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Characterization of cerebral cortical endocannabinoid levels in a rat inguinal surgery model using liquid chromatography–tandem mass spectrometry (LC–MS/MS)

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Background: The brain endocannabinoid system is believed to play significant roles in anti-nociception, fear response, anxiety, and stress. This study investigated the effects of rat inguinal surgery on the levels of endocannabinoids in the cerebral cortex.

Aim: The aim of this study was to investigate the effects of acute post-surgical pain on the levels of endocannabinoids in the cerebral cortex.

Methods: Quantitation of endocannabinoids in the rat cerebral cortex was performed by liquid chromatography–tandem mass spectrometry.

Results: There was no significant difference in the cerebral cortical levels of anandamide (AEA) and 2-arachidonoylglycerol (2-AG) between the sham and surgery experimental groups. However, there were lateralized differences in the levels of these endocannabinoids between the right and left cerebral cortices irrespective of the two groups. The concentrations of AEA and 2-AG were significantly higher in the right cerebral cortex compared to the contralateral cerebral cortex.

Conclusion: Acute post-surgical pain did not induce significant alterations in the cerebral cortical levels of endocannabinoids in this study, but the phenomenon of lateralization of the cerebral cortical AEA and 2-AG levels was observed; this latter finding may be related to the role played by endocannabinoids in fear conditioning.

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Key words: Acute post-surgical pain, endocannabinoids, fear, lateralization, liquid chromatography–tandem mass spectrometry (LC–MS/MS), rat inguinal surgery model.

Background

Endocannabinoid system and acute post-surgical pain

The endocannabinoid system is an endogenous lipid signalling system with cannabimimetic actions (Zogopoulos *et al.* 2013). It is comprised of the cannabinoid receptors: type-1 (CB1) and type-2 (CB2) (Matsuda *et al.* 1990), their endogenous lipid-based ligands: the endocannabinoids, of which anandamide (AEA) and 2-arachidonoylglycerol (2-AG) are the most studied (Devane *et al.* 1992), and the proteins that are responsible for their biosynthesis, transport, and degradation [fatty acid amide hydrolase (FAAH) and monoacylglycerol lipase (MAGL)] (Bari *et al.* 2006). The endocannabinoid system is widely expressed in the central nervous system (Finn & Chapman 2004; Marsicano & Kuner 2008). The CB1 receptor expression has been demonstrated in chief brain regions involved in nociception (Finn & Chapman 2004) and fear (Herkenham *et al.* 1991; Glass *et al.* 1997; Mailleux *et al.* 1992; Tsou *et al.* 1998). Receptor autoradiography and immunohistochemistry studies have demonstrated the presence of this subtype of cannabinoid receptor in brain regions essential for fear processing, anxiety, and stress, such as the hippocampus, amygdala, bed nucleus of stria terminalis, pre-frontal cortex (PFC), and hypothalamus (Herkenham *et al.* 1991; Glass *et al.* 1997; Mailleux *et al.* 1992; Tsou *et al.* 1998; Puente *et al.* 2010). The CB1 receptors are also expressed in other parts of the cerebral cortex, thalamus, periaqueductal gray (PAG), parabrachial nucleus, cerebellum, and brainstem regions including the rostral ventromedial medulla (RVM) (Herkenham *et al.* 1991; Glass *et al.* 1997; Mailleux *et al.* 1992; Tsou *et al.* 1998). This subtype of cannabinoid receptors is found in the dorsal horn and lamina X of the spinal cord (Pertwee 1997). They are expressed on presynaptic neurons of the central and peripheral nervous system (Rea *et al.* 2007); furthermore, a subset of sensory neurons in the dorsal root ganglia containing substance P and α-calcitonin gene-related peptide also express CB1 receptors (Rea *et al.* 2007). The CB2 receptors are chiefly expressed in tissues

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of the immune defence system (Howlett *et al.* 2002). There is, however, growing evidence that this class of cannabinoid receptor may well be expressed in the central nervous system (Van Sickle *et al.* 2005; Onaivi *et al.* 2006; Zhang *et al.* 2003; Beltramo *et al.* 2006). Both the CB1 (Matsuda *et al.* 1990) and CB2 (Munro *et al.* 1993) cannabinoid receptors are members of the Gi/o protein-coupled receptor family. They are negatively coupled to the enzyme adenylyl cyclase (Howlett *et al.* 1998) and positively coupled to mitogen-activated protein kinase (MAPK) (Bouaboula *et al.* 1995).

The endocannabinoids are believed to be biosynthesized when needed and are thought to function in a retrograde fashion, whereby they are released from the post-synaptic neuron and bind to receptors expressed pre-synaptically (Elphick & Egertova 2001; Kreitzer & Regehr 2002). The degradation of endocannabinoids occurs within the cell; there is, however, some uncertainty regarding the mechanism of reuptake into the cells (Rea *et al.* 2007). Carrier-mediated transport as a means of transporting endocannabinoids into the cell is probable. In regard to AEA transport, there is evidence for a putative AEA transport protein (Beltramo *et al.* 1997; Beltramo & Piomelli 2000). Endocannabinoids are degraded by FAAH and MAGL in the cell (Rea *et al.* 2007; Desarnaud *et al.* 1995; Deutsch & Chin 1993). The FAAH (Cravatt *et al.* 1996) and MAGL (Dinh *et al.* 2002) demonstrate selectivity for AEA and 2-AG, respectively.

