Corrigendum: Selective inhibition of 2-AG hydrolysis enhances endocannabinoid signaling in hippocampus

Judit K Makara, Marco Mor, Darren Fegley, Szilárd I Szabó, Satish Kathuria, Giuseppe Astarita, Andrea Duranti, Andrea Tontini, Giorgio Tarzia, Silvia Rivara, Tamás F Freund & Daniele Piomelli

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Our paper identified 6-methyl-2-*p*-tolylaminobenzo[*d*]oxazin-4-one (URB754; Specs) as a monoacylglycerol lipase (MGL) inhibitor that enhances hippocampal depolarization-induced suppression of inhibition (DSI). However, in subsequent tests of non-commercial URB754, we failed to replicate these results, suggesting that a bioactive impurity was present in the commercial material. We have identified this impurity as bis(methylthio)mercurane (**Supplementary Results** online). Because this compound interacts with multiple targets, we tested another MGL inhibitor, methylarachidonylfluorophosphonate (MAFP), which prolonged DSI (**Fig. 1**), confirming that monoacylglycerol lipase contributes to the termination of DSI, as others have reported[1]. Another generation of endocannabinoid metabolism inhibitors is needed to test this hypothesis further.

Note: Supplementary information is available on the Nature Neuroscience website.

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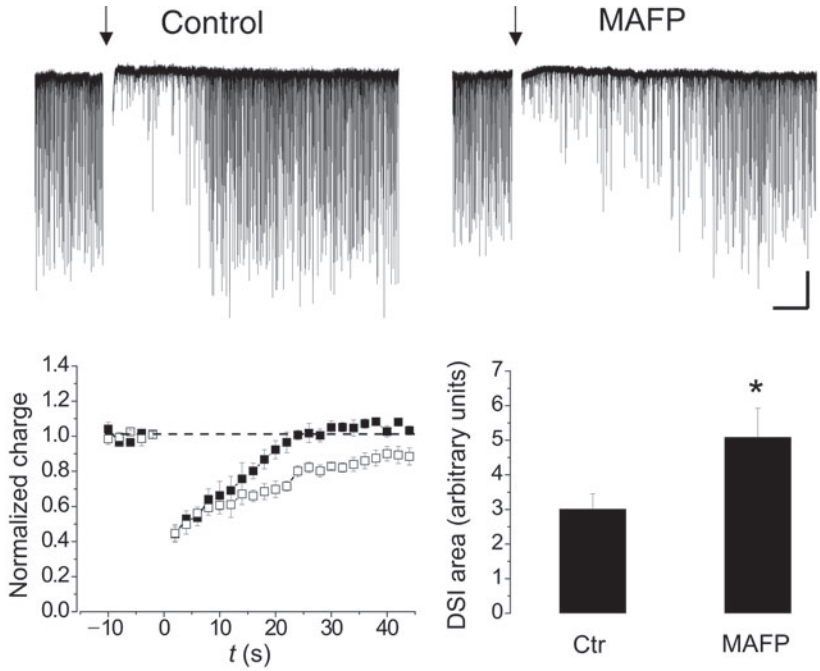

Figure 1 Effects of MAFP on DSI in hippocampal CA1 pyramidal cells. Top, traces from a representative experiment showing the effects of vehicle (ethanol, 0.00003%) or MAFP (Tocris, 45 nM) on the transient reduction of spontaneous inhibitory postsynaptic potentials (IPSCs) elicited by a depolarizing stimulus (arrow). Scale bars, 100 pA, 5 s. Bottom left, averaged time-course of DSI after administration of vehicle (solid squares) or MAFP (open squares). Bottom right, DSI area in the first 30 s after stimulus application was significantly larger in MAFP-treated than in control slices.

Erratum: Reduced sodium current in GABAergic interneurons in a mouse model of severe myoclonic epilepsy in infancy

Frank H Yu, Massimo Mantegazza, Ruth E Westenbroek, Carol A Robbins, Franck Kalume, Kimberly A Burton, William J Spain, G Stanley McKnight, Todd Scheuer & William A Catterall

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In the version of this article initially published, the acceptance date was incorrect. The paper was accepted on 2 August 2006. This error has been corrected in the PDF versions of the article.

Erratum: The many roots of aggression

Jordan Grafman, Maren Strezniak & Frank Krueger

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In the version of this article initially published, the second author's name was spelled incorrectly. The correct name should be Maren Strenziok. The error has been corrected in the HTML and PDF versions of the article.

