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The endogenous lipid agent \( N \)-arachidonoylethanolamine (anandamide), among other effects, has been shown to be involved in nociceptive processing both in the central and peripheral nervous systems. Anandamide is thought to be synthesised by several enzymatic pathways both in a \( \text{Ca}^{2+} \)-sensitive and \( \text{Ca}^{2+} \)-insensitive manner, and rat primary sensory neurons produce anandamide. Here, we show for the first time, that cultured rat primary sensory neurons express at least four of the five known \( \text{Ca}^{2+} \)-insensitive enzymes implicated in the synthesis of anandamide, and that application of 1,2-dioleoyl-\( sn \)-glycero-3-phosphoethanolamine-\( N \)-arachidonoyl, the common substrate of the anandamide-synthesising pathways, results in anandamide production which is not changed by the removal of extracellular \( \text{Ca}^{2+} \). We also show that anandamide, which has been synthesised in primary sensory neurons following the application of 1,2-dioleoyl-\( sn \)-glycero-3-phosphoethanolamine-\( N \)-arachidonoyl induces transient receptor potential vanilloid type 1 ion channel-mediated excitatory effect that is not inhibited by concomitant activation of the cannabinoid type 1 receptor. Finally, we show that sub-populations of transient receptor potential vanilloid type 1 ion channel-expressing primary sensory neurons also express some of the putative \( \text{Ca}^{2+} \)-insensitive anandamide-synthesising enzymes. Together, these findings indicate that anandamide synthesised by primary sensory neuron via a \( \text{Ca}^{2+} \)-insensitive manner has an excitatory rather than an inhibitory role in primary sensory neurons and that excitation is mediated predominantly through autocrine signalling. Regulation of the activity of the \( \text{Ca}^{2+} \)-insensitive anandamide-synthesising enzymes in these neurons may be
capable of regulating the activity of these cells, with potential relevance to controlling nociceptive processing in these neurons.

Keywords
TRPV1 - CB1 - Nociceptive processing - Pain

Footnote
A. Varga and A. Jenes contributed equally to this work.

The online version of this article (doi:10.1007/s00424-013-1360-7) contains supplementary material, which is available to authorized users.

Electronic supplementary material

ESM 1
(DOC 778 kb)
Anandamide produced by Ca\(^{2+}\)-insensitive enzymes induces excitation in primary sensory neurons

Angelika Varga · Agnes Jenes · Timothy H. Marczylo · Joao Sousa-Valente · Jie Chen · Jonathan Austin · Srikumar Selvarajah · Fabiana Piscitelli · Anna P. Andreou · Anthony H. Taylor · Fiona Kyle · Mohammed Yaqoob · Sue Brain · John P. M. White · Laszlo Csernoch · Vincenzo Di Marzo · Laki Buluwela · Istvan Nagy

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Abstract The endogenous lipid agent \(N\)-arachidonoyl-lethanolamine (anandamide), among other effects, has been shown to be involved in nociceptive processing both in the central and peripheral nervous systems. Anandamide is thought to be synthesised by several enzymatic pathways both in a Ca\(^{2+}\)-sensitive and Ca\(^{2+}\)-insensitive manner, and rat primary sensory neurons produce anandamide. Here, we show for the first time, that cultured rat primary sensory neurons express at least four of the five known Ca\(^{2+}\)-insensitive enzymes implicated in the synthesis of anandamide, and that application of 1,2-dioleoyl-\(sn\)-glycero-3-phosphoethanolamine-\(N\)-arachidonoyl, the common substrate of the anandamide-synthesising pathways, results in anandamide production which is not changed by the removal of extracellular Ca\(^{2+}\). We also show that anandamide, which has been synthesised in primary sensory neurons following the application of 1,2-dioleoyl-\(sn\)-glycero-3-phosphoethanolamine-\(N\)-arachidonoyl induces transient receptor potential vanilloid type 1 ion channel-mediated excitatory effect that is not inhibited by concomitant activation of the cannabinoid type 1 receptor. Finally, we show that sub-populations of transient receptor potential vanilloid type 1 ion channel-expressing primary sensory neurons also express some...
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**Keywords** TRPV1 · CB1 · Nociceptive processing · Pain

**Introduction**

N-Arachidonylethanolamine (anandamide) [7] is an endogenous lipid agent implicated in a variety of physiological functions and pathological processes both within, and outside, the nervous system [3, 21]. Although anandamide interacts with a variety of proteins [15], the majority of its actions are mediated via the cannabinoid type 1 (CB1) receptor and the transient receptor potential vanilloid type 1 ion channel (TRPV1) [6, 7, 9, 26, 51]. One of the most prominent functions of anandamide resides in its TRPV1- and CB1 receptor-mediated regulatory action in nociception in primary sensory neurons (PSN) [9].

Several cell types in various tissues, including PSN, produce anandamide [5, 8, 10, 44, 45], the synthesis of which is believed to occur either in a Ca\(^{2+}\)-sensitive, or Ca\(^{2+}\)-insensitive manner [22, 23, 32, 36, 41, 43, 47, 48] and through several enzymatic pathways (Fig. 1). The molecular identities and biochemical activities of six enzymes implicated in the synthesis of various N-acyltyethanolamines (NAEA) including anandamide from corresponding N-acylphatidyl-ethanolamines (NAPE) have now been established [Fig. 1] (22, 23, 32, 36, 41, 43). We have recently shown that one of these enzymes, N-acylphatidylethanolamine-selective phospholipase D (NAPE-PLD; Fig. 1), which is the only known Ca\(^{2+}\)-sensitive enzyme involved in anandamide synthesis [32, 43, 47, 48], is expressed by a sub-population of PSN [29]. However, the ability of PSN to produce anandamide in the absence of Ca\(^{2+}\) [45] indicates that, in addition to the Ca\(^{2+}\)-sensitive pathway (Fig. 1), one or more Ca\(^{2+}\)-insensitive pathways must also be present in these cells.

Anandamide synthesised in a Ca\(^{2+}\)-sensitive manner induces excitation of PSN through the activation of TRPV1 [44]. However, the effect of anandamide synthesised by Ca\(^{2+}\)-insensitive enzymes acting on NAPE in PSN is not known. Therefore, after having first examined the expression and function of putative Ca\(^{2+}\)-insensitive NAPE-converting enzymes, we also studied the effect of anandamide produced by these enzymes, in cultured rat PSN.

