THE ENDOCANNABINOID SYSTEM IN THE PRELIMBIC CORTEX MEDIATES FEAR-CONDITIONED ANALGESIA IN RATS

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Introduction

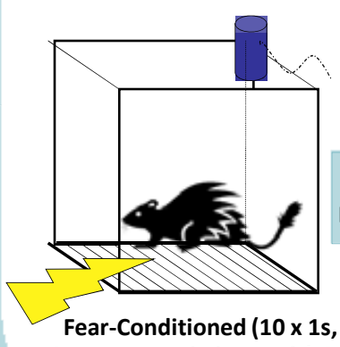
Fear-conditioned analgesia (FCA) is pain suppression expressed upon re-exposure to a neutral context previously paired with an aversive stimulus. Evidence suggests a key role for the endocannabinoid system in FCA[1,2]. The medial prefrontal cortex plays a role in FCA[3], but the role of the endocannabinoid system in the medial prefrontal cortex in FCA has not been investigated. The aim of the present study was to investigate the role of the cannabinoid$_1$ (CB_1) receptor and fatty acid amide hydrolase (FAAH), a key enzyme catalysing the degradation of the endocannabinoid anandamide and related N-acylethanolamines, in the prelimbic cortex (PrL) region of the medial prefrontal cortex, in FCA and formalin-evoked nociceptive behaviour *per se* in rats.

Methods

Schematic representation of fear-conditioned analgesia paradigm

Conditioning Day

Male Lister-Hooded rats (280-350g; n = 7-10 per group)



Fear-Conditioned (10 x 1s, 0.4mA at 1 min intervals) OR Non-Fear-Conditioned

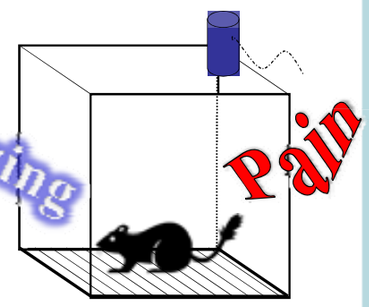
15 mins later

Vehicle (100% DMSO)
URB597 (0.1mM/0.3µL)
AM251 (2mM/0.3µL)
URB597 + AM251
INTRA-PRL MICROINJECTION

23.5 hrs later

Intraplantar Formalin (50µL, 2.5%)

Test Day



Behaviour Recorded

Composite Pain Score (CPS)

$$CPS = \frac{(CP1 + (2 \times CP2))}{\text{Trial Duration}}$$

CP1 = Duration of Paw elevation
CP2 = Duration of Licking, biting, flinching

After 30 minutes all animals sacrificed by decapitation

Brains are removed and snap-frozen for analysis of endocannabinoid levels by LC-MS/MS

