Mol Pharmacol

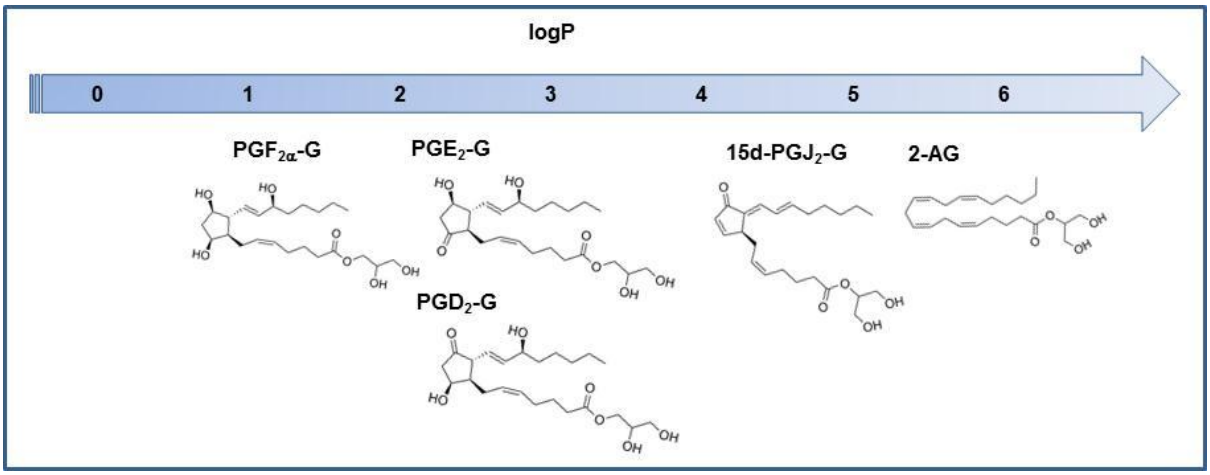
Supplemental information accompanying the manuscript

Robust hydrolysis of prostaglandin glycerol esters by human monoacylglycerol lipase (MAGL)