![Fig. 1 Putative anandamide-synthesising enzymatic pathways. A schematic representation of the putative anandamide-synthesising enzymatic pathways. The molecular identity of six enzymes has been established so far (bold). While NAPE-PLD synthesises anandamide in a Ca\(^{2+}\)-sensitive manner, other enzymes (underlined), the expression of which has been studied in these experiments, act in a Ca\(^{2+}\)-insensitive manner. Substrates are shown in italics. NAPE-PLD N-acylphatidylethanolamine phospholipase D, GDE1 glycerophosphodiester phosphodiesterase 1, ABHd4 α/β-hydrolase 4, PTPn22 protein tyrosine phosphatase, non-receptor type 22, sPLA2G1b group 1b soluble phospholipase A2, Inpp5 inositol 5'-phosphatase, NAPE N-acylphosphatidylethanolamine, PLC phospholipase C.](image-url)
Tocrisolve and ethanol were 0.05 %. The maximum final concentration of DMSO was also 0.05 %.

Cultures of dorsal root ganglia

Dorsal root ganglion (DRG) cultures were prepared as described [31]. Briefly, DRG from the first cervical to the sixth lumbar segments were collected in Ham's nutrient F12 culture medium (Sigma) supplemented with 2 % Ultroser G (BioSepa SA), 1 mM glutamine (Invitrogen), 50 IU/ml penicillin (Invitrogen) and 50 μg/ml streptomycin (Invitrogen).

Following incubation in 2,000 U/ml collagenase type IV (Worthington Biochemical Corporation) for 3 h, DRG were triturated and the cells were plated on poly-dL-ornithine (Sigma)-coated glass coverslips. Cells were grown for 24–48 h in the supplemented medium to which nerve growth factor (NGF, 50 ng/ml; Promega), was added. For Ca2+-imaging experiments, after trituration, the cell suspension was centrifuged through 15 % bovine serum albumin (Sigma) and then also supplemented with 10 μM cytosine arabinoside (Sigma).

Isolation of total RNA

The total RNA was isolated using QIA shredder and RNeasy Mini or RNeasy Plus Mini Kits (Qiagen), as described [31].

Reverse transcriptase polymerase chain reaction (RT-PCR)

Extracted RNA (1 μg) was reverse-transcribed using SuperScript II cDNA synthesis reagents (Invitrogen). Primers designed to amplify rat glycerophosphodiester phosphodies-
terase 1 (GDE1, NM_198779.1), α/β-hydrolase 4 (ABHd4, NM_001108866.1), protein tyrosine phosphatase, non-receptor type 22 (PTPn22, NM_001106460.1), group 1b secre-
tory phospholipase A2 (sPLA2G1b, NM_031585.1), inositol 3′-phosphatase (Inpp5, NM_019311.1) and the house-
keeping gene, glyceraldehyde-3-phosphate dehydrogenase (GAPDH, NC_005103), were purchased from Eurofins. The sequences of the primers, annealing temperatures and the predicted product sizes are shown in Table 1.

The PCR reaction mixture contained cDNA, forward and reverse primers, 1.5 mM MgCl2, 1× Green Go-Taq Reaction buffer (Promega), 0.2 mM deoxynucleotide mix (Promega) and 1.25 U Go-Taq DNA polymerase (Promega). The amplification reaction consisted of 30 cycles with 0.5 min of dena-
turation at 95 °C, 1 min annealing at the given temperatures (Table 1), and followed by 1 min final extension at 72 °C in a thermal cycler (Eppendorf-Mastercycler Personal; Eppendorf). For GAPDH, the amplification reaction consisted of only 25 cycles. PCR products were separated on 2 % agarose gels by electrophoresis and visualized with ethidium bromide.

Immunofluorescence staining

Cells attached to the coverslips were washed with PBS and fixed by 4 % paraformaldehyde in 0.1 M PBS (pH 7.4). Permeabilisation (0.3 % Triton-X 100), and blocking (10 % normal donkey serum) were followed by overnight incubation at 4 °C with primary antibodies at dilutions listed in Table 2, and visualisation with secondary antibodies. Coverslips were mounted on glass slides with Vectashield containing DAPI (Vector Laboratories) and examined using a Leica DMR Fluorescence microscope. Images were captured on a Hamamatsu CCD camera using the QWin software package (Leica).

On some images the brightness and contrast were digitally modified. For control, the primary antisera were omitted, or the immunoreactions were performed on sections of tissues in which the expression of the various anandamide-synthesising enzymes had been shown previously. While no staining in PSN neurons was seen when the primary antibodies were omitted, enzyme expression was seen in various tissues with already demonstrated expression of the anandamide-synthesising enzymes (data not shown).

Anandamide release

Cultures were washed twice with HBSS–HEPES buffer (in mM: NaCl 111, KCl 3, MgCl2 0.49, CaCl2 1.27, glucose 5.5, MgSO4 0.4, KH2PO4 0.44, Na2HPO4 0.33, HEPES 10, 100 mM MAFP, pH 7.4) then incubated for 5 min in HBSS–HEPES buffer or HBSS–HEPES buffer containing 20:4-NAPE (30 nM, 100 nM, 300 nM, 1 μM, 3 μM, 10 μM, 30 μM, 100 μM). In some experiments, CaCl2 was omitted from, and 10 mM ethylene glycol tetraacetic acid (EGTA, Sigma) was added to the buffer. All the washes and incubations were carried out at 37 °C unless stated otherwise.

The superfusate was collected on ice and either processed immediately for lipid extraction or stored at −80 °C for further processing. Cells were immediately scraped into ice-cold cell-
lysis buffer, used for protein quantification using BCA Protein Assay Reagent. For some anandamide measurements, only 10 % of the cells were used for protein quantification. The remaining 90 % of the cells were stored in methanol, and then added to the buffer for anandamide measurements.

Anandamide measurements

The 20:4-NAPE application-induced anandamide synthesis was measured using three slightly different methods (Fig. S1). For protocol A (Fig. S1a), lipids were extracted from the buffer into HEPES buffer containing 20:4-NAPE (30 nM, 100 nM, 300 nM, 1 μM, 3 μM, 10 μM, 30 μM, 100 μM). In some experiments, CaCl2 was omitted from, and 10 mM ethylene glycol tetraacetic acid (EGTA, Sigma) was added to the buffer. All the washes and incubations were carried out at 37 °C unless stated otherwise.

