SUPPLEMENTAL MATERIALS

Supplemental Table 1. Primary antibodies, proteases, and cathepsin substrates used in the study.

Antibody/peptide	Source	Type	Clone	Manufacturer	Application
cathepsin L	Rat		204101	R&D Systems	IHC/ICC
cathepsin L	Mouse		22	BD Transduction	IF
cathepsin L	Goat	Poly		R&D Systems	immunoblot
cathepsin B	Rat		173317	R&D Systems	immunoblot
perlecan	Rat		A7L6	Chemicon	IHC/immunoblot
perlecan	Mouse		7E12	Chemicon	immunoblot
GFAP	Rabbit	Poly		Abcam	IF/IHC
laminin	Rabbit	Poly		Abcam	IF/IHC
perlecan domain V	Mouse		268908	R&D Systems	immunoblot
collagen IV α2 chain	Rabbit	Poly		Sigma-Aldrich	immunoblot
β-actin	Mouse		AC-15	Abcam	immunoblot
cathepsin L	Human liver protease			Calbiochem	ECM assay
Z-arg-arg-MNA	substrate to cathepsin B			Enzyme Systems	activity
Z-phe-arg-MNA	substrate to cathepsin L			Enzyme Systems	activity

Supplemental Table 2. Primers for the real time RT-PCR studies.

Gene	Primer		GenBank nr
	Forward	Reverse	
MMP-2	TTTTGTGCCCAAAGAAAGG	GCCCTCCTAAGCCAGTCTCT	NM031054
MMP-9	CCACCGAGCTATCCACTCAT	CCTGTGAGTGGGTTGGATTC	NM031055
cathepsinB	GCAGGGAGTGAGGCAGATAG	CCTGTGAGTGGGTTGGATTC	X82396
cathepsinL	GGAGAGCAGTGTGGGAGAAG	GGATCTGCAGCATCAGAGGT	NM0131561

Legends: Supplemental Figures

Figure 1.

Relative gene expressions of cathepsin L and cathepsin B by primary microglia and astrocytes.

RT-PCR studies of cathepsin L and cathepsin B mRNA in primary microglia (M) and primary astrocytes (A) plated on PDL, subjected to normoxia (black bars) and to OGD (open and hatched bars). OGD stimulated a modest but significant increase in cathepsin L transcripts from microglia (*, $p = 0.012$; n = 8), but not cathepsin B (n = 8), compared to normoxia. OGD produced no effect on astrocyte gene expression of cathepsin L or B (n = 12).

Figure 2.

The effect of decreasing pH on cathepsin L generation and secretion by murine primary astrocytes.

Astrocytes produced low levels of cathepsin L and failed to secrete cathepsin L, with no increase in activity under OGD at neutral pH. Decreasing the ambient pH to 5.6 marginally increased the activity detected. Astrocytes were cultured at normoxia (closed bars) and OGD (open bars) as described in the text. At the end of the culture period, 0.5ml Ringer's solutions buffered to pH7.4 or pH5.6 replaced the culture medium of each well (6-well plate). The plates were further incubated at 37˚C for 0, 0.5, 1.0, or 2.0 hours and rotated at 80rpm. Both cell lysates and supernatants were collected from each time point and the cathepsin L activity analyzed. Samples were also measured for LDH release. Data are represented as the mean ± SD (n = 3).

Figure 3.

Increased cathepsin L activity in microglia is pH- and time-dependent.

