Agonist-Induced Desensitization of Mu-Opioid Receptors depends on their cellular localization.

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Agonist-induced desensitization of mu opioid receptors (MOPrs) is one of the leading mechanisms thought to underlie the development of tolerance to opioid drugs such as morphine and heroin. Although many studies have investigated desensitization of MOPrs located on neuronal cell bodies, few have examined nerve terminal MOPrs. In this study, we have investigated desensitization of MOPrs located at nerve terminals of GABAergic interneurones in the mouse ventral tegmental area (VTA); a brain region implicated in the rewarding properties of numerous drugs of abuse. We have previously demonstrated that although cell body MOPrs rapidly desensitized by more than 50% over a 10 minute agonist application, nerve terminal receptors in these neurones did not exhibit any desensitization over the same time period (Lowe and Bailey, 2010). We have further explored this differential regulation.

250 micron-thick horizontal VTA slices from 3-4 week old male C57Bl/6J mice were prepared. Evoked GABAergic inhibitory post-synaptic currents (eIPSCs) were recorded in dopaminergic neurones using whole-cell patch-clamp techniques as described previously (Lowe & Bailey, 2010). For all experiments, to isolate the effects of MOPrs at GABAergic nerve terminals, extracellular fluid contained kynurenic acid (2 mM), strychnine (1 µM), sulpiride (10 µM), tertiapinQ (250 nM) and barium chloride (1 mM). In most experiments, the receptor reserve was removed by pretreating slices with the MOPr irreversible antagonist β-FNA (6 µM) for 30 minutes.

In the absence of spare receptors, MOPr agonists morphine (300 µM) and DAMGO (10 µM) inhibited the amplitude of eIPSCs by 41±7% (n=5) and 50±5% (n=5), respectively. The response to either agonist did not desensitize over a 10 minute application (inhibition at 10 min: morphine, 47±8%; DAMGO, 47±7%). Because activation of PKC has been shown to enhance morphine mediated desensitization in other brain regions (Bailey et al., 2004), we treated slices with the phorbol ester PMA (1 µM). Morphine still did not appear to induce desensitization during a 10 minute application (peak inhibition: 26±7%, 10 min: 24±6%, n=5). However, PMA treatment dramatically reduced the peak morphine response compared to untreated slices suggesting that desensitization may have occurred during the slow onset of morphine action (> 5 minutes). PMA did not affect the peak response to DAMGO (47±9%, n=5), nor promote DAMGO desensitization over the 10 minute application (49±10%, n=5). Therefore, PKC selectively enabled morphine induced desensitization of nerve terminal MOPrs.

Although the high efficacy MOPr agonists DAMGO and Met-Enkephalin were unable to promote rapid desensitization of nerve terminal receptors, treating slices for 7-10 hours with 30 µM Met-Enkephalin induced a dramatic reduction in nerve terminal MOPr function (post-treatment: eIPSC inhibition produced by 30 µM Met-Enkephalin was 18±9% vs control: 69±8%, n=5-9, p=0.003 Student's t-test), even under conditions where the receptor reserve had not previously been removed.

These findings suggest that, in the mouse ventral tegmental area, MOPrs located at nerve terminals desensitize very slowly to high efficacy agonists, requiring hours of agonist activation, whereas cell body receptors within the same neurones desensitize within minutes of agonist activation. Surprisingly, only the lower efficacy agonist morphine was able to promote very rapid nerve terminal receptor desensitization, but this effect required PKC activation. The dramatic difference in the rate of regulation of nerve terminal and cell body MOPrs may have far reaching implications for the regulation of all G-protein coupled receptors located at nerve terminals.

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