IN VITRO PHARMACOLOGICAL CHARACTERIZATION OF TRANSIENT RECEPTOR POTENTIAL ANKYRIN 1(TRPA1) AGONISTS AND ANTAGONISTS

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Introduction: The transient receptor potential ankyrin 1(TRPA1) is a calcium permeable channel receptor found predominantly in primary sensory neurons. Similarly to other channels of the TRP family, TRPA1is involved in various sensory processes and diseases, including migraine. Thus TRPA1selective antagonists have great interest as innovative drugs. The aim of the present study was to set up an in vitro assay useful for the pharmacological characterization of novel molecules acting as TRPA1antagonists.

Materials and methods: TRPA1standards ligands were pharmacologically evaluated using the intracellular calcium mobilization assay in human lung carcinoma cells (A549). The agonists used were allyl isothiocyanate (AITC), benzyl isothiocyanate (BITC), cinnamaldehyde (CA), and PF-4840154. The antagonists used were HC030031, A967079, and DHC200. The antagonists were tested both at different concentrations against a fixed concentration of agonist and at a single concentration against the concentration response curve to an agonist.

Results: AITC, BITC, CA, and PF-4840154increased the intracellular calcium levels in a concentration dependent manner; PF-4840154elicited maximal effects significantly higher than the other agonists. The following rank order of potency was derived: PF-4840154>> BITC = AITC = CA. Based on these results, PF-4840154was selected as standard agonist for subsequent antagonism experiments. Increasing concentrations of antagonists were tested against PF-48401541μM. All compounds inhibited the effect of PF-4840154in a concentration dependent manner. When tested at a single concentration against the concentration response curve to PF-4840154all the antagonists elicited a rightward shifted of the curve without modifying the agonist maximal effect. The following rank order of antagonist potency was consistently derived using the two protocols: A967079≥ DHC200 > HC030031.

Discussion and conclusions: The pharmacology profile (rank order of potency of agonists and of selective antagonists) of the TRPA1receptor obtained in the present study is in line with the data reported in literature; thus, we have successfully set up the experimental conditions and protocols for studying TRPA1ligands in A549cells, using the calcium mobilization assay. In conclusion, the approach validated in this study will allow the pharmacological characterization of novel TRPA1antagonists, potentially useful as innovative anti-migraine agents.