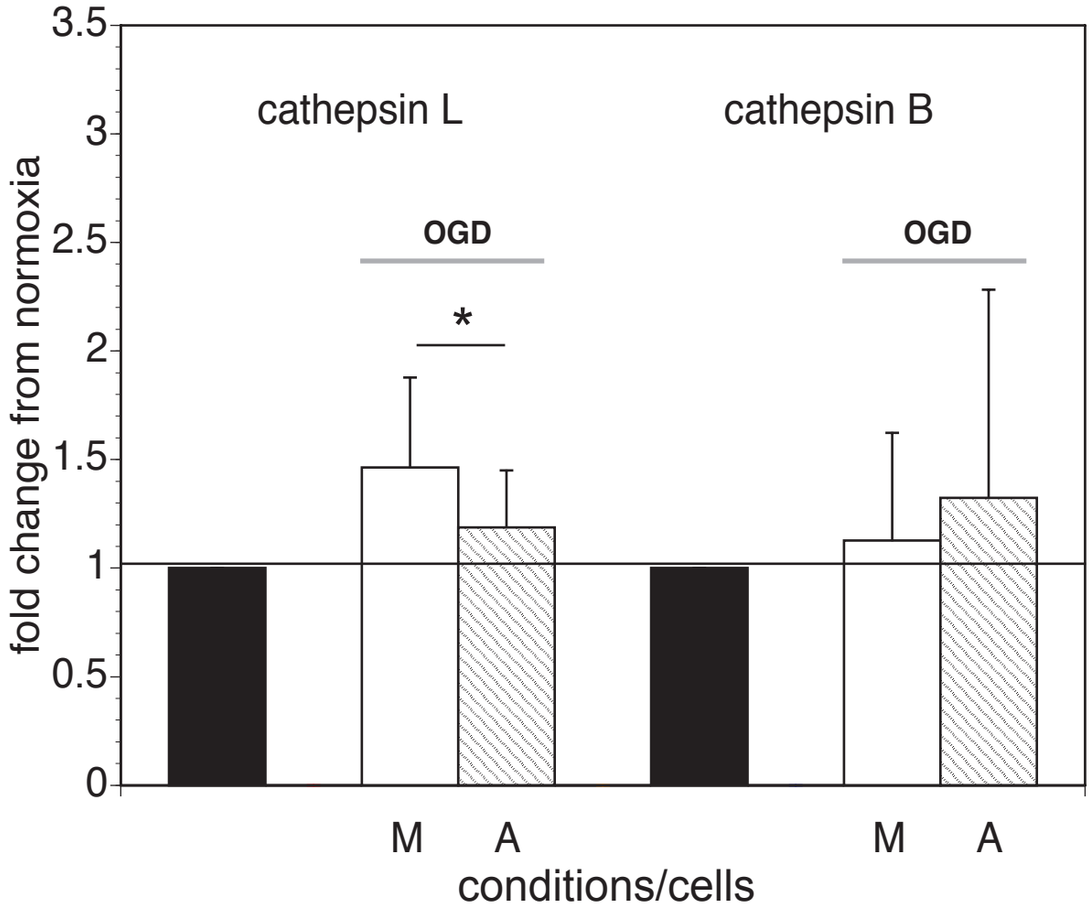
Microglia were cultured at normoxia (closed squares) and OGD (closed circles) conditions as described for **Figure 4**. Following culture microglia were subject to pH7.4, pH6.2, or pH5.6, the supernatants collected from each time point, and the cathepsin L activity analyzed. Samples were also assayed for LDH release. Data are presented as absolute cathepsin L activity (mU/μg protein ± SD), and % microglial demise based on LDH release (inset). Data are n = 6 per time point of three independent experiments conducted. General linear models (GLM)s were used to model cathepsin L activity over time under normoxia and OGD. Activities under the different pH conditions were analyzed separately. The model specifications delineated culture (normoxia or OGD) as a fixed effect, and time (0, 0.5, 1, 2 hours) as a covariate. Under pH 7.4, cathepsin L activity differed significantly over time ($F_{1,45} = 22.51$, $p < 0.0003$), but not by normoxia vs. OGD ($F_{1,45} = 2.07$, $p = 0.157$). Under pH 6.2, cathepsin L activity differed significantly over time ($F_{1,45} = 196.2$, $p < 10^{-16}$), and was significantly lower under normoxia compared to OGD ($F_{1,45} = 4.56$, $p = 0.038$). Under pH 5.6, cathepsin L activity again differed significantly over time ($F_{1,45} = 209.6$, $p<10^{-16}$), and was significantly lower under normoxia compared to OGD ($F_{1,45} = 22.96$, $p < 0.00002$).

Figure 4.