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Selective inhibition of 2-AG hydrolysis enhances endocannabinoid signaling in hippocampus

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The functions of 2-arachidonoylglycerol (2-AG), the most abundant endocannabinoid found in the brain, remain largely unknown. Here we show that two previously unknown inhibitors of monoacylglycerol lipase, a presynaptic enzyme that hydrolyzes 2-AG, increase 2-AG levels and enhance retrograde signaling from pyramidal neurons to GABAergic terminals in the hippocampus. These results establish a role for 2-AG in synaptic plasticity and point to monoacylglycerol lipase as a possible drug target.

The endocannabinoids modulate brain neurotransmission by activating CB_1 cannabinoid receptors mostly localized on axon terminals of GABAergic interneurons[1]. In the hippocampus, an endocannabinoid released from depolarized pyramidal cells transiently depresses GABA release from basket cell terminals in a retrograde signaling process called depolarization-induced suppression of inhibition (DSI)[2–4]. Although the role of CB_1 receptors in DSI is well documented, the identity of its endocannabinoid mediator remains elusive. Two molecules, anandamide and 2-AG, meet the defining criteria of an endocannabinoid. They are produced by neurons in an activity-dependent manner, they engage CB_1 receptors with high affinity and they are eliminated through regulated transport and intracellular hydrolysis[5]. In neurons, the hydrolysis of anandamide and 2-AG is catalyzed by two distinct serine hydrolases: fatty-acid amide hydrolase (FAAH), which cleaves anandamide and other lipid amides[6], and monoacylglycerol lipase (MGL), which hydrolyzes 2-AG and other 2-monoacylglycerols[7]. This catabolic segregation offers the opportunity to investigate the functions of each endocannabinoid by blocking its deactivation and thereby amplifying its actions at CB_1 receptors. Using this approach, it has been shown that inhibition of FAAH activity does not affect hippocampal DSI[8], which suggests that neither anandamide nor other FAAH substrates with cannabinoid-like activity (for example, virodhamine and *N*-arachidonoyl-dopamine)[9,10] contribute to this process.

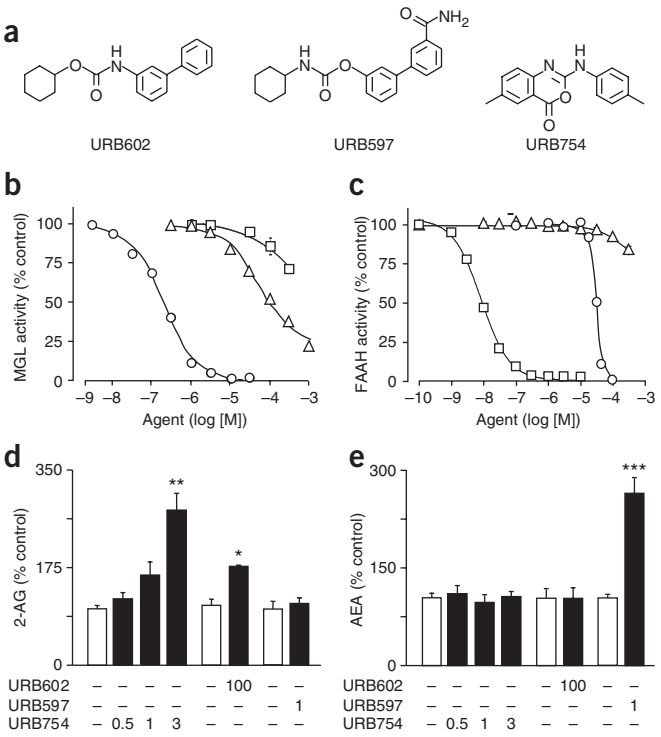

Figure 1 URB754 is a potent MGL inhibitor. (**a**) Chemical structures of MGL inhibitors URB602 (left), URB754 (right) and FAAH inhibitor URB597 (center). (**b**) Effects of URB754 (circles), URB602 (triangles) and URB597 (squares) on activity of recombinant rat brain MGL expressed in HeLa cells. (**c**) Effects of URB597, URB754 and URB602 on native rat brain FAAH activity. (**d**) URB754 (0.5–3 μM; 10 min) and URB602 (100 μM) elevated 2-AG levels in rat forebrain slice cultures, whereas URB597 (1 μM) did not. (**e**) URB597 elevated anandamide (AEA) levels in the same cultures, whereas URB754 and URB602 did not. Changes in endocannabinoid levels are expressed as percentage of control values, which were (in pmol mg^{-1} protein) 221.5 ± 67.6 (**d**) and 3.4 ± 1.9 (**e**). *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$, one-way ANOVA followed by Dunnett's test ($n = 6–8$). Enzyme assays, culture conditions and endocannabinoid measurements were conducted as outlined in **Supplementary Methods**. All experimental procedures were in accordance with Society for Neuroscience and European Union guidelines and were approved by the institutional animal care and use committees.

