Lack Of Agonist-Induced Desensitization Of Mu-Opioid Receptors Located At Nerve Terminals

Janet Lowe, Chris Bailey

University of Bath, Department of Pharmacy & Pharmacology, BA2 7AY, Bath, United Kingdom

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 cell bodies, few have examined MOPrs located at nerve terminals. In the ventral tegmental area (VTA), a brain region implicated in the rewarding properties of numerous drugs of abuse, MOPrs are located on GABAergic interneurones both somatodendritically (i.e. on cell bodies and dendrites) and on nerve terminals that innervate dopaminergic neurones. Using brain slice electrophysiological methods, we have investigated agonist-induced desensitization of both populations of MOPrs.

We prepared 250 micron-thick horizontal VTA slices from 3-4 week old male C57Bl/6J mice in ice cold cutting ACSF and then incubated and recorded from slices in ACSF at 32° using standard whole-cell patch-clamp electrophysiological methods as described previously (Ford et al., 1996): intracellular fluid (10 mM NaCl, 20 mM KCl, 85 mM K-methanesulfonate, 10 mM HEPES, 2 mM MgCl2, 10 mM phosphocreatine, 2 mM MgATP, 0.25 mM Na2GTP). To measure responses from MOPrs located at nerve terminals of GABAergic interneurones, we recorded miniature GABAergic inhibitory post synaptic currents (mIPSCs) from dopaminergic cells using a holding potential of -80 mV in the presence of tetrodotoxin (1 µM), kynurenic acid (2 mM), strychnine (1 µM), and sulpiride (10 µM). Dopaminergic cells were identified by the presence of an Ih current and a response to exogenously-applied dopamine. To measure responses from MOPrs located somatodendritically in GABAergic interneurones, MOPr-evoked K+ currents were measured in response to hyperpolarizing pulses from -60 mV to -120 mV in a high potassium altered ACSF (10 mM KCl, 118.5 mM NaCl).

Both morphine (30 µM) and DAMGO (10 µM) inhibited the frequency of mIPSCs (by 54.3±4.5%, n=5 and 64.4±7.2%, n=4, respectively). This inhibition was sustained over 10 minutes of morphine or DAMGO application (61.1±3.9% and 61.0±7.8%, respectively). To ensure that the presence of spare receptors did not mask desensitization, the receptor reserve was removed by treating slices with the irreversible MOPr antagonist β-FNA (6 µM) for 30 minutes, which significantly decreased the peak DAMGO inhibition of mIPSC frequency to 34.9±9.5% (n=5; t-test, p=0.03). Even after β-FNA treatment (i.e. in conditions where there was no receptor reserve), neither morphine nor DAMGO inhibition of mIPSC frequency desensitized over the 10 minute application period (morphine: 28.6±11.8% to 31.7±14.6%, n=10, DAMGO: 34.9±9.5% to 27.7±14.9%, n=5).

In contrast, MOPrs at the cell bodies of GABAergic neurones rapidly desensitized. DAMGO (10 µM) induced a K+ current that rapidly desensitized during a 10 minute application (137.7±14.0 pA to 67.7±7.9 pA after 10 minutes (n=3; t-test, p=0.006)).

These findings suggest that, in the mouse ventral tegmental area, MOPrs located at nerve terminals do not readily desensitize, or do so by different mechanisms to those located at cell bodies.


Funded by MRC