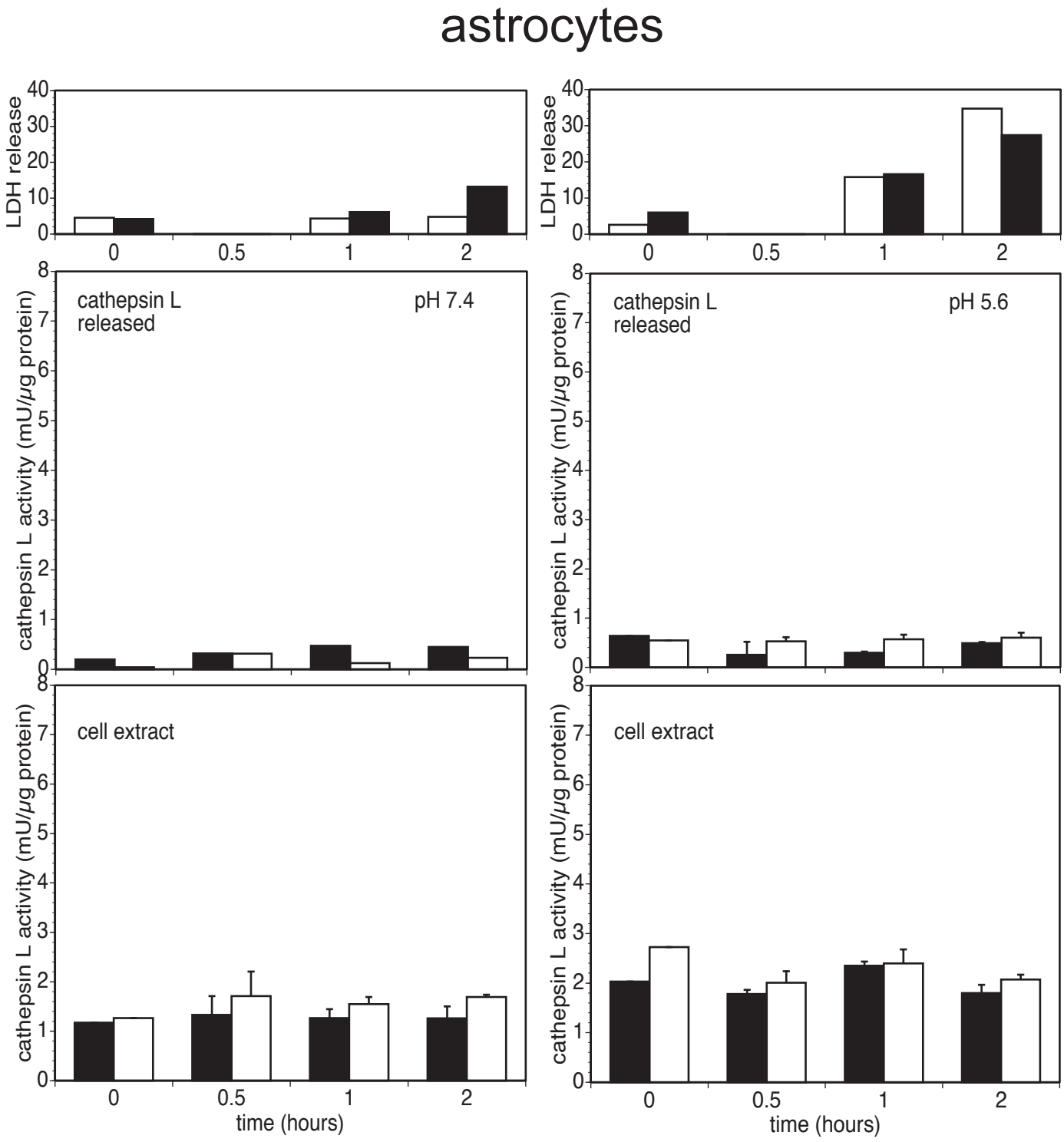
Effects of the inhibition of matrix protease activity on ECM release/degradation by secretion products of microglia grown under normoxia.

Release of perlecan (**A**), the domain V of perlecan (**B**), and the α2 chain of collagen IV (**C**) by microglia secretion products. No release/degradation of perlecan, perlecan domain V, or collagen IV was detected when mouse cortical ECM was incubated with buffer. However, microglia secretion products produced activity when cultured under normoxia that could release and/or degrade perlecan, perlecan domain V, or collagen IVα2 chain from mouse cortical ECM. E64d inhibited released activities against all ECM substrates, whereas inhibitors of cathepsin L and MMP-like activity partly blocked the generation of perlecan domain V and of the collagen IVα2 chain from mouse cortical ECM. Data are n = 6 for each condition. There were no statistically significant differences

