Effects of fatty acid amide hydrolase (FAAH) inhibitors on dorsal root afferent-mediated synaptic transmission in dorsal horn spinal cord neurones of adult rats *in vitro*



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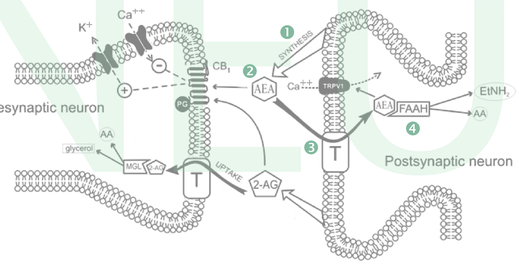
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❶ INTRODUCTION

Cannabinoid receptor agonists reduce allodynia and hyperalgesia associated with nerve injury-induced models of neuropathic pain. Both synthetic and endogenous cannabinoids (endocannabinoids), such as arachidonoyl ethanolamide (anandamide, AEA) and 2-arachidonoyl glycerol (2-AG), exert anti-allodynic, anti-hyperalgesic and anti-inflammatory actions in these chronic pain models via activity at CB1 and CB2 receptors.

The actions of endocannabinoids are terminated by removal from the extracellular space (anandamide via an anandamide membrane transporter), then subsequent enzymatic degradation. Two enzymes that metabolise endocannabinoids, are fatty-acid amide hydrolase (FAAH) and monoglyceride lipase (MGL), which preferentially degrade anandamide and 2-AG, respectively. Inhibition of the degradation of endogenous cannabinoids through the use of Fatty Acid Amide Hydrolase (FAAH) enzyme inhibitors to prolong activity of endocannabinoids, therefore constitutes an alternative strategy for cannabinoid-mediated analgesia without the motor and psychotropic CB1 receptor-mediated side effects associated with cannabinoid receptor agonists.

In view of this, in the present study we examined the effects of three FAAH inhibitors, URB597, OL-135 and Ab-2 on dorsal root afferent-mediated synaptic transmission in Lamina I/II neurones in the dorsal horn of adult rat spinal cord slice preparations.



❶ Endocannabinoids are synthesised in and released from the membrane of post-synaptic neurones on demand after calcium influx . ❷ This activates pre-synaptic CB1 receptors and inhibits neuronal activity. ❸ Anandamide (AEA) is removed from the synaptic cleft by re-uptake into the post-synaptic cleft. ❹ Once within the neurone, AEA undergoes hydrolysis by FAAH.

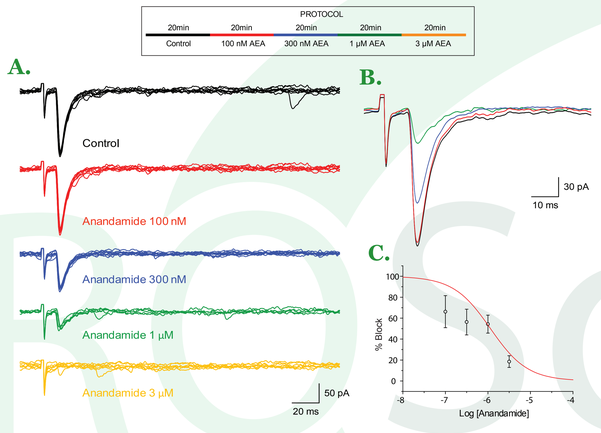
❷ METHODS

Adult male Sprague-Dawley rats (> 7 weeks) were terminally anaesthetised with isoflurane, cervically dislocated and decapitated and whole-cell patch-clamp recordings were performed at 32-35 °C from dorsal horn neurones in spinal cord slices using an Axopatch 1D amplifier. A bipolar concentric stimulating electrode, was placed on the dorsal root or dorsal root entry zone to evoke EPSCs in recorded neurones (3-30 V, 0.1 ms, 0.1 Hz).

The following drugs were used at the final concentrations indicated: Ab-2 (50 nM), OL-135 (1 µM), URB597 (100 nM), R(+)-Methanandamide (150 nM), O-2050 (100 nM). All drugs were administered to the slice by perfusion from 50 ml syringes arranged in line with the main aCSF reservoir by a series of three-way valves. The reported agonist final concentrations represent the concentrations within the perfusion system and do not take into account dilution within the recording chamber.