There is increasing evidence for the involvement of brain cannabinoid receptors in pain modulation (Rea *et al.* 2007). The inhibitory effects of the cannabinoid receptor agonist WIN 55,212-2 (an aminoalkylindole derivative) on spinal neuronal pain-evoked responses were ablated upon transection of the spinal cord, suggesting that the descending inhibitory pathways do have a significant role to play in mediating antinociception induced by cannabinoids (Hohmann *et al.* 1999). The WIN 55,212-2 was also shown to have anti-nociceptive effect in the tail flick test upon the injection of this cannabinoid receptor agonist into the amygdala, thalamus, PAG matter, and rostral ventral medulla (Martin *et al.* 1999). Among other supra-spinal structures and brain nuclei, the rostral ventral medulla, and the PAG matter, both constituent parts of the descending pain pathway have been shown to be significantly implicated in cannabinoid-induced antinociception (Martin *et al.* 1998; Meng *et al.* 1998; Walker *et al.* 2002; Lichtman *et al.* 1996). There is also compelling evidence for the involvement of the endocannabinoid system in regulation of fear, anxiety, and stress (Ruehle *et al.* 2012; Mechoulam & Parker 2013; Lafenêtre *et al.* 2007; Akirav 2011; Hill *et al.* 2009).

This research project employed a rat inguinal surgery model to investigate the effect of post-surgical pain on the levels of brain endocannabinoids.

The rat inguinal surgery model as a model of acute post-surgical pain

The rat inguinal surgery model is an animal model of post-surgical pain developed by Dr David Finn and colleagues at the National University of Ireland, Galway. This model of post-surgical pain is largely an adaptation of the Lichtenstein technique of inguinal hernia repair in humans. The Lichtenstein hernia repair technique happens to be the first tension-free technique of inguinal hernia repair (Martin *et al.* 2004). It was pioneered by Dr Irving Lichtenstein (LeBlanc 2003). The Lichtenstein technique was introduced in 1986 (LeBlanc 2003); it relies on the use of a synthetic mesh trimmed to match the inguinal canal floor size (Amid *et al.* 1996), thus reinforcing the fascia transversalis (Amid *et al.* 1996).

The possible effects of acute post-surgical pain on the levels of endocannabinoids in the cerebral cortex were assessed. The investigation encompassed rat inguinal surgery, brain dissection, and quantitation of endocannabinoids in the cerebral cortex using capillary liquid chromatography coupled to tandem mass spectrometry (LC–MS/MS).

Hypothesis

Post-surgical pain arising from rat inguinal surgery is associated with alterations in the levels of endocannabinoids (AEA and 2-AG) in the cerebral cortex, with the character of these alterations being consistent with the anti-nociceptive role thought to be played by these compounds in pain modulation.

Objectives of the research project

The principal aim of this research project was to investigate the effect of inguinal surgery on the levels of AEA and 2-AG in the rat cerebral cortex.

Materials

Animal husbandry materials included adult male Lister-Hooded rats (225–300 g; Charles River, UK), rat cages (North Kent Plastics, UK), water bottles (North Kent Plastics, UK), absorbent padding Vlesi bed sheet (Fleming Medical, Ireland), rat chow (Harlan Teklad, UK), temperature/humidity monitor (Radionics Ltd, Ireland), and weighing scales (Mason Technology, Ireland).

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Dissection materials included dissection kit (Fannin Medical Co., Ireland), scalpel blades (Swann-Morton, UK), and syringes (BD Microlance, UK).

Computer software included Microsoft Office (Microsoft Ireland, Ireland), SPSS/PASW (versions 15–18; SPSS Inc., USA), and Graph Pad Prism (version 5; Graph Pad Software Inc., USA).

Methods

Animals

Experiments were carried out in adult male Lister-Hooded rats (225–300 g on arrival; Charles River, UK) maintained at a constant temperature (21 ± 2 °C) under standard lighting conditions (12:12 hours light:dark, lights on from 08:00 to 20:00 hours). Experiments were undertaken during the light phase between 08:00 and 17:00 hours. Food and water were available *ad libitum*. The experimental protocol was carried out following approval from the Animal Care and Research Ethics Committee, National University of Ireland, Galway, under license from the Department of Health and Children of the Republic of Ireland and in compliance with the EU Directive 86/609.

Experimental procedure

The experiment consisted of two phases: an *in vivo* phase and an *ex vivo* phase. Male Lister-Hooded rats were randomly assigned to one of two groups: a sham group ($n = 10$) and a surgery group ($n = 9$). The sham and the surgery groups, respectively, constituted the control and test groups in this experiment.

In vivo phase

The *in vivo* phase of the experiment consisted of the following procedures or interventions: sham procedure, rat inguinal surgery, animal sacrifice (decapitation), brain and spinal cord removal, and storage. The sham procedure entailed anaesthetizing the experimental animals belonging to the sham group with isoflurane in conjunction with pure oxygen: isoflurane 3% was used for induction, and isoflurane 1.4–2% was used for maintenance in 0.5 l/min of oxygen. The sham procedure lasted for 1 hour duration. Inguinal hernia surgery by way of the Lichtenstein technique constituted the surgical procedure. This phase of the experiment was undertaken under license from Dara Bree and Orla Moriarty.

Animal sacrifice

Experimental animals were removed from their home cages and sacrificed by decapitation 2 hours after the sham and surgical procedures. Brains and spinal cords of the animals were removed rapidly, snap-frozen on dry ice, and stored at −80 °C prior to dissection.

Ex vivo phase

Rat brain dissection and grinding of rat brain tissue regions

The frozen rat brains were allowed to thaw for 12–15 minutes. The brain regions of interest were then rapidly dissected out with reference to a rat brain atlas (Paxinos & Watson 1997) on an ice-cold plate with the aid of a scalpel blade and a razor blade, alternating between rats from the sham and surgery groups. The duration of each dissection was approximately 13 minutes. The harvested brain regions included in order of dissection: PFC, hypothalamus, left amygdala, right amygdala, left cortex, right cortex, left striatum, right striatum, left hippocampus, right hippocampus, thalamus, cerebellum, PAG matter, and RVM. The brain regions were weighed into 1.5 ml microfuge tubes on an accurate balance after their removal. Following dissection, all of these brain regions were stored at −80 °C prior to tissue extraction and quantitation of the levels of endocannabinoids.

Brain tissue grinding was done on dry ice using a mortar and pestle; relevant brain regions were crushed to powder. The resulting powdered tissue was aliquoted out into microfuge tubes and stored at −80 °C. Prior to their storage, the tissues were weighed; tubes were pre- and post-weighed frozen.