The superfusate was collected on ice and either processed immediately for lipid extraction or stored at −80 °C for further processing. Cells were immediately scraped into ice-cold cell-
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Table 1 Sequences, annealing temperature and expected product size of primers used for RT-PCR

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Table 2 List of primary and secondary antibodies used for immunostaining

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<td>AF-568 donkey anti-rabbit IgG (1:1,000)</td>
</tr>
<tr>
<td>sPLA2G1b (Millipore)</td>
<td>1:150</td>
<td>AF-568 donkey anti-mouse IgG (1:1,000)</td>
</tr>
<tr>
<td>NeuN (Millipore)</td>
<td>1:1,000</td>
<td>AF-488 donkey anti-mouse IgG (1:1,000)</td>
</tr>
<tr>
<td>TRPV1 (different suppliers*)</td>
<td>1:2,000</td>
<td>AF-488 donkey anti-goat IgG (1:10,000)</td>
</tr>
</tbody>
</table>

*Either guinea pig anti-TRPV1 antibody (Neuromics, Edina, MN, USA) or rabbit anti-TRPV1 antibody was used. The latter one was a kind gift from Dr. Antonio Avelino (Institute of Histology and Embryology, Faculty of Medicine, University of Porto, Porto, Portugal) and tested intensively including on dorsal root ganglia of TRPV1+/− mice.

Anandamide concentrations were derived from the relative response against authentic standards using Analyst 1.5.1 (ABSciex). The threshold of quantitation was 0.01 nM.

For protocol B (Fig. S1b), lipids were extracted from the superfusate using solid-phase extraction as described [19]. Briefly, superfusates, buffers and cell-control free pools were supplemented with octa-deuterated anandamide (Cayman Chemicals, 2.5 pmol/ml) and loaded onto a pre-conditioned Oasis HLB 1 cm³, 30 mg cartridge (Waters). Cartridges were washed with 40 % aqueous methanol and then eluted with acetonitrile (1 ml, Fisher Scientific). Eluants were dried under constant N₂ stream and then re-suspended in acetonitrile (80 μl). Quantification of anandamide was done by UHPLC-ESI-MS/MS as described [25] using an Acquity UPLC BEH C18 (2.1×50 mm) column (Waters) maintained at 40 °C and an Acquity UHPLC system (Waters). Mobile phases comprised A (2 mM ammonium acetate containing 0.1 % formic acid and 5 % acetonitrile) and B (acetonitrile containing 0.1 % formic acid) and the gradient conditions were as follows: 0–0.5 min, 20 % B; 0.5 min, 50 % B; 2.5 min, 100 % B; 3.5 min, 20 % B then re-equilibrated at 20 % B until 4.0 min. Samples were maintained at 4 °C all throughout. Analytes were detected using tandem ESI-MS/MS in positive ion mode (Quattro Premier tandem mass spectrometry) and multiple reaction monitoring with transitions of m/z 348.5>63.0 and m/z 57.25.5>63.0 for anandamide and m/z 260.420>116.200 for propranolol, used as internal standard (Fig. S1a). Anandamide
anandamide were assessed by liquid chromatography-atmospheric pressure-mass spectrometry (LC-APCI-MS) conducted as described [25]. Analyses were carried out in the selected ion-monitoring mode using m/z values of 356 and 348 (molecular ions +1 for deuterated and undeuterated anandamide), using a Shimadzu HPLC apparatus (LC-10ADVP; Shimadzu Scientific Instruments) coupled to a Shimadzu (LCMS-2020) quadrupole MS via a Shimadzu APCI interface (Shimadzu Scientific Instruments; Fig. S1c). The threshold of quantitation was 0.005 nM.

Cobalt uptake

Cobalt uptake was assessed as described [24, 34, 38, 39]. Briefly, cells attached to the coverslips were washed in a buffer containing (in mM): NaCl 57.5, KCl 5, MgCl₂ 2, HEPES 10, glucose 12, sucrose 139 (pH 7.4) then incubated in the presence of cobalt (5 μM) put into the buffer with or without 20:4-NAPE (100 nM, 1 μM, 10 μM, 100 μM,) for 5 min at 37 °C. The cobalt taken up by the cells was precipitated by 2.5 % β-mercaptoethanol (Sigma). Cells were then fixed in 70 % ethanol and the coverslips were mounted on glass slides with glycerol. The mean gray value of more than 100 cells which were chosen randomly but systemically was established by a Leica light microscope attached to a PC running the QWin software package (Leica), and analysed with the ImageJ software package (NIH, USA) as described [24, 34, 38, 39].

Whole-cell voltage-clamp recordings

An Axopatch 200B amplifier and a Digidata 1200 digitizer (Molecular Devices, UK) were used to record whole-cell currents from cultured PSN as described [31]. Borosilicate glass micropipettes (4–6 MΩ) were pulled on a DMZ puller (DMZ), and filled with the following solution (concentrations in mM): NaCl 5, KCl 150, MgCl₂ 2, HEPES 10, EGTA 1; pH 7.4. The extracellular buffer contained (in mM): NaCl 150, KCl 5, MgCl₂ 2, CaCl₂ 2, HEPES 10, glucose 10; pH 7.4. All recordings were done at 37 °C and collected with the pClamp 8 software package (Molecular Devices) with 1 kHz sampling rate and 5 kHz filtering. The holding potential was −60 mV. Recordings were analysed offline by the ClampFit 8.0 software package (Molecular Devices).

Ca²⁺ imaging

Cells were loaded with Fura-2 acetoxymethyl ester (Fura-2 AM, 5 μM; Molecular Probes) in the presence of 2 mM probenecid (Molecular Probes) for 60 min at 37 °C in a HEPES-buffered saline (in mM): NaCl, 122; KCl, 3.3; CaCl₂, 1.3; MgSO₄, 0.4; KH₂PO₄, 1.2; HEPES, 25; glucose, 10; adjusted with NaOH to pH 7.3. Coverslips were superfused with extracellular solution (in mM: NaCl, 160; KCl, 2.5; CaCl₂, 1; MgCl₂, 2; HEPES, 10; glucose, 10; pH 7.3) in a laminar flow perfusion chamber (Warner Instrument Corporation). The following test solutions were applied subsequently: 20:4-NAPE (50 μM), capsaicin (1 μM) and KCl (50 mM) (timing is indicated in the corresponding figures). For studying the involvement of TRPV1 or the CB1 receptor in the 20:4-NAPE-evoked responses, either capsazepine (5 μM), or rimonabant (200 nM) was used to inhibit TRPV1 or the CB1 receptor, respectively. The antagonists were applied for 60 s prior the application of 20:4-NAPE together with capsazepine or rimonabant. For control purposes, following the application of 20:4-NAPE together with capsazepine or rimonabant, the antagonists were not removed from the bath and capsazepine with capsazepine or rimonabant was applied. Only KCl-sensitive cells (neurons) were involved in subsequent analyses. At the end of some experiments, mustard oil (50 μM; Sigma) or/and ionomycin (5 μM, Sigma) was also applied.

