Unraveling the Role of Astroglia in the Mechanism of Antidepressant Action of Ketamine

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AIM

Ketamine (KM), an anaesthetic and psychomimetic drug, exerts rapid, potent and long-lasting antidepressant effect, albeit the cellular and molecular mechanisms of this action are incompletely understood. Besides targeting neuronal NMDARs, KM also modulates the function of astroglia. We thus elucidated the effect of (sub)anaesthetic doses of KM on stimulus-evoked calcium (Ca^{2+}) signalling, fusion pore activity of vesicles and mobility of vesicles carrying the inward rectifying potassium chanel (Kir4.1) in astroglia.

METHODS

The effect of KM on ATP-evoked Ca^{2+} signalling was examined by measuring the Fluo-4 fluorescence in KM-treated and non-treated astrocytes. High-resolution patch-clamp membrane capacitance measurements were used to determine the fusion pore activity of vesicles. The spontaneous mobility and plasmalemmal localization of Kir4.1-EGFP in pKir4.1-EGFP-transfected astrocytes labeled by styril dye FM4-64 was determined by confocal microscopy.

RESULTS

The ATP-evoked peak Ca^{2+} responses were diminished in KM-treated astrocytes with ATP mobilizing ~3.3-fold less Ca^{2+} than in nontreated controls ($P < 0.001$) (Figure 1). Ketamine-evoked increase in vesicle bursting activity correlated well with a decrease in irreversible vesicle fission from the plasmalemma (R=0.93 for increasing KM incubation time and R=0.99 for increasing [KM]) (Figure 2). Already short, 30 min KM treatment reduced directional mobility of Kir4.1-positive vesicles. The apparent surface localization of Kir4.1 at astroglial plasmalemma decreased from 56% in non-treated controls to 43% ($P < 0.05$) and 33% ($P < 0.05$) in astrocytes treated with 2.5 and 25 µM KM, respectively (Figure 3).

CONCLUSION

Diverse but not mutually exclusive mechanisms of ketamine action may synergistically evoke changes in synaptic functional plasticity, leading to sustained strengthening of excitatory synapses, necessary for antidepressant effects.

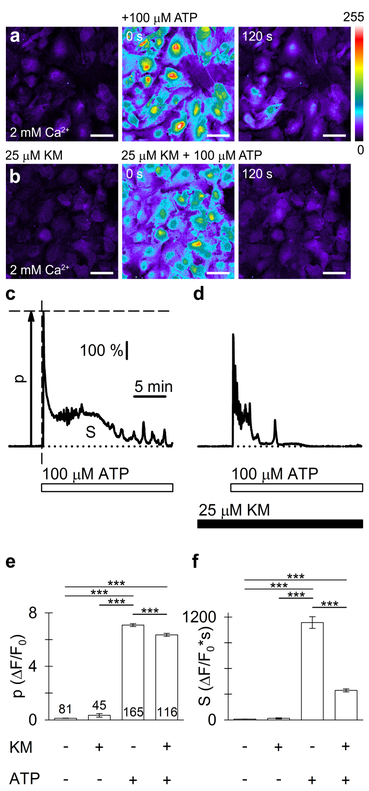

Fig. 1 Ketamine treatment attenuates purinergic Ca^{2+} signalling in rat astroglia. a, b Confocal images of fluorescent Ca^{2+} indicator Fluo-4 loaded in non-treated, control astrocytes (a) or ketamine-treated (25 µM) astrocytes (b) stimulated with 100 µM ATP to increase intracellular Ca^{2+} activity (0 s at peak Ca^{2+} response and 120 s later). Increases in $[Ca^{2+}]_i$ are indicated by the pseudocolour intensity scale (right, 0–255intensity levels). Scale bars: 50 µm. c, d The ATP-evoked increases in $[Ca^{2+}]_i$ quantified by the peak $[Ca^{2+}]_i$ (p, mean ± SEM) relative to the baseline fluorescence (dotted line, F0) and the time-integrated $[Ca^{2+}]_i$ (S). Note that ATP evoked a smaller increase in $[Ca^{2+}]_i$ in astrocytes treated with ketamine. e, f The ATP-evoked peak (p) calcium responses and the time-integrated calcium activity (S) were diminished in ketamine-treated astrocytes. Ketamine did not evoke a significant increase in $[Ca^{2+}]_i$. The numbers at the bottom of the bars indicate the number of cells analysed. ***$P < 0.001$ versus respective comparison (Mann–Whitney U test). Reproduced with permission from Stenovec et al.