Quantitation of endocannabinoids in rat cerebral cortex by LC–MS/MS

Quantitation of endocannabinoids by LC–MS/MS was undertaken as described previously (Butler *et al.* 2011; Ford *et al.* 2011; Olango *et al.* 2012). This study focused on quantitation of the levels of endocannabinoids in the cerebral cortex. Tissue extraction was undertaken by way of a lipid extraction method as follows: each brain tissue sample was first homogenized for approximately 4 seconds in 400 µl 100% acetonitrile containing known fixed amounts of deuterated internal standards (0.014 nmol AEA-d8, 0.48 nmol 2-AG-d8, 0.016 nmol PEA-d4, 0.015 nmol OEAd2) using an ultrasonic homogenizer/sonicator (Mason, Ireland). Homogenates were then centrifuged at 14 000 rpm for a duration of 15 minutes at a temperature of 4 °C, and the supernatant was collected and evaporated to dryness in a centrifugal evaporator; the heat time was 120 minutes, and the duration of supernatant evaporation was 120 minutes at a temperature of 45 °C. Following evaporation to dryness, lyophilized samples were resuspended in 40 µl 65% acetonitrile, and 2 µl was injected onto a Zorbax C-18 column (150 × 0.5 mm internal diameter)

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Table 1. *Precursor and product ion mass-to-charge (m/z) ratios for all the analytes and their corresponding deuterated forms*

Precursor	Product ion mass-to-charge (m/z) ratios	Retention times (minutes)
AEA	348.3–62.1	12.2
AEAd8	356.3–63.1	12.1
2-AG	379.3–287.4	13.2
2-AG-d8	387.3–294.2	13.3
PEA	300.3–62.1	16.0
PEA-d4	304.3–62.1	15.9
OEA	326.3–62.1	16.6
OEA-d2	328.3–62.1	16.5

from a cooled autosampler maintained at 4 °C (Agilent Technologies Ltd, Ireland). Mobile phases consisted of A [high-performance liquid chromatography (HPLC) grade water with 0.1% formic acid] and B (acetonitrile), with a flow rate of 12 µl/min. Reversed-phase gradient elution began initially at 65% B, and over 10 minutes was ramped linearly up to 100% B. At 10 minutes, the gradient was held at 100% B up to 20 minutes. At 20.1 minutes, the gradient returned to initial conditions for a further 10 minutes to re-equilibrate the column. The total runtime was 30 minutes. Under these conditions, AEA, 2-AG, PEA, and Oleoylethanolamine were eluted at the following retention times: 12.2, 13.2, 16.0, and 16.6 minutes, respectively. Analyte detection was undertaken in electrospray-positive ionization mode on an Agilent 1100 HPLC system coupled to a triple quadrupole 6460 mass spectrometer (Agilent Technologies Ltd, Ireland). Instrument conditions and source parameters such as fragmentor voltage and collision energy were optimized for each analyte of interest before assaying samples; standards were infused separately. Quantitation of target endocannabinoids was accomplished by positive ion electrospray ionization and multiple reaction monitoring (MRM) mode, allowing for simultaneous detection of the protonated precursor and product molecular ions [M+.H+] of the analytes of interest and the deuterated forms of the internal standards. Precursor and product ion mass-to-charge (m/z) ratios for all analytes and their corresponding deuterated forms are shown in Table 1, see the supplementary data Fig. 1. The quantitation of each analyte was performed by determining the peak area response of each target analyte against its corresponding deuterated internal standard. This ratiometric analysis was performed by the Masshunter Quantitative Analysis Software (Agilent Technologies Ltd, Ireland). The amount of analyte in unknown samples was calculated from the analyte/internal standard peak area response ratio with an 11-point calibration curve constructed from a range of concentrations of the nondeuterated form of each analyte and a fixed amount of deuterated internal standard. The values obtained from the Masshunter Quantitative Analysis Software were originally expressed in ng per mg of tissue by dividing the weight of the crushed tissue. To express values as nmol or pmols per mg, the corresponding values were then divided by the molar mass of each analyte expressed as ng/nmol or pg/pmol. Linearity (regression analysis determined R^2 values of 0.99 or greater for each analyte) was determined over a range of 19.67 ng–75 fg for all the analytes except for 2-AG, which was 196.68 ng–750 fg, see supplementary data Fig. 2. The limit of quantification was 1.32, 12.1, 1.5, and 1.41 pmol/g for AEA, 2-AG, PEA, and OEA, respectively

Statistical analyses

The SPSS statistical software package (SPSS version 15.0 for Microsoft Windows; IBM, USA) was used to analyse all data. Parametric neurochemical data were analysed using independent samples *t*-test and two-way analysis of variance (ANOVA). Non-parametric data were analysed using Kruskal–Wallis non-parametric test. Post-hoc test for parametric and non-parametric data was undertaken using Fisher's LSD and Mann–Whitney *U* post-hoc tests, respectively. Data were considered significant when $p < 0.05$. Results are expressed as group mean ± standard error of the mean (S.E.M).

Results

Effect of surgery on the levels of AEA, 2-AG, PEA, and OEA in the cerebral cortex

The concentrations of AEA, 2-AG, PEA, and OEA were measured in the animal cerebral cortical tissues following surgery and the sham procedure (animals were sacrificed 2 hours after both interventions) to characterize the potential changes in the levels of these endocannabinoids resulting from acute post-operative pain. There was no significant difference in the levels of AEA (t (17) = 0.016, $p > 0.05$) and 2-AG (t (17) = 0.224, $p > 0.05$) between the sham and surgery groups. Figure 1 shows the effect of surgery on the cortical levels of AEA and 2-AG when the levels for the right and left cerebral cortical tissues were pooled.

Similarly, there was no significant difference in the levels of PEA (t (17) = 1.366, $p > 0.05$) and OEA (t (17) = 0.053, $p > 0.05$) between the sham and surgery groups. Figure 1 shows the effect of surgery on the cortical levels of PEA and OEA when levels for ipsilateral and contralateral tissues were pooled.