Application of drugs was controlled manually with one visual field being tested per coverslip. Experiments were performed at 37 °C, except where otherwise indicated.

Images were captured with a Peltier element-cooled slow scan charge-coupled camera system (PTI). Following subtraction of the background fluorescence, the ratio of fluorescence intensity at the two wavelengths as a function of time (rate 1 Hz) was automatically calculated \((R = F_{340}/F_{380})\) and the resultant graphs analysed using the ImageMaster 5.0 software package (PTI) and the Microsoft Excel software package. Only recordings with a stable baseline before 20:4-NAPE application were included in the analyses. First, the noise and baseline before the first drug application were established. Then, the maximum amplitude of the 340/380 ratio was established in defined sections of recordings. Each of these defined sections started at the beginning of a drug application and lasted until the beginning of the next drug application. An increase of more than 10% in the 340/380 ratio was then regarded as a response, if, during visual verification, the change was clearly associated with the drug application. Based on our pilot data, 2.5 times of the standard deviation of the amplitude of the baseline noise equals with a maximum of 9.5–10 % increase in the 340/380 ratio.

Statistics

Data of repeated measurements were averaged. Differences between the averaged values were analysed by Student’s t-test, one-way analysis of variance (ANOVA) or repeated measure multivariate ANOVA as appropriate. In ANOVA, following checking the normality of data, post hoc analysis for the statistically significant differences among the treatment groups was performed by the Fischer's test. Significant differences in the proportion of neurons responding to various drugs were assessed by Fisher's exact test. Data are shown as tmean ± standard error of mean. A difference between two values was
considered to be significant if $p < 0.05$. The $n$ values refer to the number of repeated experiments (number of cultures used for immunohistochemistry, anandamide release measurements and cobalt uptake, and the number of cells measured in whole-cell recordings and calcium imaging) and $p$ values are given as $<0.0001$ if the actual value was smaller than that.

**Results**

Several Ca$^{2+}$-insensitive enzymes, which are implicated in anandamide synthesis, are expressed in rat cultured primary sensory neurons.

RT-PCR analysis showed gene expression for all five Ca$^{2+}$-insensitive enzymes that are known to be implicated in the synthesis of NAEA, including anandamide, from the corresponding NAPEs in cultures prepared from rat DRG (Figs. 1 and 2a). In order to study the expression of the enzymes at a cellular level, immunostaining was performed on cultured rat PSN using antibodies raised against the enzymes together with an anti-NeuN-antibody, which identifies neurons. By analysing the staining in at least 100 neurons in each culture, we found that sub-populations of PSN express ABHD4, GDE1, Inpp5, and PTPn22 (Fig. 2b; for the proportions of cells expressing these enzymes please see Table 3). The analysis also revealed that while only neurons express ABHD4, Inpp5 and PTPn22, neurons and some non-neuronal cells express GDE1 (Fig. 2b).

The antibody raised against sPLA2G1b did not produce staining in the cultures. However, the same antibody produced staining in the pancreas (data not shown). The anti-ABHD4-, anti-Inpp5-, anti-PTPn22- and anti-GDE1 antibodies also produced staining in tissues in which the expression of these enzymes has been reported previously (data not shown).

When the primary antibodies were replaced with normal serum, no staining was seen either in cultured PSN or in any tissues which we processed (data not shown). Application of 20:4-NAPE induces anandamide production in cultured PSN.

20:4-NAPE is the substrate for anandamide synthesis used by all the known enzymatic pathways (Fig. 1) [20, 22, 23, 32, 36, 37, 41, 43]. Therefore, to ascertain whether the enzymes found to be expressed in cultured rat PSN form any functional anandamide-synthesising pathways, the level of 20:4-NAPE application-induced anandamide synthesis was measured.

Exposure of the cells to 100 μM 20:4-NAPE significantly increased the anandamide content of the buffer from a level below the quantitation threshold in the control to 8.86±3.44 pmol/ml ($n=4$; Student's $t$-test, $p=0.01$; Protocol B) at 37°C. After normalisation to the protein content, the anandamide concentration was 13.25±4.39 ng/mg protein ($n=4$; Fig. 3a).

Incubation of the cells in the vehicle resulted in an anandamide content of 0.01±0.01 ng/mg (n=3) protein in the superfusate (data not shown). This value was not significantly different from that measured in the control of this experiment (0.04±0.04 ng/mg protein, $n=3$; $p=0.5$, Student's $t$-test). Incubation
Table 3 Relative number of cultured rat primary sensory neurons exhibiting immunopositivity for various enzymes, which are implicated in Ca\(^{2+}\)-insensitive anandamide synthesis, and for TRPV1

<table>
<thead>
<tr>
<th></th>
<th>ABHd4</th>
<th>GDE1</th>
<th>Inpp5</th>
<th>PTPn22</th>
<th>sPLA2G1b</th>
</tr>
</thead>
<tbody>
<tr>
<td>Relative number of cells exhibiting immunopositivity</td>
<td>29.8±1.9</td>
<td>40.27±9.94</td>
<td>44.1±1.9</td>
<td>48.5±10.04</td>
<td>n. d.</td>
</tr>
<tr>
<td>Relative number of TRPV1-expressing cells</td>
<td>50.63±3.84</td>
<td>46.6±3.46</td>
<td>56.2±4.77</td>
<td>42.4±2.37</td>
<td>n. q.</td>
</tr>
<tr>
<td>Relative number of TRPV1-expressing neurons which also exhibit enzyme-immunopositivity</td>
<td>23.11±8.55</td>
<td>25.9±2.68</td>
<td>64.93±10.9</td>
<td>17.93±7.58</td>
<td>n. d.</td>
</tr>
<tr>
<td>Relative number of enzyme-expressing neurons which also express TRPV1</td>
<td>13.25±4.5</td>
<td>21.8±4.21</td>
<td>51.27±8.33</td>
<td>16.4±5.49</td>
<td>n. d.</td>
</tr>
</tbody>
</table>