To test the alternative hypothesis that 2-AG mediates DSI, we first used the compound URB602, a non-competitive MGL inhibitor that blocks 2-AG hydrolysis in rat brain slices without affecting FAAH-catalyzed anandamide degradation[11] (**Fig. 1**). Depolarization

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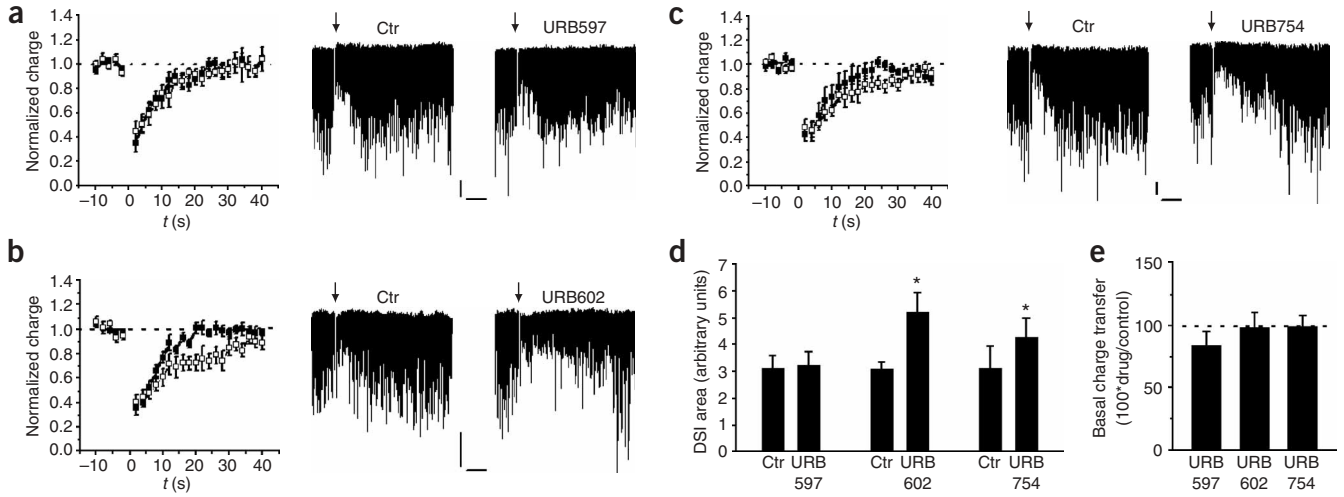

Figure 2 Inhibition of MGL, but not FAAH, activity prolongs DSI in CA1 pyramidal cells. (**a**–**c**) Time course of DSI measured in the same cell before (filled squares) and after (open squares) treatment with (**a**) URB597 (0.1 μM; $n = 7$); (**b**) URB602 (100 μM; $n = 8$) or (**c**) URB754 (0.5 μM; $n = 7$). Panels on the right show representative DSI traces from individual experiments before and after drug application. Arrows indicate the depolarizing stimuli used to elicit DSI (from –60 mV to 0 mV, 1 s). Scale bars, 100 pA, 10 s. (**d**) Application of URB754 (0.5 μM) and URB602 (100 μM), but not URB597 (0.1 μM), led to greater DSI area than in control (Ctr). (**e**) The drugs did not influence basal charge transfer. *, $P < 0.05$, Student's *t*-test.