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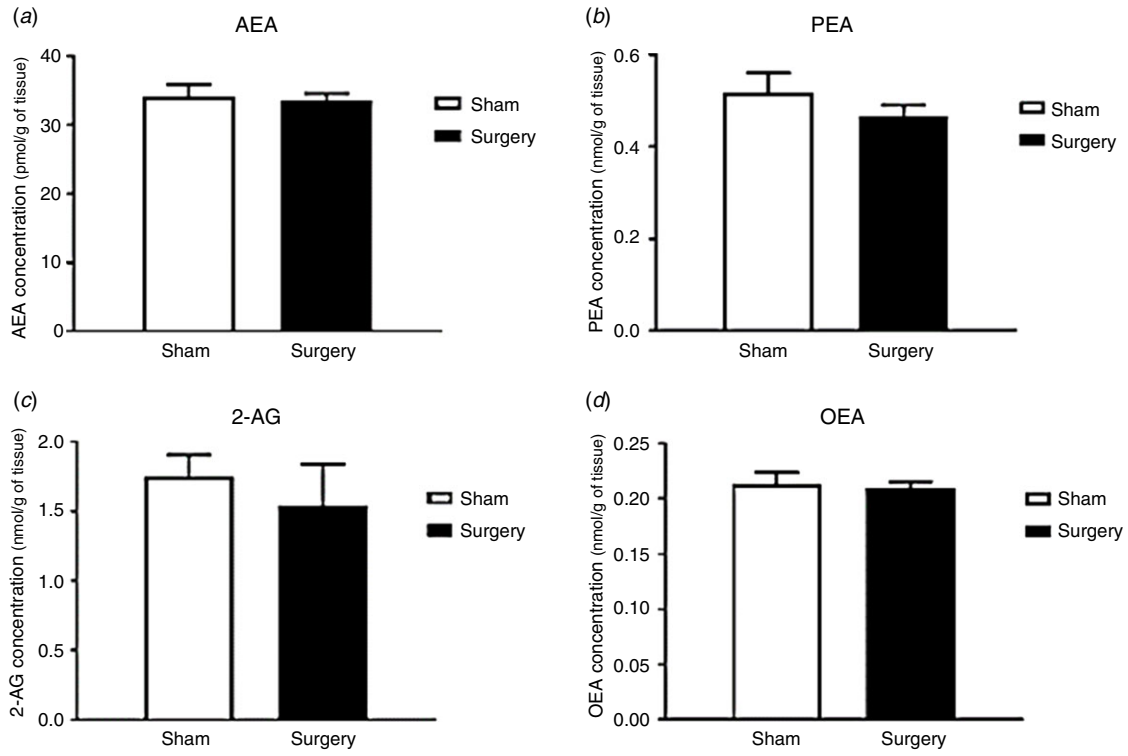

Fig. 1. Effect of surgery on the levels of (*a*) AEA, (*b*) 2-AG, (*c*) PEA, and (*d*) OEA in the cerebral cortex. Data are presented as mean ± s.e.m ($n = 9$–10), sham ($n = 10$), and surgery ($n = 9$). There was no significant difference in the levels of AEA, 2-AG, PEA, and OEA between the sham and surgery groups. See text for details of statistical analysis.

Lateralization of cerebral cortical AEA and 2-AG levels

In both the sham and surgery groups, there was lateralization of AEA and 2-AG in the cerebral cortex such that the levels of these two analytes were significantly higher in the right side compared to the contralateral (left) side, see Fig. 2. A two-way ANOVA revealed no significant effect of procedure on the levels of AEA (F (1, 35) = 0.009, $p = 0.926$; $p > 0.05$) but did reveal a significant effect of side (F (1, 35) =15.965, $p = 0.000$; $p < 0.001$). Fisher's LSD post-hoc test confirmed that levels of AEA were significantly higher in the right cortex of sham and surgery compared with levels in the contralateral (left) cortex ($p < 0.05$). There was no significant side × procedure interaction (F (1, 35) = 0.338, $p = 0.565$; $p > 0.05$). Kruskal–Wallis non-parametric test revealed a significant difference in the levels of 2-AG ($K = 29.125$, $p < 0.001$) between groups (i.e. left cortex sham, left cortex surgery, right cortex sham, and right cortex surgery); Mann–Whitney U post-hoc tests revealed significantly higher levels of 2-AG in the right cortex of sham compared to the levels in the left side ($U = 0.000$, $p < 0.001$) and in the right cortex of surgery compared with levels in the contralateral side ($U = 0.000$, $p < 0.001$).

Lateralization of cerebral cortical PEA

The concentration of PEA was significantly higher in the left cerebral cortex in both the sham and surgery groups, see Fig. 3. Kruskal–Wallis non-parametric test revealed a significant difference in the levels of cortical PEA ($K = 29.185$, $p < 0.001$) between groups (i.e. contralateral (left) cortex sham, contralateral (left) cortex surgery, ipsilateral (right) cortex sham, and ipsilateral (right) cortex surgery groups); Mann–Whitney U post-hoc test showed significant differences in levels of PEA between the contralateral (left) cortex sham and ipsilateral (right) cortex sham groups ($U = 0.000$, $p < 0.001$), with PEA being significantly higher in the left cortex of sham, and between the contralateral (left) cortex surgery and ipsilateral (right) cortex surgery groups ($U = 0.000$, $p < 0.001$), with PEA being significantly higher in the left cortex of surgery.