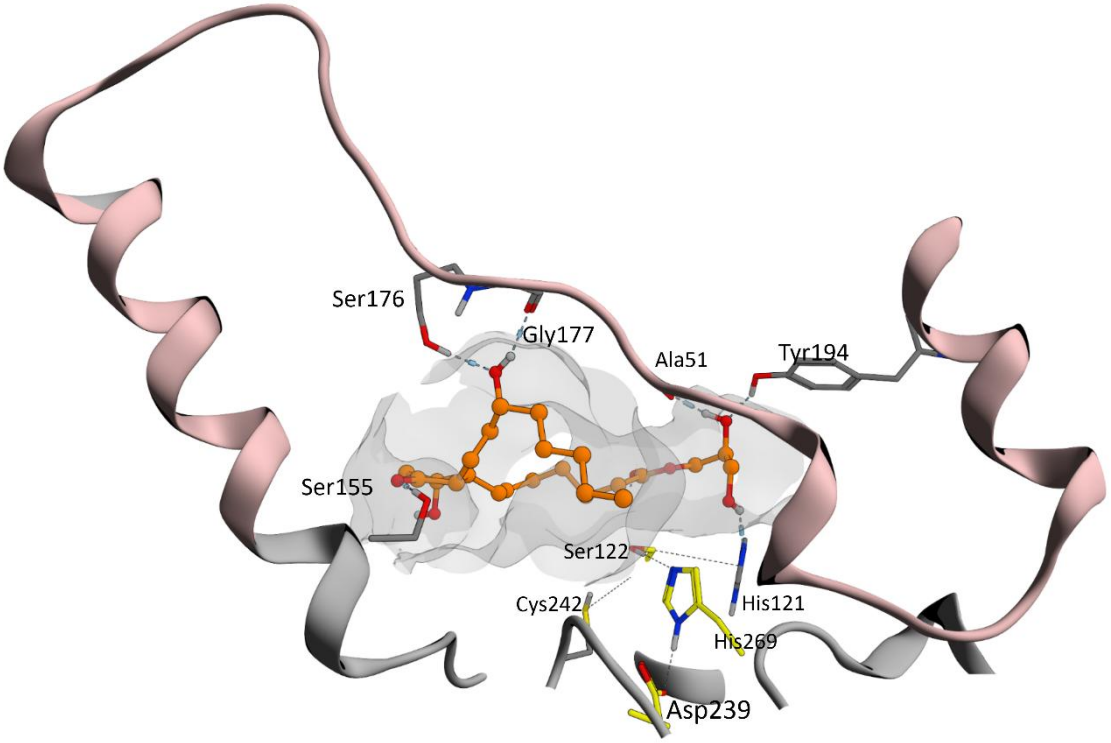
by

Juha R. Savinainen, Emilia Kansanen, Tatu Pantsar, Dina Navia-Paldanius, Teija Parkkari, Marko Lehtonen, Tuomo Laitinen, Tapio Nevalainen, Antti Poso, Anna-Liisa Levonen and Jarmo T. Laitinen