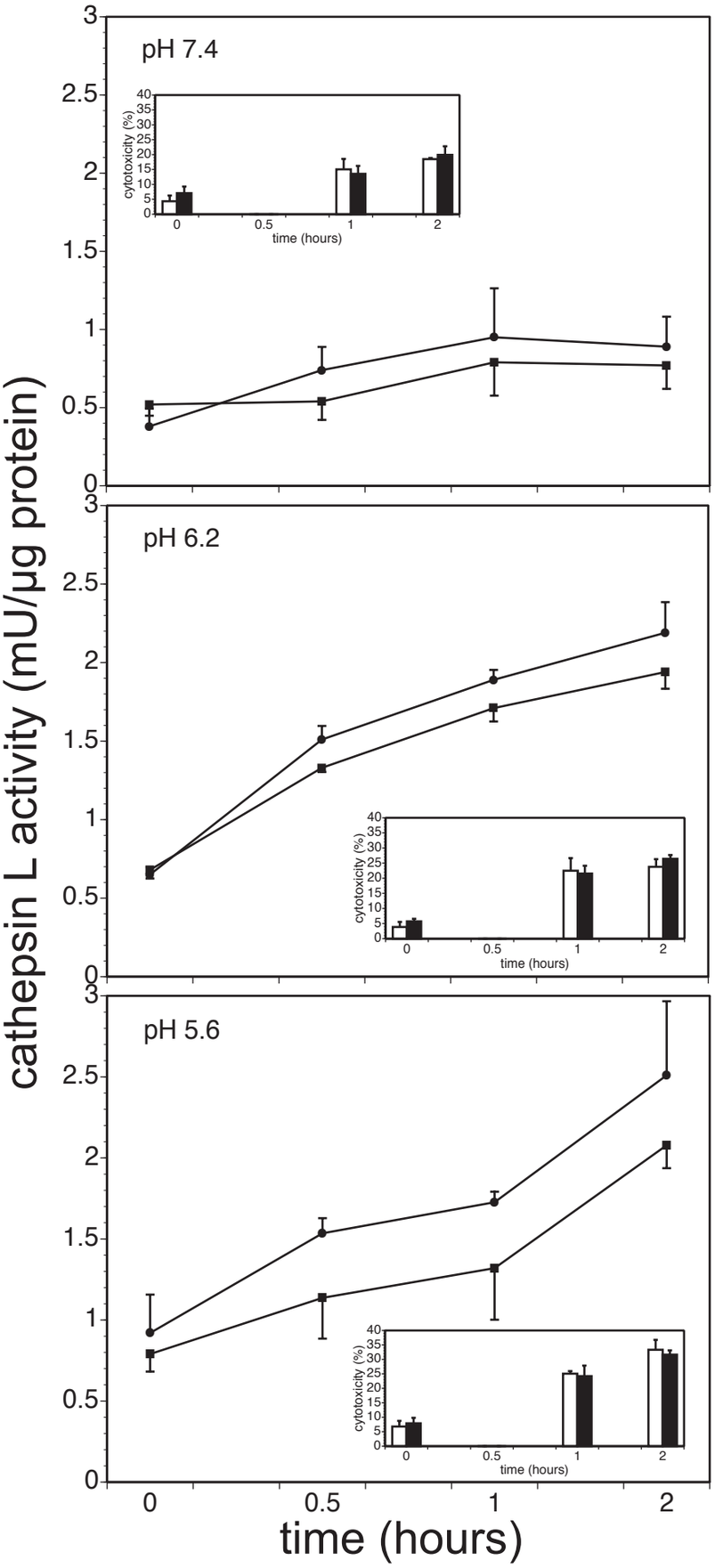
in the release of perlecan (**A**) under E64d, CAA, or GM ($F_{2,15}$=1.60, p = 0.23). In contrast, the release of the domain V of perlecan (**B**) under E64d, CAA, or GM differed significantly ($F_{2,15}$=10.58, p = 0.0014). Subsequent pairwise multiple comparisons via Tukey's HSD procedure revealed that release of the domain V of perlecan did not differ significantly under E64d or CAA, but was significantly higher under GM, at an overall experiment-wise α level of 0.05. The release of the α2 chain of collagen IV (**C**) also differed significantly under E64d, CAA, or GM ($F_{2,15}$=8.51, p = 0.0034). Subsequent pairwise multiple comparisons via Tukey's HSD procedure revealed two homogeneous subsets in which the mean releases of the α2 chain of collagen IV were not significantly different from one another at the overall experimentwise α = 0.05 level: (CAA, E64d) and (E64d, GM).