Lateralization of cerebral cortical OEA in the surgery group

There was also right lateralization of cortical OEA in the surgery group, see Fig. 4. A two-way ANOVA revealed no significant difference in the levels of OEA (F (1, 35) = 0.01, $p = 0.920$; $p > 0.05$) between the procedure

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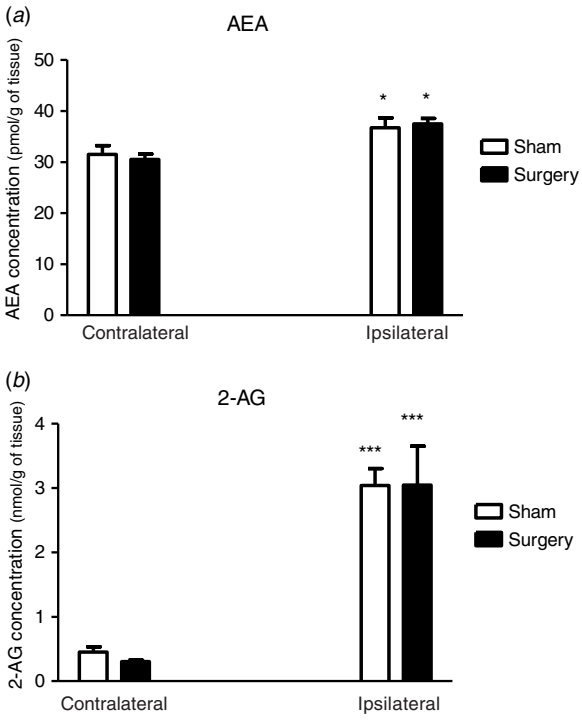

Fig. 2. Lateralization of (*a*) AEA and (*b*) 2-AG levels in the cerebral cortex. Data are presented as mean ± SEM ($n = 9$–10), sham ($n = 10$), and surgery ($n = 9$). There were significant differences in the levels of AEA between the contralateral (left) cortex sham and ipsilateral cortex (right) sham groups ($p < 0.05$), and between the contralateral (left) cortex surgery and ipsilateral (Right) cortex surgery groups ($p < 0.05$). There were also significant differences in the levels of 2-AG between the contralateral (left) cortex sham and ipsilateral (right) cortex sham groups ($p < 0.001$), and between the contralateral (left) cortex surgery and ipsilateral (right) cortex surgery groups ($p < 0.001$). * = $p < 0.05$ and *** = $p < 0.001$. See text for details of statistical analysis.

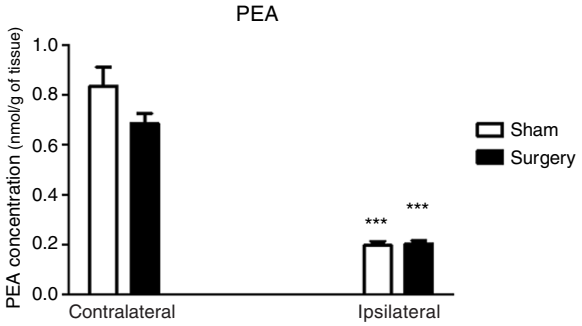

Fig. 3. Lateralization of PEA levels in the cerebral cortex. Data are presented as mean ± s.e.m ($n = 9$–10), sham ($n = 10$), and surgery ($n = 9$). There were significant differences in the levels of PEA between the contralateral (left) and ipsilateral (right) sham groups ($p < 0.001$), and between the contralateral (left) and ipsilateral (right) surgery groups ($p < 0.001$). ***=$p <$ 0.001. See text for details of statistical analysis.

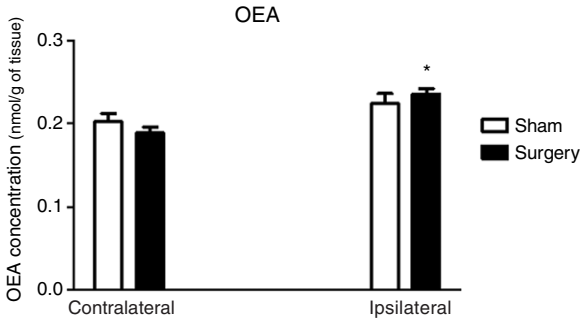

Fig. 4. Lateralization of cortical OEA levels in the surgery group. Data are presented as mean ± s.e.m ($n = 9$–10), Sham ($n = 10$), and surgery ($n = 9$). There was no significant difference in the levels of OEA between the contralateral (left) cortex sham and ipsilateral (right) cortex sham groups ($p > 0.05$), but there was a significant difference between the contralateral (left) cortex surgery and ipsilateral (right) cortex surgery groups ($p < 0.05$). * = $p < 0.05$. See text for details of statistical analysis.

groups (i.e. sham and surgery groups), there was a significant difference in the levels of OEA ($F(1, 35) = 12.734$, $p = 0.001$; $p < 0.05$) between the sides, that is contralateral (left) and ipsilateral (right) sides; Fisher's LSD post-hoc test showed no significant difference in the levels of OEA between the contralateral (left) cortex sham and ipsilateral (right) cortex sham groups ($p > 0.05$) but revealed a significant difference in the cortical levels of OEA between the contralateral (left) cortex surgery and ipsilateral (right) cortex surgery groups ($p < 0.05$), with OEA being significantly higher in the right cortex of surgery.

Discussion

The role of the endocannabinoid system in the modulation of acute post-surgical pain is yet to be fully elucidated. In the current study, we used a rat inguinal surgery model to assess the dynamics of cortical endocannabinoids in the immediate post-operative period. There were no significant differences in the cerebral cortical levels of AEA and 2-AG between the sham and surgery experimental groups. The perception of pain occurs in the cerebral cortex (Guyton & Hall 2010; Barrett *et al.* 2012). The cingulate cortex, primary somatosensory cortex, and insular cortex are all parts of the cerebral cortex and play significant roles in pain perception (Barrett *et al.* 2012). These different anatomical and functional parts of the cerebral cortex could not be easily delineated and were, therefore, considered together in this study. This may have had some effect on the final outcome of the cerebral cortical endocannabinoid levels. Using a paw incision model of post-operative pain, Alkaitis et al. previously demonstrated that the levels of AEA in the

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ipsilateral and contralateral portions of the lumbar spinal cord were significantly lower in the surgery group at 24 hours and 72 hours after surgery compared to basal concentrations in naïve controls (Alkaitis *et al.* 2010). In the same study, these authors further demonstrated that the levels of 2-AG were significantly higher in the contralateral spinal cord at 24 hours and in the ipsilateral spinal cord at 72 hours after surgery compared to the concentrations in the controls. We speculate that the time frame of 2 hours after surgery adopted in the present study to evaluate the dynamics of the cortical endocannabinoids may not have been sufficient to allow for observation of any significant differences between the sham and surgery groups.