n number of cultures, n.q. not quantified, n.d. not detected

of the cells in 100 μM 20:4-NAPE at room temperature also significantly increased the anandamide concentration in the buffer (from 0.06±0.03 ng/mg protein to 1.17±0.03 ng/mg protein, n=3; Student’s t-test, p=0.009; Fig. 3b). However, the 20:4-NAPE application-induced increase in the anandamide content of the buffer at room temperature was significantly less than that measured at 37°C (Student’s t-test, p=0.03) indicating that the production of anandamide depends on enzyme activity. To make sure that no 20:4-NAPE hydrolysing activity in the buffer contributed to the increase in the anandamide levels, we also quantified the anandamide content of cell-free superfusate containing 100 μM 20:4-NAPE. The buffer was kept either at room temperature or 37°C up to 4 h. We measured the lowest and highest anandamide concentration at 2.5 h at 37°C (0.023 ng/ml) and at 5 min at 37°C (0.15 ng/ml), respectively. We found no apparent relationship between the anandamide content and the time or temperature of incubation (data not shown). These data indicated that cultured rat PSN has the ability to convert 20:4-NAPE into anandamide.

The 20:4-NAPE application-evoked increase in the anandamide content of the supernatant at 37°C was concentration-dependent (Fig. 3c; Protocol A). The lowest concentration of 10 μM 20:4-NAPE at which the anandamide content of the superfusate was significantly different from that of the control was 10 μM (ANOVA followed by Fisher’s test, p=0.03).

Fig. 3 20:4-NAPE application induces anandamide production in cultured rat primary sensory neurons. a Incubation of rat cultured PSN in the presence of 100 μM 20:4-NAPE for 5 min at 37 °C results in a significant increase in the anandamide content of the superfusate when compared to the basal anandamide level. The measurement was done by ultra-high performance liquid chromatography-electrospray-tandem mass spectrometry following the extraction of lipids from the superfusate. Spectrograms of anandamide analysis are shown in Fig. S1. (n=3, asterisk indicates significant difference.) b Incubation of rat cultured PSN in the presence of 100 μM 20:4-NAPE for 5 min at room temperature also increases the anandamide content of the superfusate. However, the anandamide content following incubation of the neurons at room temperature is significantly smaller than following incubation of the neurons at 37 °C (please note the difference in the scale of the y-axis in a and b). Spectrograms of anandamide analysis are shown in Fig. S1. (n=3, asterisk indicates significant difference from the basal anandamide level measured at room temperature.) c Incubation of rat cultured PSN in 30 nM–100 μM 20:4-NAPE for 5 min at 37 °C results in a concentration-dependent increase in the anandamide content of the superfusate. The lowest 20:4-NAPE concentration that induces significant increase of the anandamide content of the superfusate is 10 μM. (n=3 for each data point, * indicates significant difference.) (D) Withdrawal of Ca\(^{2+}\) from the superfusate does not change either the basal anandamide content of the superfusate or the anandamide content of the superfusate following the addition of 100 μM 20:4-NAPE for 5 min (n=3 for each data point, asterisk indicates significant difference)
Among the known anandamide-synthesising enzymes, NAE-PLD activity is significantly enhanced in the presence of Ca²⁺ [32, 42, 43, 47, 48]. To ascertain whether or not during 20:4-NAPE application, any Ca²⁺ influx increases the intracellular Ca²⁺ concentration [Ca²⁺], to an extent which is enough to contribute NAE-PLD to anandamide synthesis, we applied 20:4-NAPE to cultured PSN either with Ca²⁺ present or absent in the buffer. In the presence of Ca²⁺, the anandamide content of the superfusate incubated with vehicle was 0.62±0.35 ng/mg protein (n=3), whereas in the absence of Ca²⁺, it was 0.98±0.12 ng/mg protein (n=3; Protocol A). These values did not differ significantly from each other (Student’s t-test, p=0.4; Fig. 3d). Application of 20:4-NAPE (100 μM) resulted in a significant increase in the anandamide content of the superfusate in both the presence and absence of Ca²⁺ (9.57±2.58 ng/mg protein (n=3) and 11.47±0.92 ng/mg protein (n=3); Student’s t-test, p=0.01 in the presence and p=0.0001 in the absence of Ca²⁺; Fig. 3d). Thus, the anandamide content of the 20:4-NAPE-containing, and Ca²⁺-free, superfusates did not differ significantly from each other after the application of this substrate (Student’s t-test, p=0.5).

We also compared the anandamide content of the superfusate to that of 90% of the cells together with the superfusate (the other 10% of the cells was used for protein measurements). The anandamide content of the cells and superfusate together under control conditions (incubation with vehicle) was 0.09 ng/mg protein (n=2; Protocol C). Following 100 μM 20:4-NAPE application for 5 min, the anandamide content of the cells and superfusate increased to 28.03 ng/mg protein (n=2; data not shown).

Together, these data indicate that the 20:4-NAPE-evoked anandamide synthesis in cultured rat PSN is not affected by the removal of Ca²⁺ from the extracellular buffer. These data also show that about 2/3 of anandamide produced in PSN following 20:4-NAPE application, similar to anandamide produced by increasing the [Ca²⁺] in central neurons [8], is retained in the cells.

20:4-NAPE application induces inward currents in a sub-population of cultured rat PSN

Anandamide synthesised in PSN by increasing the [Ca²⁺] induces TRPV1-mediated excitation [44]. TRPV1 activation by anandamide results in cobalt accumulation in a sub-population of PSN [24, 34, 38, 39]. Therefore, to test whether endogenous anandamide synthesised following the application of 20:4-NAPE has similar effect to that synthesised in a Ca²⁺-sensitive manner, we studied cobalt accumulation during 20:4-NAPE application. The efficacy of this technique for assessing TRPV1-activity has been consistently demonstrated [24, 30, 34, 38, 39, 49, 50].

While application of the vehicle did not (2.63±0.34%, n=3; p=0.44, ANOVA followed by Fisher’s test), application of 20:4-NAPE significantly increased the proportion of labelled neurons and this effect was concentration-dependent (Fig. 4a–c). The lowest concentration of 20:4-NAPE which produced a significant increase in the proportion of labelled cells was 0.1 μM (6.44±1.16%, n=4 cultures; ANOVA followed by Fisher’s test, p=0.01; Fig. 4c). The EC₅₀ of 20:4-NAPE was 7.41±1.4 μM. The majority of the cobalt-labelled cells were small diameter neurons (Fig. 4d). These observations suggest that the cobalt influx occurred in nociceptive neurons, the great majority of which express TRPV1 [6, 31].