Data were analysed using 2-way ANOVA followed by Fisher's LSD post-hoc test

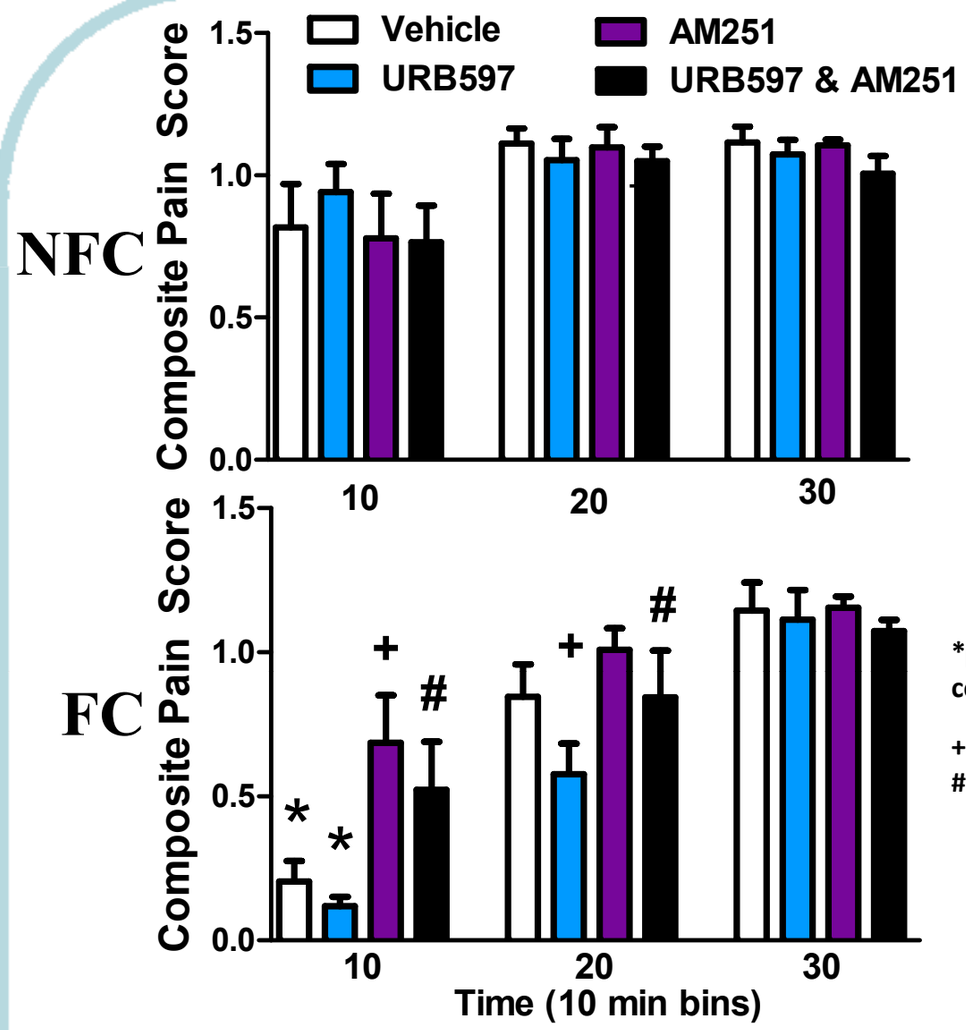
All data are expressed as mean ± SEM
P value < 0.05 deemed significant

Summary and Conclusions

- Formalin-evoked nociceptive behaviour was suppressed in the first 10 minutes of the trial, confirming expression of FCA.
- Duration of freezing was increased in the first 5 minutes of re-exposure to the context paired previously with footshock.
- Intra-PrL administration of AM251 significantly attenuated FCA in the first 10 minutes of the trial. Freezing in the first 5 minutes of the trial was also attenuated by AM251.
- FCA was significantly enhanced/prolonged by intra-PrL administration of URB597 at the 10-20 minute time point, an effect blocked by co-administration of AM251.
- There was no effect of AM251 and/or URB597 on formalin-evoked nociceptive behaviour or freezing in non-fear-conditioned animals.
- There was no significant effect of intra-PrL URB597 administration on AEA, 2-AG, OEA and PEA levels in the PrL of both fear conditioned and non-fear conditioned animals.

In conclusion, these data suggest that CB1 receptors in the PrL mediate FCA and that a FAAH substrate in the PrL enhances/prolongs FCA via CB_1 receptor activation. The results suggest an important role for the endocannabinoid system in the PrL in this potent form of endogenous analgesia.