of CA1 pyramidal neurons elicited DSI in ~50% of the cells tested. Peak DSI was reduced by 55.0 ± 2.6% compared with control ($n = 37$) and was followed by a complete recovery of inhibitory activity (**Fig. 2a–c**). As previously reported, superfusion with the CB_1 antagonist AM251 (2 μM, 8–12 min) reduced peak DSI[2–4] from 52.5 ± 10.2% to 13.4 ± 3.4% ($P < 0.05$; $n = 4$), whereas 0.1 μM of FAAH inhibitor URB597 (refs. 8,12; **Fig. 1a**) had no effect on either DSI (charge transfer reduction, measured as DSI area in the first 30 s after the depolarizing stimulus, was 3.1 ± 0.5 for control and 3.2 ± 0.5 for URB597; **Fig. 2a,d**; $P > 0.79$, $n = 7$) or basal charge transfer (84.0 ± 10.9% of control, **Fig. 2e**; $P > 0.19$; $n = 7$). In contrast, the MGL inhibitor URB602 (100 μM) delayed the recovery from DSI and increased charge transfer reduction (control: 3.1 ± 0.2, URB602: 5.2 ± 0.7; **Fig. 2b,d**; $P < 0.05$; $n = 8$). This effect was blocked by the CB_1 antagonist AM251 (**Supplementary Fig. 1**) and was not accompanied by changes in amplitude and decay time constant of depolarization-evoked Ca^{2+} signals, as measured by two-photon microscopy (**Supplementary Fig. 2**). Furthermore, though prolonged in the presence of URB602, DSI returned to control levels by the time of the next stimulation, indicating that occlusion did not occur at the drug concentration used in these experiments (**Fig. 2e**; basal charge transfer: 98.6 ± 11.9%, $P > 0.91$).

URB602 inhibits 2-AG hydrolysis selectively, but with low potency[11]. To discover more potent and selective agents, we screened a focused library of serine hydrolase inhibitors with electrophilic carbonyl groups in different chemical environments, whose shape, size and lipophilicity were comparable to those of URB602. This screening led to the identification of URB754 (**Fig. 1a**), a benzoxazin-4-one related to known elastase and intestinal lipase inhibitors[13,14]. URB754 inhibited MGL activity with a half-maximal concentration (IC_{50}) of 200 ± 16 nM ($n = 3$), about two orders of magnitude more potently than did URB602 ($IC_{50} = 75 \pm 7$ μM in rat brain MGL expressed in HeLa cells (**Fig. 1b**) and 28 ± 4 μM in native rat brain MGL[11]). Kinetic analyses indicated that inhibition occurred through a noncompetitive mechanism (**Supplementary Table 1**). Moreover, overnight dialysis did not restore activity in MGL preparations incubated with URB754 (1 μM), suggesting that inhibition was irreversible (data not shown). In contrast, much higher concentrations of URB754 were needed to affect rat brain FAAH activity ($IC_{50} = 31.8 \pm 3.8$ μM; $n = 4$; **Fig. 1c**), binding of [^{3}H]-Win-55212-2 to rat cerebellar CB_1 receptors ($IC_{50} = 10 \pm 3.8$ μM, $n = 4$) and cyclooxygenase (Cox)-1 or Cox-2 activities ($IC_{50} >$ 100 μM). Consistent with these results, incubation with URB754 (0.1–3 μM) reduced MGL activity in intact rat forebrain slice cultures ($IC_{50} = 450 \pm 7$ nM, $n = 6$) and increased 2-AG accumulation (**Fig. 1d**) but did not change the levels of anandamide (**Fig. 1e**) or other FAAH substrates (**Supplementary Fig. 3**). URB602 (100 μM) produced a similar, albeit weaker, effect (**Fig. 1d**), whereas the FAAH inhibitor URB597 (1 μM) acted in the opposite manner, preventing anandamide degradation without influencing 2-AG levels (**Fig. 1d,e**). Finally, incubation of acutely prepared hippocampal slices with URB754 (0.5 μM) significantly prolonged DSI (**Fig. 2c**), increasing charge transfer reduction from 3.1 ± 0.8 to 4.3 ± 0.7 (**Fig. 2d**, $P < 0.05$; $n = 7$) without occlusion (**Fig. 2e**, basal charge transfer: 98.5 ± 9.3% of control, $P > 0.87$). However, at 3 μM, URB754 elicited a gradual occlusion of DSI that was blocked by AM251 (**Supplementary Fig. 4**), suggesting that at this concentration the inhibitor caused a marked accumulation of non-metabolized 2-AG (**Fig. 1d**).

The functions of 2-AG in synaptic plasticity have been previously inferred from experiments with non-specific biosynthesis inhibitors[5] or Cox-2 inhibitors[8], whose effects on brain endocannabinoid metabolism are unknown. Our results, showing that blockade of intracellular MGL activity selectively increases brain 2-AG levels and prolongs hippocampal DSI, provide unambiguous evidence that 2-AG mediates this form of retrograde signaling. Potent and selective MGL inhibitors designed on the scaffolds of URB602 and URB754 will provide a valuable tool to explore the physiological roles of 2-AG and validate MGL as a drug target.

Note: Supplementary information is available on the Nature Neuroscience website.

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COMPETING INTERESTS STATEMENT

The authors declare competing financial interests (see the *Nature Neuroscience* website for details).

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