Interestingly, differences in the levels of endocannabinoids were observed between the right and left cerebral cortices irrespective of sham or surgical treatment. Quantitation of the cerebral cortical AEA and 2-AG levels revealed the presence of higher levels of these analytes in the right cerebral cortex compared to the contralateral cerebral cortex. This lateralization of endocannabinoids may be related to fear conditioning. Previous studies have reported that MAPK/extracellular-signal-regulated kinase (ERK) activation in the amygdala is essential for acquiring and strengthening conditioned fear (Di Benedetto *et al.* 2008). Indeed, conditioned fear has been shown to significantly increase the expression of phospho-Erk1 (pErk1) in the right basolateral amygdala (BLA) of rats treated with both saline and formalin (Di Benedetto *et al.* 2008). An increase in pErk expression (which is cannabinoid receptor type 1, CB1 dependent) in the amygdala (BLA) has been previously associated with fear conditioning (Cannich *et al.* 2004). It is likely that an increase in the levels of the CB1 receptor agonist AEA during conditioned fear results in CB1 receptor activation, which in turn leads to an increase in the expression of pErk1 in the right BLA (Olango 2012). The frontal, ventromedial, and cingulate cortices are also implicated in fear conditioning (Curzon *et al.* 2009). It is possible that a similar mechanism accounting for the lateralized upsurge in the levels of endocannabinoids in the amygdala may be in operation in the cerebral cortex. Right lateralization of cortical OEA is demonstrated in the surgery group. In the sham group, while the level of OEA in the cerebral cortex was higher in the right side compared to the left, this was not statistically significant. The OEA and PEA are known to have an 'entourage effect' (Bradshaw & Walker 2005). Indeed, fear-mediated increase in pErk1 has been shown to be related to increased OEA levels occurring in association with a potentiation of AEA in the right BLA (Olango 2012). It is possible that a similar mechanism may account for the right cortical lateralization of OEA in the surgery group.

Further studies will be needed to determine whether alterations occur in the concentrations of these lipid-based compounds at the synaptic level, and a time-course study for the levels of the endocannabinoids will also be necessary. Pharmacological studies involving drugs targeting the endocannabinoid system and their effects on surgery-induced behavioural deficits are another necessary future study. It will be interesting to measure the levels of endocannabinoids in additional brain regions involved in pain perception and in the descending modulation of pain, including the relevant thalamic nuclei, the relevant sub-nuclei of the cerebral cortex, the PAG matter, and the rostral ventral medulla. Finally, establishing whether these lateralized differences in the concentration of endocannabinoids in the cerebral cortex are present in naïve animals or are the result of common procedures performed on both the sham and surgery group animals would require further investigation.

Conclusion

In conclusion, acute post-surgical pain (rat inguinal surgery model)-induced alterations in the cerebral cortical levels of endocannabinoids (AEA and 2-AG) were not observed in this study. Lateralized differences in the concentration of these lipid-based compounds between the right and left cerebral cortices were observed irrespective of the sham and surgery groups; this latter finding may be related to the role played by endocannabinoids in fear conditioning, anxiety, and stress coping.

Limitations

Given that the primary aim of this research project was to determine the effects of inguinal hernia repair surgery on the brain levels of endocannabinoids, an experimental naïve group was not included in the experimental design.

Ethical standards

The authors assert that all procedures contributing to this work comply with the ethical standards of the relevant national and institutional committee on human experimentation with the Helsinki Declaration of 1975, as revised in 2008. The authors assert that ethical approval for publication of this study has been provided by their local ethics committee.

Consent for publication

Not applicable.

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Availability of data and materials

All data generated or analysed during this study are included in this published article and its supplementary information files.

Competing interests

The authors have no competing interests. Dara Brea and Orla Moriarty were employees of Covidien.

Financial support

Funding for this research project was provided by Covidien.

Authors' contributions

MI was responsible for rat brain dissection, brain tissue preparation, extraction and quantitation of endocannabinoids, study design, data analysis and interpretation, and the writing of the manuscript. MI undertook this research project in partial fulfilment of the requirements for the Degree of Master of Science in Neuropharmacology at the National University of Ireland Galway.

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Supplementary material

To view supplementary material for this article, please visit https://doi.org/10.13039/501100002736

References

Akirav I (2011). The role of cannabinoids in modulating emotional and non-emotional memory processes in the hippocampus. *Frontiers in Behavioral Neuroscience* **5**, 34.

Alkaitis MS, Solorzano C, Landry RP, Piomelli D, DeLeo JA, Romero-Sandoval EA (2010). Evidence for a role of endocannabinoids, astrocytes and p38 phosphorylation in the resolution of postoperative pain. *PLoS One* **5**(5): e10891.

Amid PK, Shulman AG, Lichtenstein IL (1996). Open. "tension-free" repair of inguinal hernias: the Lichtenstein technique. *European Journal of Surgery* **162**, 447–453.

Bari M, Battista N, Fezza F, Gasperi V, Maccarrone M (2006). New insights into endocannabinoid degradation and its therapeutic potential. *Mini-Reviews in Medicinal Chemistry* **6**, 109–120.

Barrett KE, Barman SM, Boitano S, Brooks H (2012). *Ganong's Review of Medical Physiology*, 24th edn., McGraw Hill Professional: New York.

Beltramo M, Bernardini N, Bertorelli R, Campanella M, Nicolussi E, Fredduzzi S, Reggiani A (2006). CB2 receptor-mediated antihyperalgesia: possible direct involvement of neural mechanisms. *European Journal of Neuroscience* **23** (6), 1530–1538.