20:4-NAPE application induces inward currents in a sub-population of cultured rat PSN

To confirm the excitatory effect of 20:4-NAPE application, we recorded whole cell currents from cultured rat PSN. In total, 22 cells were found that responded with inward currents to 50 μM 20:4-NAPE which is the near maximal concentration for this agent to induce cobalt uptake (Fig. 5a). The peak amplitude of the response was –0.41±0.06 nA (n=22).

All nine of the 20:4-NAPE-responsive neurons that we tested also responded to 500 nM capsaicin (Fig. 5a and b). The average amplitude of the 20:4-NAPE-evoked currents (–0.35±0.08 nA, n=9) was significantly smaller than that of the capsaicin-evoked currents (–2.36±0.55, n=9; Student’s t-test, p=0.002; Fig. 5c). However, in addition to the double-responsive cells, 27 neurons which responded to capsaicin but not to 20:4-NAPE application were also found (data not shown). In the control experiments, no neurons produced any noticeable responses to the vehicle (n=11, data not shown).

20:4-NAPE application increases the [Ca²⁺], in a capsaizpine- and rimonabant-sensitive manner

In order to study the pharmacological properties of the 20:4-NAPE application-evoked excitatory responses, we assessed changes in [Ca²⁺], produced by the application of 50 μM 20:4-NAPE. Application of the vehicle for a minute did not produce any increase in the [Ca²⁺], in any of the 384 KCl-responsive neurons, whereas 234 of these 384 responded to 1 μM capsaicin application (data not shown). Application of 20:4-NAPE, however, increased [Ca²⁺], in 189 of 546 KCl-responsive neurons (34.6%; Fig. 6a and b). All the neurons that responded to 20:4-NAPE application were sensitive to capsaicin (Fig. 6a and b). In addition to the double-responsive neurons, 273 of the 546 neurons (50%) responded only to capsaicin (Fig. 6a and b). Hence, the total number of capsaicin-responsive neurons was 462 of 546 (84.6%).
Fig. 6b), which was significantly higher than the proportion of capsaicin-sensitive neurons measured when only the vehicle (p < 0.0001; Fischer's exact test) or the buffer (224 of 343 cells) was applied before capsaicin application. The 20:4-NAPE application-evoked changes in [Ca²⁺]i activated more slowly than those evoked by capsaicin (Fig. 6a). Furthermore, the amplitude of the 20:4-NAPE application-evoked responses were significantly smaller than those evoked by capsaicin when normalised to the KCl-evoked responses (20:4-NAPE/KCl=0.15±0.01, Student's t-test, p < 0.0001; Fig. 6a). Interestingly, the great majority of neurons, which generated responses to 20:4-NAPE application, exhibited sustained/very slowly inactivating Ca²⁺ transients to capsaicin application. On the other hand, the great majority of capsaicin-responsive neurons, which did not respond to 20:4-NAPE application, exhibited fast inactivation of responses during capsaicin application (Fig. 6a).

In the presence of capsazepine (5 μM), 403 KCl-responding neurons exhibited a stable base line before 20:4-NAPE application. Of these, 26 responded to 20:4-NAPE and capsaicin (6.45 %; Fig. 6b). In addition to these neurons, 16 cells responded only to 20:4-NAPE application, and 21 cells responded only to capsaicin. Thus, the overall proportions of both the 20:4-NAPE (42 of 403, 10.4 %) and capsaicin-responding neurons (47 of 403, 11.7 %) in the presence of capsazepine were significantly smaller than that measured in the absence of capsazepine ( Fisher's exact test, p < 0.0001 for both 20:4-NAPE and capsaicin; Fig. 6b).

In the presence of rimonabant, 20:4-NAPE application increased [Ca²⁺]i in 105 of 498 KCl-responding neurons with a stable base line before 20:4-NAPE application (21.08 %; Fig. 6b). This proportion was significantly smaller from that measured in the control ( Fisher's exact test; p < 0.0001). Rimonabant, however, had no significant effect on the overall number of capsaicin-responding neurons (421 of 498 cells, 84.54 %, p = 1, Fisher's exact test; Fig. 6b). Rimonabant significantly reduced the amplitude of both the 20:4-NAPE application-evoked (to 20:4-NAPE/KCl=0.12±0.009, Student's t-test, p = 0.02), and the amplitude of the capsaicin-induced responses (to capsaicin/KCl=0.61±0.018, Student's t-test, p < 0.0001). These data suggest that the 20:4-NAPE application-evoked excitatory effect is mediated by TRPV1, and that 20:4-NAPE application, when assessed average...
responses, does not result in a CB1 receptor mediated inhibitory effect.

The 20:4-NAPE application-evoked increase in [Ca^{2+}]_{i} in mouse PSN is mediated by TRPV1.

In order to confirm the role of TRPV1 in 20:4-NAPE application-evoked responses, we also studied the effect of this agent on PSN isolated from WT and TRPV1 \(^{-/-}\) (KO) mice. Ten of 68 KCl-responsive neurons (14.71 \%) from WT mice were regarded as responsive to 20:4-NAPE (50 μM) application (Fig. 7a). As in experiments using rat PSN cultures, all the 20:4-NAPE-responsive neurons were responsive to capsaicin (1 μM; Fig. 7a) and the 20:4-NAPE application-evoked responses activated more slowly than the capsaicin-evoked responses (Fig. 7a). As with cultures prepared from rat DRG, cultures prepared from WT mouse DRG also had neurons, which responded only to capsaicin (32 of 68 [48.53 \%]; Fig. 7a).

In contrast, none of the KCl-responsive neurons (n = 59) responded to 20:4-NAPE application in cultures prepared from DRG of KO mice (Fig. 7b). As expected, none of the neurons in the cultures prepared from DRG of KO mice responded to capsaicin (Fig. 7b). However, 22 of the 59 KCl-responding cells responded to mustard oil, showing that the cells were healthy and responsive to agents for which they expressed receptors (Fig. 7b). Therefore, our findings on neurons of...
Sub-populations of cultured rat primary sensory neurons co-express TRPV1 and enzymes implicated in Ca\(^{2+}\)-insensitive anandamide synthesis. Cultured rat PSN were incubated in antibodies raised against TRPV1 (green) and ABHd4, GDE1, Inpp5 or PTPn22. For quantitative data, please see Table 3. Scale bar=25 μm.