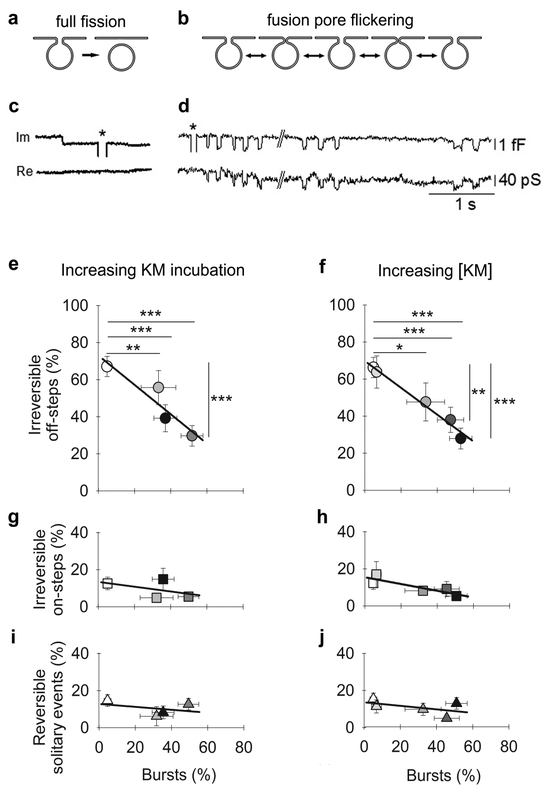

Fig. 2 Ketamine-evoked increase in astroglial vesicle bursting activity correlates with a decrease in irreversible fission of vesicles from the plasmalemma. a A schematic representation of an irreversible off step (full vesicle fission) and b a burst of off and on steps (closing and opening of the fusion pore) observed in membrane capacitance (Cm) recordings (below). c Irreversible off step in the imaginary component of the admittance signal (Im), proportional to membrane capacitance in a control cell. d Burst consisting of reversible step-like events starting with an off step in Im in a ketamine-treated cell. *Calibration pulses. e–j The correlation between the percentage of bursts and other event types within each cell plotted for increasing incubation period with 25 µM ketamine (KM; e, g, i) or [KM] (30 min) (f, h, j). Controls are depicted in white followed by increasing KM incubation periods (acute, 30 min, 24 h incubation) or [KM] (0.025, 0.25, 2.5, 25 µM) represented in incrementing grey scale intensity. Linear functions (black lines) fit to data in (e) irreversible off steps (circles), y = 0.74 − 0.79x (R = 0.93); (f) irreversible off steps, y = 0.71 − 0.72x (R = 0.99); (g) irreversible on steps (squares), y = 0.13 − 0.13x (R = 0.48); (h) irreversible on steps, y = 0.15 − 0.18x (R = 0.87); (i) reversible solitary steps (triangles), y = 0.13 − 0.08x(R = 0.38); (j) reversible solitary steps, y = 0.13 − 0.10x (R = 0.52). In both (e) and (f), the regression line slopes for irreversible off steps are significantly different (P < 0.001, one-way ANCOVA) from the slopes of irreversible on steps (g, h) and reversible solitary steps (i,j). *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$. Reproduced with permission from Lasic and colleagues.

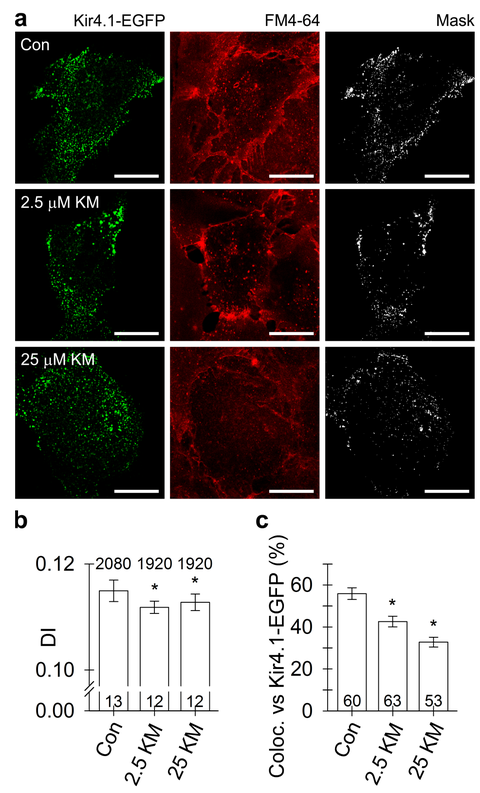

Fig. 3 Ketamine treatment reduces directional mobility of Kir4.1-positive vesicles and apparent plasmalemmal localization of Kir4.1 in astroglia. a Confocal images of transfected astrocytes (not treated with ketamine (top), treated with 2.5 µM (middle) or 25 µM (bottom) ketamine) containing Kir4.1-EGFP-positive vesicles (numerous green fluorescent puncta, left) and plasmalemma labelled with the membrane styril dye FM4-64 (middle); the mask images (white, right) display co-localized pixels. Scale bars: 20 µm. b Directional mobility of Kir4.1-EGFP-positive vesicles is diminished in ketamine-treated astrocytes. c Quantitative co-localization (mean ± SE; %) of FM4-64 versus Kir4.1-EGFP fluorescence indicates reduced appearance of Kir4.1-EGFP at FM4-64-stained plasmalemma in astrocytes treated with 2.5 µM or 25 µM ketamine (2.5 or 25 KM, respectively). The numbers above the top and bottom of the bars indicate the number of cells (b, c) and vesicles (b) analysed, respectively. *$P < 0.05$ (ANOVA on ranks followed by Dunn's test; b,c)

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