Beltramo M, Piomelli D. (2000). Carrier-mediated transport and enzymatic hydrolysis of the endogenous cannabinoid 2- arachidonylglycerol. *Neuroreport* **11**, 1231–1235.

Beltramo M, Stella N, Calignano A, Lin SY, Makriyannis A, Piomelli D (1997). Functional role of high-affinity anandamide transport, as revealed by selective inhibition. *Science* **277**, 1094–1097.

Bouaboula M, Poinot-Chazel C, Bourrié B, Canat X, Calandra B, Rinaldi-Carmona M, Le Fur G, Casellas P (1995). Activation of mitogen-activated protein kinases by stimulation of the central cannabinoid receptor CB1. *Biochemical Journal* **312**, 637–641.

Bradshaw HB, Walker JM (2005). The expanding field of cannabimimetic and related lipid mediators. *British Journal of Pharmacology* **144**, 459–465.

Butler RK, Ford GK, Hogan M, Roche M, Doyle KM, Kelly JP, Kendall DA, Chapman V, Finn DP (2011). Fear-induced suppression of nociceptive behaviour and activation of Akt signalling in the rat periaqueductal grey: role of fatty acid amide hydrolase. *Journal of Psychopharmacology* **26**, 83–91.

Cannich A, Wotjak CT, Kamprath K, Hermann H, Lutz B, Marsicano G (2004). CB1 cannabinoid receptors modulate kinase and phosphatase activity during extinction of conditioned fear in mice. *Learning & Memory* **11** (5), 625–632.

Cravatt BF, Giang DK, Mayfield SP, Boger DL, Lerner RA, Gilula NB (1996). Molecular characterization of an enzyme that degrades neuromodulatory fatty-acid amides. *Nature* **384**, 83–87.

Curzon P, Rustay NR, Browman KE (2009). Cued and contextual fear conditioning for rodents. In *Methods of Behavior Analysis in Neuroscience* (ed. J. J. Buccafusco) 2nd edn. CRC Press/Taylor & Francis: Boca Raton (FL). Chapter 2. (https://www.ncbi.nlm.nih.gov/books/NBK5223/)

Desarnaud F, Cadas H, Piomelli D (1995). Anandamide amidohydrolase activity in rat-brain microsomes – identification and partial characterization. *Journal of Biological Chemistry* **270**, 6030–6035.

Deutsch DG, Chin SA (1993). Enzymatic-synthesis and degradation of anandamide, a cannabinoid receptor agonist. *Biochemical Pharmacology* **46**, 791–796.

Devane WA, Hanus L, Breuer A, Pertwee RG, Stevenson LA, Griffin G, Gibson D, Mandelbaum A, Etinger A, Mechoulam R (1992). Isolation and structure of a brain constituent that binds to the cannabinoid receptor. *Science* **258** (5090), 1946–1949.

https://doi.org/10.1017/ipm.2019.29 Published online by Cambridge University Press

Di Benedetto B, Kallnik M, Weisenhorn DMV, Falls WA, Wurst W, Holter SM (2008). Activation of ERK/MAPK in the lateral amygdala of the mouse is required for acquisition of a fear-potentiated startle response. *Neuropsychopharmacology* **34** (2), 356–366.

Dinh TP, Freund TF, Piomelli D (2002). A role for monoglyceride lipase in 2-arachidonoylglycerol inactivation. *Chemistry and Physics of Lipids* **121**, 149–158.

Elphick MR, Egertova M (2001). The neurobiology and evolution of cannabinoid signalling. *Philosophical Transactions of the Royal Society of London Series B-Biological Sciences* **356**, 381–408.

Finn DP, Chapman V (2004) Cannabinoids as analgesic agents: evidence from in vivo studies. *Current Neuropharmacology* **2** (1), 75–89.

Ford GK, Kieran S, Dolan K, Harhen B, Finn DP (2011). A role for the ventral hippocampal endocannabinoid system in fear-conditioned analgesia and fear responding in the presence of nociceptive tone in rats. *PAIN* **152**, 2495–2504.

Glass M, Dragunow M, Faull RLM (1997). Cannabinoid receptors in the human brain: a detailed anatomical and quantitative autoradiographic study in the fetal, neonatal and adult human brain. *Neuroscience* **77**, 299–318.

Guyton AC, Hall JE (2010). *Guyton and Hall Textbook of Medical Physiology*, 12th edn. Elsevier Health Sciences: Philadelphia.

Herkenham M, Lynn AB, Johnson MR, Melvin LS, De Costa BR, Rice KC (1991). Characterization and localization of cannabinoid receptors in rat brain: a quantitative in vitro autoradiographic study. *Journal of Neuroscience* **11**, 563–83.

Hill MN, McLaughlin RJ, Morrish AC, Viau V, Floresco SB, Hillard CJ, Gorzalka BB (2009). Suppression of amygdalar endocannabinoid signaling by stress contributes to activation of the hypothalamic-pituitary-adrenal axis. *Neuropsychopharmacology* Dec, **34** (13), 2733–45.

Hohmann AG, Tsou K, Walker JM (1999). Cannabinoid suppression of noxious heat-evoked activity in wide dynamic range neurons in the lumbar dorsal horn of the rat. *Journal of Neurophysiology* **81**, 575–583.

Howlett AC, Barth F, Bonner TI, Cabral G, Casellas P, Devane WA, Felder CC, Herkenham M, Mackie K, Martin BR, Mechoulam R, Pertwee G (2002). International Union of Pharmacology. XXVII. Classification of cannabinoid receptors. *Pharmacological Reviews* **54**, 161–202.

Howlett AC, Song C, Berglund BA, Wilken GH, Pigg JJ (1998). Characterization of CB1 cannabinoid receptors using receptor peptide fragments and site-directed antibodies. *Molecular Pharmacology* **53**, 504–510.

Kreitzer AC, Regehr WG (2002). Retrograde signaling by endocannabinoids. *Current Opinion in Neurobiology* **12**, 324–330.