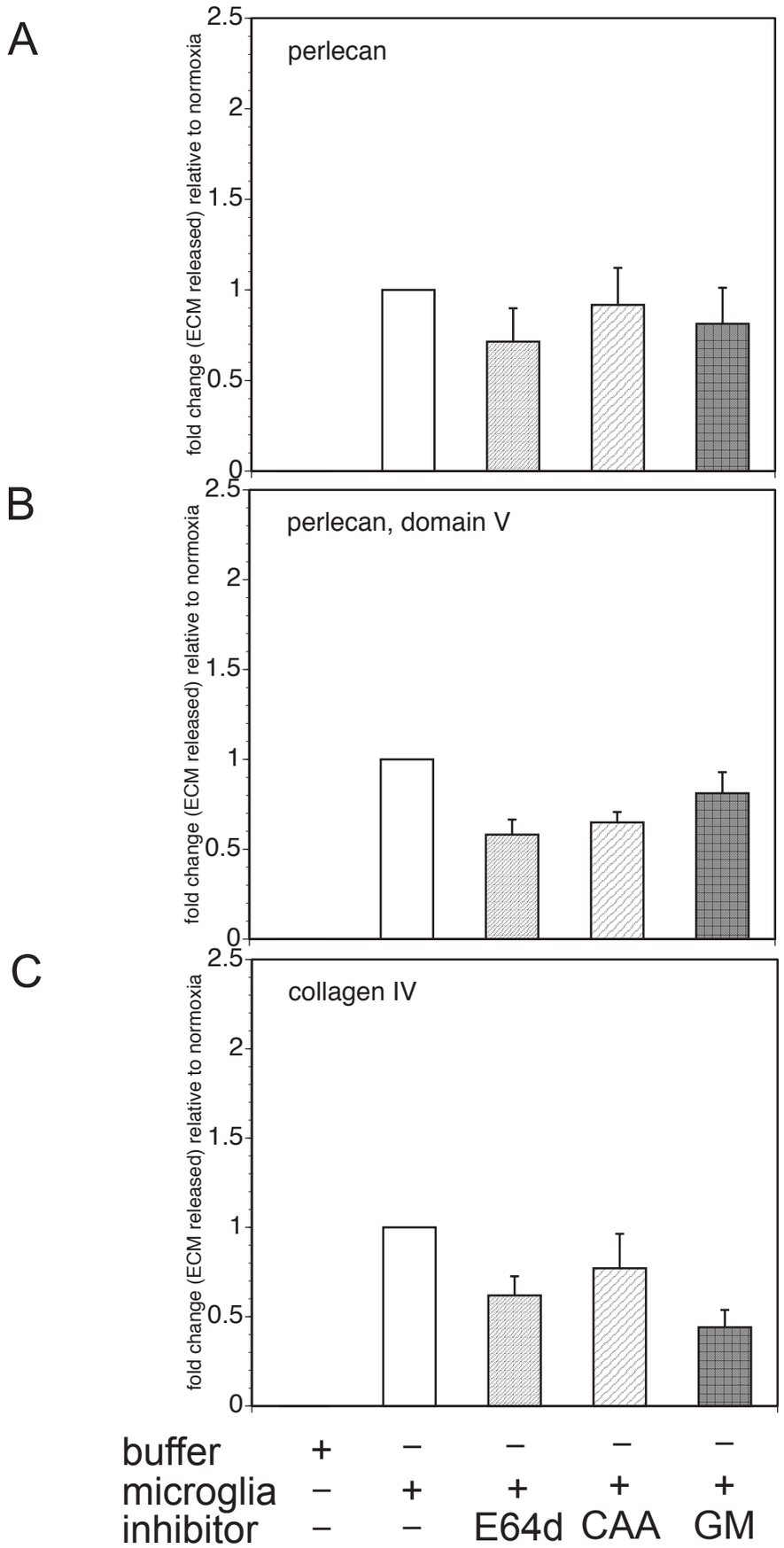
Supplemental Figure 1



Supplemental Figure 2



Supplemental Figure 3



Supplemental Figure 4