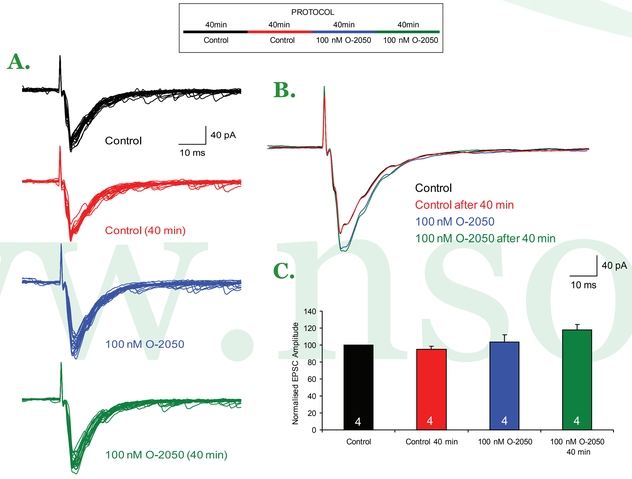
❸ Anandamide decreased evoked EPSCs in dorsal horn neurones

(**A**) Whole-cell voltage-clamp recordings (Vh -60 mV) showing the concentration-dependent decrease in electrically evoked EPSC amplitude upon application of anadamide (AEA). (**B**) Superimposed plot of the effects on AEA. Each trace is the average of 10 consecutive sweeps. (**C**) Concentration-inhibition curve showing the effect of AEA on dorsal root evoked EPSCs in dorsal horn neurones. (n=6). The calculated IC50 value was 156 ± 36 nM



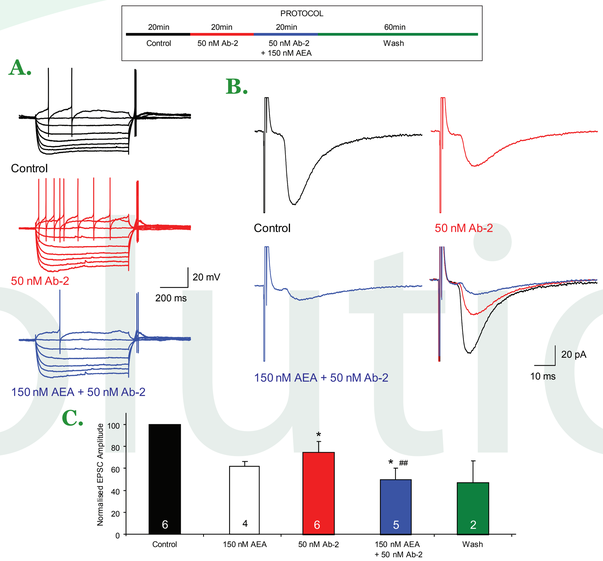
❹ CB1 receptor antagonism enhances evoked EPSCs

(**A**) Whole-cell voltage-clamp recordings (Vh -60 mV) showing the increase to 110.0 ± 12.0% of control values in electrically evoked EPSC amplitude upon application of the CB1-receptor antagonist O-2050 (n=4). (**B**) Superimposed plot of the effects of O-2050 with accompanying summary bar charts (**C**). Each trace is the average of 10 consecutive sweeps.