**Discussion**

We have shown in the present study that four of the five enzymes (ABHd4, GDE1, Inpp5 and PTPn22) which have been implicated previously in Ca\(^{2+}\)-insensitive anandamide synthesis from 20:4-NAPE [22, 23, 32, 36, 41, 43], are found at transcript level (please see also Fig. S2) in cultures prepared from rat DRG, and at protein level in cultured rat (and mouse, see Fig. S3) PSN. The GDE1 protein is also expressed by non-neuronal cells as well as by PSN. The anti-sPLA2G1b antibody, which we used in this study, does not produce any staining in rat PSN cultures, although it labels cells in acini in the pancreas. RT-PCR shows weak expression of sPLA2G1b mRNA in PSN cultures. Hence, one cannot exclude the possibility that this enzyme may be expressed under the detection threshold of the immunostaining technique in some neurons or non-neuronal cells.

20:4-NAPE application to PSN cultures increases the anandamide concentration in the cells and the superfusate. The finding that application of 20:4-NAPE to cell-free superfusate does not increase the anandamide content of the buffer, together with the finding that the anandamide content of the cells and superfusate is greater than that of the superfusate alone indicates that 20:4-NAPE passes the cells membrane and anandamide is synthesised intracellularly. Indeed, all NAPE-converting enzymes are intracellular and transiently associated with membranes to act on NAPEs embedded in those [4, 22, 23, 32, 36, 41, 43]. Therefore, the conversion of 20:4-NAPE to anandamide in an enzymatic way, as we have shown here, can be only through the rapid incorporation of this phospholipid into the membranes and its hydrolysis by some of the enzymes we investigated here. The rate of anandamide production induced by 100 μM 20:4-NAPE...
application (~38 pmol/5 min/mg protein) is well within the
range of 20:4-NACE hydrolysis (up to ~40 pmol/min/mg
protein) measured in brain homogenates [20, 36, 37]. The
distribution of enzymes across PSN and non-neuronal cells
indicates that, while non-neuronal cells may produce some
anandamide, the greatest amount of this endocannabinoid/
edoovanilloid is likely to be synthesised in PSN.

Calcium regulates the synthesis of anandamide mostly
through the NACE-synthesising enzyme known as Ca²⁺-de-
dependent N-acetyltransferase, which catalyses the formation
of 20:4-NACE [4, 8]. However, when this rate-limiting step in
anandamide and NAEA biosynthesis is bypassed, as in our
case, the only way for anandamide to be produced in a Ca²⁺-
sensitive manner is via NAC-PLD action on 20:4-NACE [4,
22, 23, 32, 36, 41–43] (Fig. 1). Indeed, in the absence of Ca²⁺,
NAC-PLD activity is negligible even in the presence of the
substrate [42, 47], whereas increasing the [Ca²⁺] alone results
in anandamide production [8, 44]. Although, application of
20:4-NACE induces some increase in the [Ca²⁺], the finding
removal of Ca²⁺ from the extracellular buffer does not
change the anandamide concentration in the superfuse sug-
gests that Ca²⁺ influx during 20:4-NACE application is not
sufficiently high to induce NAC-PLD activity. Hence, follow-
ing 20:4-NACE application, at least the great majority of anan-
damide is synthesised by PSN by Ca²⁺-insensitive pathways.
Consequently, some of the Ca²⁺-insensitive anandamide-
synthesising enzymes present in the cultures prepared from
rat DRG form functional pathways, which may also include
the Ca²⁺-independent NAC biosynthesising enzyme previ-
ously identified by Jin and colleagues [17]. However, the exact
identity of the pathway(s) is not known and attempts to dissect
tem would produce ambiguous results at present because of
the unavailability of selective and specific blockers of the
enzymes.

Application of 20:4-NACE results in cobalt-influx in a sub-
population of small diameter cultured PSN, the majority of
which express TRPV1 [6, 28, 31]. While the lowest concentra-
tion of 20:4-NACE which induces a significant increase in the
number of cobalt-labelled neurons is 0.1 µM, the lowest con-
centration of 20:4-NACE which results in a significant increase
in the anandamide content of the superfuse is 10 µM. We
have shown here, however, that PSN — similar to central
neurons [8] — retain the majority of anandamide. Hence, the
concentration of anandamide at the intracellular anandamide-
binding site of TRPV1 [18], is probably considerably higher
than that in the superfuse. This difference renders any direct
comparison between minimally effective concentrations of
20:4-NACE at increasing the anandamide concentration in the
superfusate and at evoking Ca²⁺ uptake very difficult.

In addition to inducing cobalt accumulation, application of
20:4-NACE also increases the [Ca²⁺], and induces whole-cell
currents. All the cells which respond to 20:4-NACE applica-
tion with increased [Ca²⁺], or inward currents, in control
conditions, also respond to the archetypal TRPV1 agonist,
capsaicin. Taken together, these data suggest that the 20:4-
NACE application-evoked excitatory effect is mediated
through TRPV1 in PSN. Indeed, the TRPV1 antagonist
capsazepine, which we applied at a concentration known to
have little effect on other molecules than TRPV1 [11], signif-
ificantly reduces the proportion of neurons responding to
20:4-NACE application. However, the inhibitory effect of
capsazepine might be different in species other than rat [46].

Nevertheless, the finding that cultured PSN prepared from
TRPV1−/− mouse DRG do not, whereas a sub-population of
cultured PSN prepared from WT mouse DRG does, respond
to 20:4-NACE application provides evidence that, in PSN, the
20:4-NACE application-evoked excitatory effect is indeed
mediated through TRPV1.

