Supplementary Material For:

Cold-adapted protease enables quantification of surface proteins in the absence of membrane trafficking

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Previously, proteolytic analysis of surface expression of glutamate receptors was done using chymotrypsin at 37°C (1–4). Hence, proteolytic activity of cod trypsin on ice was compared with that of a mammalian chymotrypsin (bovine; Cat. no. C4129; Sigma-Aldrich, Steinheim, Germany) both on ice and at 34°C using a decrease in GluA4 immunoreactivity in brain slices as a measure of the extent of proteolytic cleavage of surface proteins. As can be observed in Supplementary Figure S1A, there was only a slight activity of bovine chymotrypsin on ice with the immunoactivity of GluA4 decreasing to 88.4 ± 1.1% (n = 2) compared with the control. On the other hand, cod trypsin activity on ice was as efficient as chymotrypsin activity at 34°C, as assessed by a decrease in GluA4 amount to 63.8 ± 5.5% and 55.2 ± 3.8% (n = 2 for each) after treatment with cod trypsin on ice and bovine chymotrypsin at 34°C, respectively. However, the case for KCC2 was different (Supplementary Figure S1B). For KCC2, there was a massive decrease in the immunoreactivity of the full-length protein (denoted by block arrow) in slices treated with bovine chymotrypsin at 34°C, with a concomitant increase in a cleaved fragment (denoted by arrowhead). Note that chymotrypsin cleavage product of KCC2 was detected at a different size from the cod trypsin cleavage product (denoted by arrow). Thus, while the surface expression of KCC2 as assessed by cod trypsin treatment on ice was 16.2 ± 2.0% (n = 2), the surface expression determined by chymotrypsin treatment at 34°C was 82.7 ± 13.6% (n = 2). The difference in proteolytic extent of KCC2 by bovine chymotrypsin at 34°C and cod trypsin on ice was evidently due to the fast recycling of a membrane pool of KCC2 (5) at mammalian physiological temperatures, which was inhibited by performing proteolysis of surface proteins with cod trypsin on ice.

References