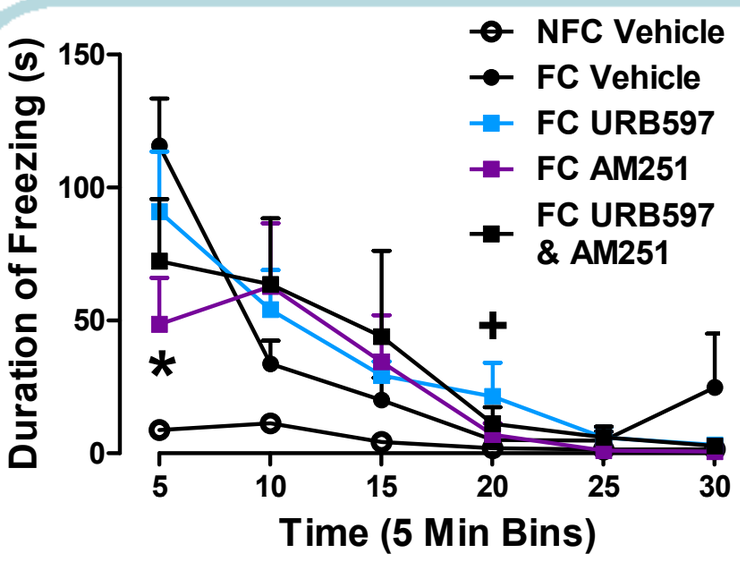
Results



*p<0.05 *vs* NFC counterparts

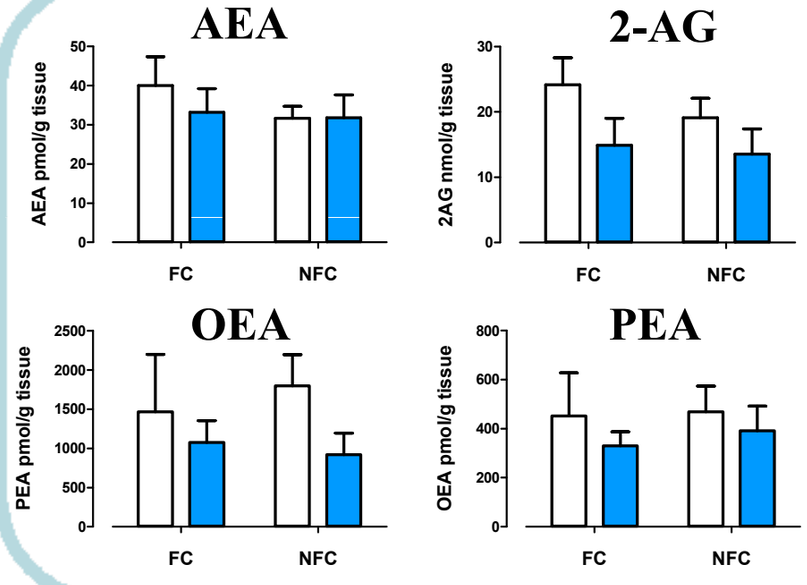
+ p<0.05 *vs* FC Vehicle
# p<0.05 *vs* FC URB597

Fear-conditioned rats exhibited significantly less formalin-evoked nociceptive behaviour over the first 10 minutes of the trial, confirming the expression of FCA. Intra-PrL AM251 significantly attenuated FCA in the first 10 minutes of the trial. Intra-PrL URB597 significantly enhanced/prolonged FCA from 10-20 min, an effect blocked by co-administration of AM251. There was no effect of AM251 and/or URB597 on formalin-evoked nociceptive behaviour in non-fear-conditioned animals. FC: Fear conditioned.



* p<0.01 for FC AM251 *vs* FC Vehicle
\+ p=0.05 for FC URB597 *vs* FC Vehicle

Fear –conditioned rats exhibited an increased duration of freezing in the first 5 minutes of the trial. Freezing in the first 5 minutes of the trial was also attenuated by AM251. Intra-PrL URB597 significantly enhanced duration of freezing from 15-20 min.



There was no significant effect of intra-PrL URB597 on AEA, 2-AG, OEA and PEA levels in the PrL of either fear conditioned or non-fear conditioned animals.