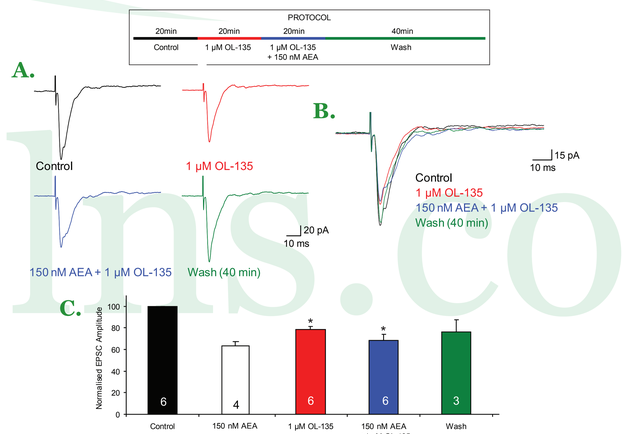
❺ Effects of the FAAH inhibitor Ab-2

(**A**) Superimposed samples of a continuous whole-cell current clamp recording showing the effect of 50 nM AB-2. (**B**) Whole-cell voltage-clamp recordings (Vh -60 mV) showing the decrease in electrically evoked EPSC amplitude to 76.9 ± 6.4% of control levels upon application of Ab-2 (n=6) and further decrease in amplitude to 54.5 ± 7.3% of control levels in the presence of AEA + Ab-2 with accompanying summary bar charts (**C**). Each trace is the average of 10 consecutive sweeps. (* $P < 0.05$ vs. control, ## $P < 0.01$ vs. AEA).



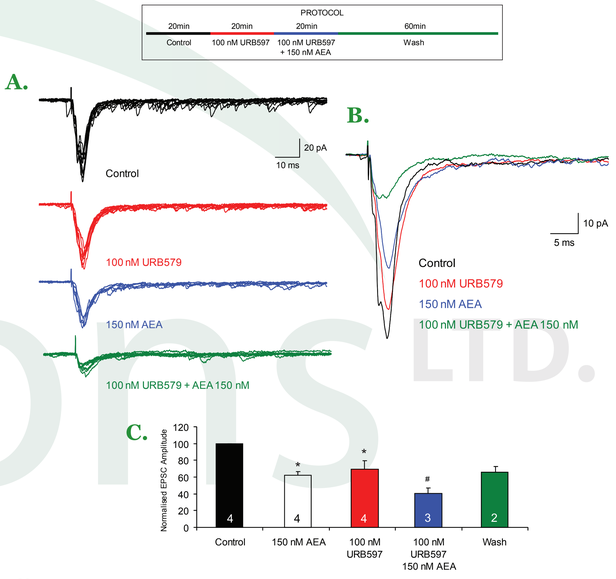
❻ Effects of the FAAH inhibitor OL-135

(**A**) Whole-cell voltage-clamp recordings (Vh -60 mV) showing the decrease in electrically evoked EPSC amplitude to 82.0 ± 3.1% of control levels upon application of OL-135 (n=6) and further decrease in amplitude to 72.0 ± 4.8% in the presence of AEA + OL-135 with accompanying summary bar charts (**C**). Each trace is the average of 10 consecutive sweeps. (* $P < 0.05$ vs. control).



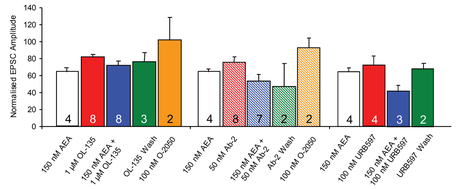
❼ Effects of the FAAH inhibitor URB597

(**A**) Whole-cell voltage-clamp recordings (Vh -60 mV) showing the decrease in electrically evoked EPSC amplitude to 70.± 9.7% of control levels upon application of URB597 (n=4) and further decrease in amplitude to 40.6 ± 6.5% in the presence of AEA + URB597 with accompanying summary bar charts (**C**). Each trace is the average of 10 consecutive sweeps. (* $P < 0.05$ vs. control, # $P < 0.05$ vs. URB597).



❽ FAAH inhibitor summary

Summary graph comparing the effects of OL-135, Ab-2 and URB597 on dorsal root-evoked EPSC amplitude in dorsal horn neurones of rat spinal cord.



❾ CONCLUSIONS

The effects of the endogenous endocannabinoid, Anandamide and three FAAH inhibitors were tested on electrically evoked excitatory post-synaptic currents (EPSCs) in dorsal horn neurones of adult rat spinal cord slices. Both anandamide and the three FAAH inhibitors decreased electrically evoked excitatory postsynaptic currents in these neurones while CB1 receptor antagonists enhanced these currents.

These data suggest a role for endocannabinoids in modulating sensory input to dorsal horn neurones and suggest a tonic endocannabinoid tone exists within these neurones. These results suggest modulating the endogenous cannabinoid system by targeting FAAH may be a useful strategy to treat pain.

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