Proceedings of the British Pharmacological Society at http://www.pA2online.org/abstracts/Vol18Issue1abst030P.pdf

Analogue A54: a novel competitive antagonist of Englerin A

H. N. Rubaiy[1], T. Seitz[2], S. Hahn[2], A. Choidas[3], P. Habenberger[3], B. Klebl[3], K. Dinkel[3], P. Nussbaumer[3], H. Waldmann[4], M. Christmann[2], D. J. Beech[1]. [1]School of Medicine, LICAMM, University of Leeds, Leeds, United Kingdom, [2]Institute of Chemistry and Biochemistry, Freie Universität Berlin, Berlin, Germany, [3]Lead Discovery Center GmbH, Dortmund, Germany, [4]Max-Planck-Institut für Molekulare Physiologie, Dortmund, Germany.

Introduction: Transient Receptor Potential Canonical 4 and 5 (TRPC4 and TRPC5) proteins assemble as homomers or heteromerize with TRPC1 to allow Ca^{2+} and Na^{+} entry into cells. It is suggested that TRPC1/4/5 channels are involved in cancer, epilepsy, innate fear, pain, rheumatoid arthritis, and adverse cardiac remodeling but lack in pharmacological tools to study these channels have had restricted progress[1]. The compound (-)-Englerin A (EA) is a very potent and selective inhibitor of renal cancer cell growth and also, a potent and specific agonist of TRPC1/4/5 channels[1, 2, 3, 4].

Method: Renal cell carcinoma A498 cells and human embryonic kidney 293 cells overexpressing TRPC4 or TRPC5 were studied by intracellular Ca^{2+} measurement or whole-cell patch-clamp electrophysiology. The EA analogue A54 was generated by total synthesis[1, 2, 4. 5].

Results: Analogue A54 had weak or no agonist activity at endogenous TRPC4/TRPC1 channels in A498 cells (n/N = 7/21, $p<0.001$, one-way ANOVA and post-test Bonferroni) or TRPC4 or TRPC5 homomeric channels overexpressed in HEK 293 cells. A54 strongly inhibited EA-mediated activation of TRPC4/TRPC1 (A498, IC_{50} = 62 ± 17 nM, slope = 0.8 ± 0.01) (n/N = 5/15, mean ± SEM) or TRPC5 (n=5 independent recordings, $p<0.001$) and weakly inhibited activation of TRPC4 (n/N = 5/15, $p<0.05$). Studies of TRPC5 showed that A54 right-shifted the EA concentration-response curve without changing its slope, EA EC_{50s} were 1.7 nM (0 nM A54), 4.2 nM (10 nM A54) and 14.3 nM (50 nM A54) and EA slope values were 1.4 (0 nM A54), 1.3 (10 nM A54), and 1.3 (50 nM A54), respectively (n/N = 6/24), consistent with competitive antagonism[5]. In contrast, TRPC5 activated by Gd^{3+} (n=5 independent recordings, $p<0.05$) and TRPC4 activated by sphingosine-1-phosphate were not inhibited but potentiated by A54[5] (n/N = 5/15, $p<0.05$). A54 did not activate TRPC3 channels or affect activation of these channels by the agonist 1-oleoyl-2-acetylglycerol (n/N = 6/18, $p=NS$)

Conclusions: This study for first time reports on a competitive antagonist, analogue A54, of EA in A498 cells. Perhaps, this new pharmacological tool of TRPC1/4/5 channels permits a better understanding of TRPC1/4/5 research, channel activation, and binding sites.

References:

1. *Rubaiy et al. (2017). J. Biol. Chem.* ***292****: 8158-8173*
2. *Ludlow et al. (2017). J. Biol. Chem.* ***292****: 723-731*
3. *Rubaiy HN et al. (Commentary, (2017) Channels,* ***11(5)****:362-364)*
4. *Rubaiy HN (Review, (2017) J Pharm Pharm Sci. (2017)* ***20****:48-67)*
5. *Rubaiy et al. (2017) Br J Pharmacol.* [Epub ahead of print]