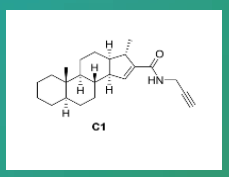

Fig.2: Our C1 compound: N-(prop-2-ynyl)-carboxamido steroid (with unnatural backbone).

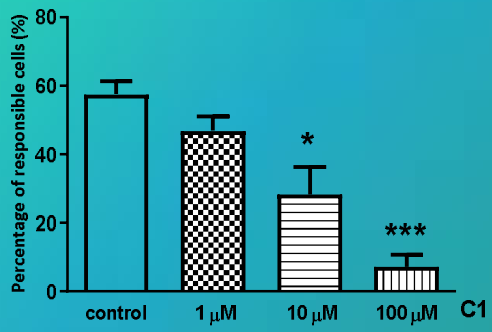

Fig.3: Inhibitory effect of C1 compound on TRPV1 receptor-activation on TRG neurons by fluorescent Ca-imaging. Percentage of responsible cells (one way Anova *p < 0.05, ***p < 0.001).

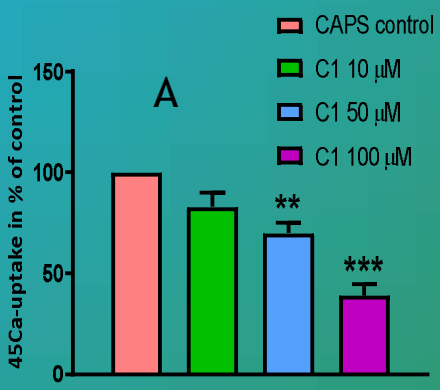

Fig.5: Inhibitory effect of C1 compound of TRPV1 (A) and TRPA1 (B) ion channels on receptor-expressing CHO cell line (one-way Anova *p < 0.05, **p<0,01, ***p < 0.001).

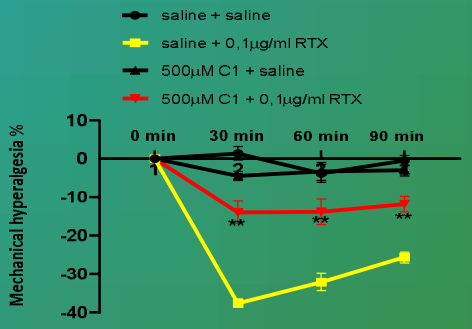

Fig.6: C1 treatment significantly reduced the decrease of the RTX-evoked mechanical hyperalgesia (p<0,01; vs. RTX-treated group; Two-way ANOVA, Bonferroni post hoc test).**

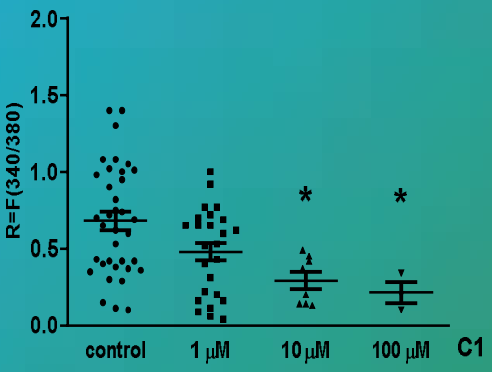

Fig.4: Inhibitory effect of C1 compound on TRPV1 receptor-activation on TRG neurons by fluorescent Ca-imaging. R values of capsaicin-induced Ca^{2+}-influx (one way Anova *p < 0.05).

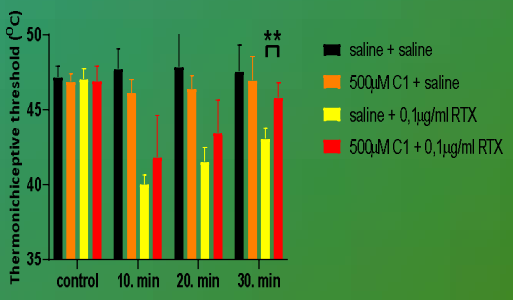

Fig.7: C1 treatment significantly reduced the decrease of the RTX-evoked thermonociceptive threshold (p<0,01; vs. RTX-treated group; Two-way ANOVA, Bonferroni post hoc test).**

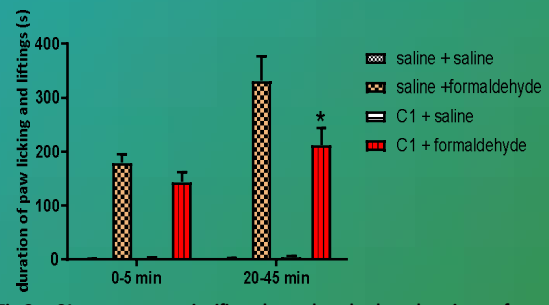

Fig.8: C1 treatment significantly reduced the duration of formaldehyde-induced hyperalgesia (*p<0,05; vs. formaldehyde-treated group; Two-way ANOVA, Bonferroni post hoc test).

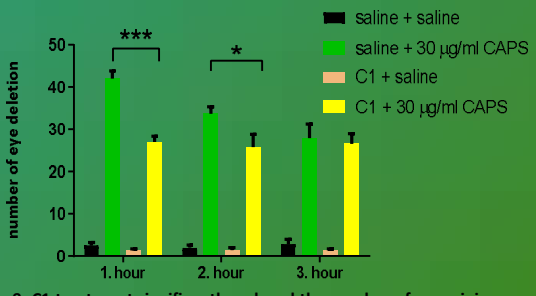

Fig.9: C1 treatment significantly reduced the number of eye wiping movements in capsaicin induced acut chemonociception test (*p<0,05, ***p<0,001 saline + CAPS- vs C1 + CAPS-treated group; Two-way ANOVA, Bonferroni post hoc test).

CONCLUSION

Our results provide the first evidence that disruption of lipid rafts by C1 have analgesic effect in in vivo mouse models. Our in vitro and in vivo findings suggest that the hydrofobic interactions between the TRP channel and lipid raft interfaces modulate the opening properties of these channels and therefore, targeting this interaction might be a promising tool for drug developmental purposes.

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