Lafenêtre P, Chaouloff F, Marsicano G (2007). The endocannabinoid system in the processing of anxiety and fear and how CB1 receptors may modulate fear extinction. *Pharmacol Res* Nov, **56** (5), 367–81.

LeBlanc KA (2003). *Laparoscopic Hernia Surgery: An Operative Guide*. Distributed in the U.S.A. by Oxford University Press: London: Arnold; New York, NY.

Lichtman AH, Cook SA, Martin BR (1996). Investigation of brain sites mediating cannabinoid-induced antinociception in rats: evidence supporting periaqueductal gray involvement. *Journal of Pharmacology and Experimental Therapeutics* **276**, 585–593.

Mailleux P, Parmentier M, Vanderhaeghen JJ (1992). Distribution of cannabinoid receptor messenger RNA in the human brain: an in situ hybridization histochemistry with oligonucleotides. *Neuroscience Letters* **143**, 200–204.

Marsicano G, Kuner R (2008). In: *Anatomical Distribution of Receptors, Ligands and Enzymes in the Brain and in the Spinal Cord: Circuitries and Neurochemistry in Cannabinoids in the Brain*. (ed. A. Köfalvi), pp. 161–201. Boston, MA: Springer.

Martin TJ, Buechler NL, Kahn W, Crews JC, Eisenach JC (2004). Effects of laparotomy on spontaneous exploratory activity and conditioned operant responding in the rat: a model for postoperative pain. *Anesthesiology* **101** (1), 191–203.

Martin WJ, Coffin PO, Attias E, Balinsky M, Tsou K, Walker JM (1999). Anatomical basis for cannabinoid-induced antinociception as revealed by intracerebral microinjections. *Brain Research* **822**, 237–242.

Martin WJ, Tsou K, Walker JM (1998). Cannabinoid receptor mediated inhibition of the rat tail-flick reflex after microinjection into the rostral ventromedial medulla. *Neuroscience Letters* **242**, 33–36.

Matsuda LA, Lolait SJ, Brownstein MJ, Young AC, Bonner TI (1990). Structure of a cannabinoid receptor and functional expression of the cloned cDNA. *Nature* **346**, 561–564.

Mechoulam R, Parker LA (2013). The endocannabinoid system and the brain. *Annual Review of Psychology* **64**, 21–47.

Meng ID, Manning BH, Martin WJ, Fields HL (1998). An analgesia circuit activated by cannabinoids. *Nature* **395**, 381–383.

Munro S, Thomas KL, Abu-Shaar M (1993). Molecular characterization of a peripheral receptor for cannabinoids. *Nature* **365**, 61–65.

Olango WM (2012). Investigating the role of the endogenous cannabinoid system in emotional modulation of pain: neurochemical and molecular mechanisms. PhD Thesis, NUI Galway.

Olango WM, Roche M, Ford GK, Harhen B, Finn DP (2012). The endocannabinoid system in the rat dorsolateral periaqueductal grey mediates fear-conditioned analgesia and controls fear expression in the presence of nocicpetive tone. *British Journal of Pharmacology* **165**, 2549–2560.

Onaivi ES, Ishiguro H, Gong JP, Patel S, Perchuk A, Meozzi PA, Myers L, Mora Z, Tagliaferro P, Gardner E, Brusco A, Akinshola BE, Liu QR, Hope B, Iwasaki S, Arinami T, Teasenfitz L, Uhl GR (2006). Discovery of the

https://doi.org/10.1017/ipm.2019.29 Published online by Cambridge University Press

presence and functional expression of cannabinoid CB2 receptors in brain. *Annals of the New York Academy Sciences* **1074**, 514–536.

Paxinos G, Watson C (1997). *The Rat Brain in Stereotaxic Co-ordinates*. Academic Press: San Diego.

Pertwee RG (1997). Pharmacology of cannabinoid CB1 and CB2 receptors. *Pharmacology and Therapeutics* **74**, 129–180.

Puente N, Elezgarai I, Lafourcade M, Reguero L, Marsicano G, Georges F, Manzoni OJ, Grandes P (2010). Localization and function of the cannabinoid CB1 receptor in the anterolateral bed nucleus of the stria terminalis. *PLoS One* Jan 25, **5** (1), e8869.

Rea K, Roche M, Finn DP (2007). Supraspinal modulation of pain by cannabinoids: the role of GABA and glutamate. *British Journal of Pharmacology* **152** (5), 633–648.

Ruehle S, Rey AA, Remmers F, Lutz B (2012). The endocannabinoid system in anxiety, fear memory and habituation. *Journal of Psychopharmacology* Jan **26** (1), 23–39.

Tsou K, Brown S, Sanudo-Pena MC, Mackie K, Walker JM (1998). Immunohistochemical distribution of cannabinoid CB1 receptors in the rat central nervous system. *Neuroscience* **83**, 393–411.

Van Sickle MD, Duncan M, Kingsley PJ, Mouihate A, Urbani P, Mackie K, Stella N, Makriyannis A, Piomelli D, Davison JS, Marnett LJ, Di Marzo V, Pittman QJ, Patel KD, Sharkey KA (2005). Identification and functional characterization of brainstem cannabinoid CB2 receptors. *Science* **310** (5746), 329–332.

Walker JM, Krey JF, Chu CJ, Huang SM (2002). Endocannabinoids and related fatty acid derivatives in pain modulation. *Chemistry and Physics of Lipids* **121**, 159–172.

Zhang J, Hoffert C, Vu HK, Groblewski T, Ahmad S, O'Donnell D (2003). Induction of CB2 receptor expression in the rat spinal cord of neuropathic but not inflammatory chronic pain models. *European Journal of Neuroscience* **17** (12), 2750–2754.

Zogopoulos P, Vasileiou I, Patsouris E, Theocharis SE (2013). The role of endocannabinoids in pain modulation. *Fundamental & Clinical Pharmacology* **27**, 64–80.

https://doi.org/10.1017/ipm.2019.29 Published online by Cambridge University Press