Several findings indicate that the 20:4-NACE application-
induced excitatory effects are exerted indirectly, through anan-
damide production. First, both the anandamide production,
and the 20:4-NACE application-induced cobalt accumulation,
depend on the concentration of the substrate. Second, if 20:4-
NACE activated TRPV1 directly all capsaicin-sensitive neu-
rons would be expected to respond. However, we found that
only ~40 % of capsaicin-responsive neurons exhibit responses
to 20:4-NACE application. Third, if 20:4-NACE activated
TRPV1 directly, a similar pattern of temperature-dependent
change in the responsiveness of neurons to 20:4-NACE and
capsaicin is expected to occur. However, while application of
20:4-NACE, which induces responses in about a third of the
neurons at 37 °C, fails to produce responses in any neurons at
room temperature (Fig. 4a and b). At the same time, the
proportion of capsaicin-responsive neurons is the same at
37 °C and room temperature (Fig. 4b). Fourth, the lack of
responsiveness to 20:4-NACE at room temperature co-occurs
with a 10-fold reduction in the anandamide content of the
superfusate following the application of 20:4-NACE to the
cells. Fifth, direct TRPV1 activators, such as capsaicin and
anandamide [18], activate TRPV1 with fast kinetics. Howev-
er, the kinetics of 20:4-NACE-evoked responses are signifi-
cantly slower than those of the capsaicin-evoked responses.
Sixth, the amplitude of whole-cell currents produced by 20:4-
NACE application, which results in about 30 nM anandamide
when the concentration is measured in the cells and
superfusate together, is compatible with the amplitude of
whole-cell currents evoked by 100 nM anandamide included
into the recording electrode [12]. These findings collectively
provide evidence that the 20:4-NACE-evoked effects depend
on enzyme activity and at least the majority, if not all, of
the 20:4-NACE application-induced TRPV1-mediated excitation
is produced indirectly, via the conversion of 20:4-NACE to
anandamide. The involvement of 20:4-NACE-derived metab-
olites other than anandamide is highly unlikely because thin
layer chromatography shows the presence of no other lipids
than anandamide and 20:4-NACE in the superfusate (Fig. S5).

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The limited responsiveness of capsaicin-sensitive neurons to 20:4-NAPE may appear in contrast with the co-expression pattern of TRPV1 and the four enzymes we examined in this study. However, each of these four enzymes constitutes only a member of specific enzymatic pathways (Fig. 1), none of which is selective for the synthesis of anandamide [22, 23, 32, 36, 41, 43]. Due to the unknown molecular identity of some enzymes, at present, it is impossible to establish the proportion of neurons which express (or co-express) a complete anandamide-synthesising pathway(s). Nevertheless, our findings suggest that less than half of TRPV1-expressing neurons may express pathways, which are able to produce anandamide in a Ca\(^{2+}\)-insensitive manner. These findings, together with the concentration of anandamide in the superfusate, following 20:4-NAPE application, being well below the minimum concentration of exogenous anandamide needed for TRPV1 activation [1, 12, 14, 38, 51] also suggest that anandamide synthesised in a Ca\(^{2+}\)-insensitive manner, similar to anandamide produced through a Ca\(^{2+}\)-sensitive pathway [44], activates TRPV1 through autocrine signalling. Notably, the overall proportion of capsaicin-responsive cells is increased from ~60 % to above 80 % when 20:4-NAPE is applied before capsaicin. Furthermore, more dual-responsive than capsaicin-only-responsive neurons exhibit an increased/slowly inactivating capsaicin-induced increase in the [Ca\(^{2+}\)]\(_i\). Hence, the autocrine signalling by anandamide synthesised in a Ca\(^{2+}\)-insensitive manner by PSN seems to have some sensitising effect on TRPV1-mediated responses, which results in exhibiting such responses above the detection threshold in neurons which do not produce detectable responses to capsaicin in control conditions.

While exogenous anandamide induces a CB1 receptor-mediated inhibition on TRPV1-mediated responses in PSN [2, 14, 24, 27, 33], the selective and specific CB1 receptor antagonist, rimonabant, reduces the proportion of neurons responding to, and the amplitude of responses induced by, 20:4-NAPE application. These data may suggest that exogenous anandamide, and anandamide produced from 20:4-NAPE via Ca\(^{2+}\)-insensitive pathways may exert their actions through not only completely overlapping mechanisms (i.e., while exogenous anandamide induces a CB1 receptor-mediated inhibitory effect, endogenous anandamide does not induce such an effect). However, the effect of exogenous anandamide on TRPV1 activity depends on its concentration and the availability of the CB1 receptor. Hence, exogenous anandamide above 1 μM induces larger responses in TRPV1-CB1 receptor co-expressing human embryonic kidney 293 cells than in such cells when they express TRPV1 alone [16]. Consistently, the exogenous anandamide-evoked excitatory effect is reduced by rimonabant in cultured rat PSN [35]. Furthermore, like the effect of anandamide on TRPV1, the effect of capsaicin is also reduced when the CB1 receptor is not available either due to the blocking CB1 receptor activity with rimonabant [35] or to the deletion of the CB1 receptor [13]. In agreement with findings in CB1\(^{-/-}\) mice [13], we found here that rimonabant reduces the amplitude of capsaicin-evoked responses, but not the proportion of neurons responding to capsaicin. Therefore, the inhibitory effect of rimonabant on the proportion of neurons responding to 20:4-NAPE by increased [Ca\(^{2+}\)]\(_i\) is consistent with the proposed constitutive sensitising action of the CB1 receptor on TRPV1, which may occur under certain conditions [13, 16]. Hence, the effects of exogenous anandamide above 1 μM and the effects of endogenous anandamide synthesised from 50 μM 20:4-NAPE in a Ca\(^{2+}\)-insensitive manner, at least as far as activating TRPV1 and not producing CB1 receptor-mediated inhibitory effects are concerned, are similar in PSN. Together these findings indicate that anandamide that is synthesised in a Ca\(^{2+}\)-insensitive manner from 50 μM 20:4-NAPE has an excitatory rather than an inhibitory effect in PSN.

In conclusion, results of the present study show that: (a) a sub-population of PSN has at least one enzymatic pathway which synthesises anandamide from 20:4-NAPE in a Ca\(^{2+}\)-insensitive manner; (b) the synthesis of anandamide from exogenous 20:4-NAPE primarily, if not exclusively, occurs via Ca\(^{2+}\)-insensitive pathways; and (c) anandamide of PSN origin synthesised from 20:4-NAPE in a Ca\(^{2+}\)-insensitive manner produces an autocrine TRPV1-mediated excitation, which may complement the previously reported excitatory effects on TRPV1 of anandamide synthesised in a Ca\(^{2+}\)-sensitive manner in PSN [13]. The effect of anandamide of PSN origin together with the effects of exogenous anandamide on PSN [1, 12, 14, 24, 33] emphasises the high degree of flexibility of action of this important lipid mediator, and its multifaceted role in controlling nociceptive processing [40]. Therefore, a better understanding of the expression and function of the anandamide-synthesising enzymes in PSN may allow us to control the activity of those cells, and hence develop more effective treatments of somato- and viscerosensory disturbances outside the central nervous system.

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References


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