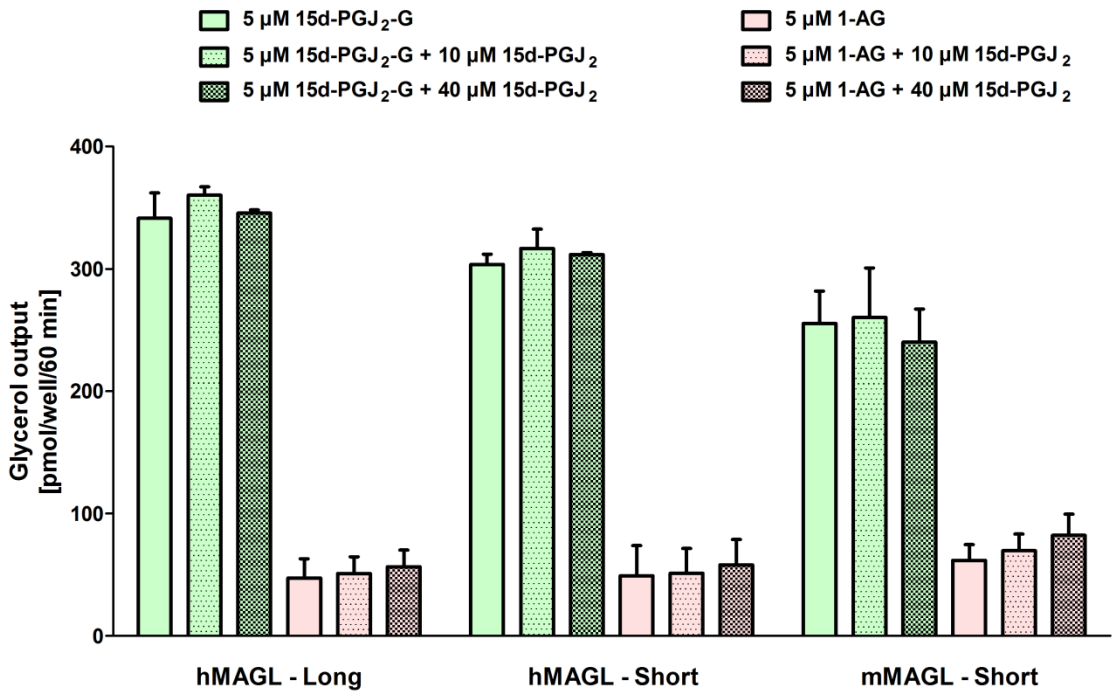
Inventory of Supplemental Information



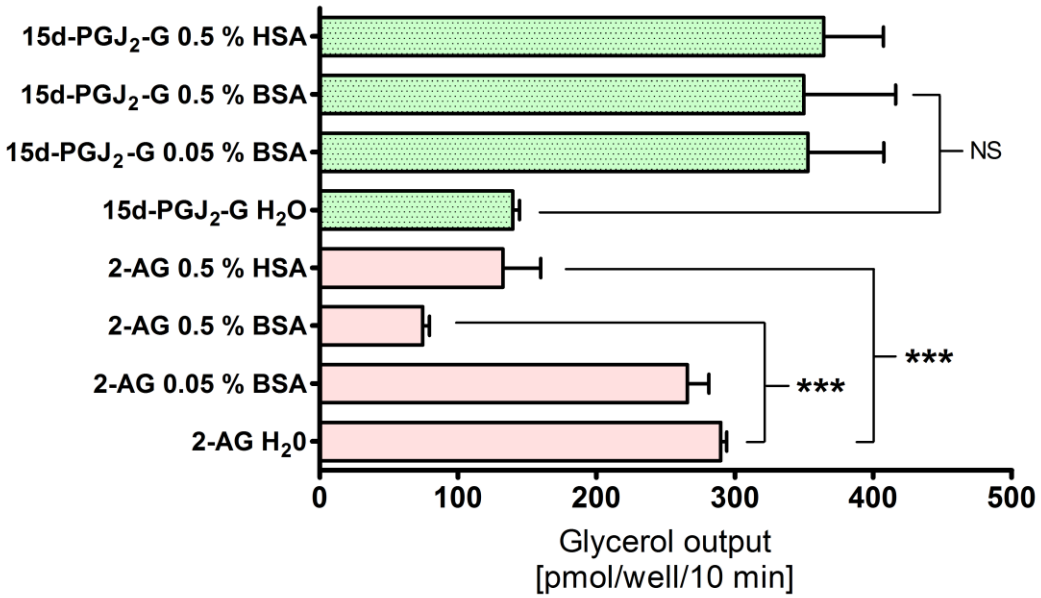
Supplemental Figure 1. Chemical structures and calculated logP values of the PG-G species tested in this study. The logP values were estimated using ChemAxon Marvin 6.0 software. Note that 15d-PGJ_2-G represented the 2-isomer while PGD_2-G, PGE_2-G and $PGF_{2\alpha}$-G are 1(3)-isomers.



Supplemental Figure 2. Location of additional hydrogen bond interaction between PGD_2-G (orange) and the LID domain of hMAGL (pink). The view is from the direction of the substrate access site. In contrast to PGD_2, 15d-PGJ_2-G is unable to form additional hydrogen bonds between the 15d-PGJ_2 moiety and the lid domain (see Figure 5A).



Supplemental Figure 3. No inhibition of MAGL isoforms by the 15d-PGJ_2-G hydrolysis end-product 15d-PGJ_2. Lysates (0.3 µg/well) of HEK293 cells transiently overexpressing human MAGL-long (313 aa), human MAGL-short (303 aa) and mouse MAGL-short (303 aa) isoforms were incubated together with the indicated substrates (15d-PGJ_2-G or 1-AG, 5 µM final concentration) in the presence or absence of the indicated concentrations of 15d-PGJ_2. Glycerol output was determined at time-point 60 min. Data are mean + S.D. of duplicate wells from two independent experiments. There were no statistically significant differences in the substrate utilization in the absence or presence of 15d-PGJ_2 (one-way analysis of variance followed by Tukeys multiple comparisons).



Supplemental Figure 4. Substrate availability for MAGL-catalyzed hydrolysis in incubations with or without bovine (BSA) or human serum albumin (HSA). Lysates (0.3 µg/well) of HEK293 cells transiently overexpressing hMAGL (long isoform) were incubated together with the indicated substrates (25 µM final concentration) in the presence or absence (H_2O) of the indicated albumin concentrations (w/v). Glycerol output was determined at time-point 10 min. Data are mean + S.E.M. of duplicate wells and combined from three independent experiments. Statistical comparisons were done using one-way analysis of variance, followed by Tukeys multiple comparisons and the significance indicated with an asterix (*** $p<0.001$).