References

1. Finn *et* al., (2004) Eur J Neurosci., 20, 848-852.
2. Butler *et* al., (2008) Pain., 140,491-500
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The endocannabinoid system in the prelimbic cortex mediates fear-conditioned analgesia in rats

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Fear-conditioned analgesia (FCA) is pain suppression expressed upon re-exposure to a neutral context previously paired with an aversive stimulus. Evidence suggests a key role for the endocannabinoid system in FCA [1,2]. The medial prefrontal cortex plays a role in FCA [3], but the role of the endocannabinoid system in the medial prefrontal cortex in FCA has not been investigated. The aim of the present study was to investigate the role of the cannabinoid$_1$ (CB_1) receptor and fatty acid amide hydrolase (FAAH), a key enzyme catalysing the degradation of the endocannabinoid anandamide and related *N*-acylethanolamines, in the prelimbic cortex (PrL) region of the medial prefrontal cortex, in FCA and formalin-evoked nociceptive behaviour *per se* in rats.

Male Lister-Hooded rats (280–350g; n=7–10 per group) were bilaterally implanted with guide cannulae 1mm above the PrL under 2–3% isoflurane/O_2 anaesthesia. 6–7 days post-surgery, fear-conditioned animals received 10 × 1s footshocks (0.4mA, 1min intervals) in a Perspex arena (the context). Non-footshock controls were included. 23.5 hours later, animals received intra-plantar injection of formalin (50µL, 2.5%) into the right hindpaw under brief isoflurane anaesthesia. 15 minutes later, animals received bilateral intra-PrL microinjection of either the CB_1 receptor antagonist AM251 (2mM/0.3µL), the FAAH inhibitor URB597 (0.1mM/0.3µL), co-administration of both drugs, or vehicle (100% DMSO). Animals were then returned to their homecage before re-exposure to the context 15 minutes later. Formalin-evoked nociceptive behaviour and contextually induced freezing were assessed for 30 minutes by an experimenter blind to treatment, after which rats were euthanized and brains harvested for injection site verification. Only those animals with microinjections placed accurately and bilaterally in the PrL were included in the data analysis. Data were analysed by repeated measures or two-way ANOVA followed by Fisher's LSD post-hoc test ($p \leq 0.05$ significant). Data are expressed as mean±SEM.

Fear conditioning significantly increased the duration of freezing (NoFC-Veh 24.32±9.31s *vs.* FC-Veh 169.5±18.15s; $p<0.01$) and decreased the formalin-evoked composite pain score (NoFC-Veh 0.90±0.11 *vs.* FC-Veh 0.34±0.09; $p<0.01$) over the first 15 minutes of the trial, confirming the expression of FCA. Intra-PrL administration of AM251 significantly attenuated FCA in the first 15 minutes of the trial (FC-Veh 0.34±0.09 *vs.* FC-AM251 0.76±0.14; $p<0.05$). Freezing in the first 5 minutes of the trial was also attenuated by AM251 (FC-Veh 115.7±17.69s *vs.* FC-AM251 46.06±17.97s; $p<0.01$). Intra-PrL URB597 significantly enhanced FCA from 10–20min (FC-Veh 0.84±0.11 *vs.* FC-URB597 0.57±0.10; $p=0.05$), an effect blocked by co-administration of AM251 (FC-AM251+URB597 0.84±0.16 *vs.* FC-URB597 0.57±0.10; $p<0.05$). There was no effect of AM251 and/or URB597 on formalin-evoked nociceptive behaviour or freezing in non-fear-conditioned animals.

In conclusion, these data suggest that CB_1 receptors in the PrL mediate FCA and that a FAAH substrate in the PrL enhances FCA via CB_1 receptor activation. The results suggest an important role for the endocannabinoid system in the PrL in this potent form of endogenous analgesia.

1. Finn, D.P., Beckett, S.R., Richardson, D., Kendall, D.A., Marsden, C.A., Chapman, V. 2004 Evidence for differential modulation of conditioned aversion and fear-conditioned analgesia by CB1 receptors. Eur J Neurosci, 20, 848–852.

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