N-Arachidonoyl Dopamine: A Novel Endocannabinoid and Endovanilloid with Widespread Physiological and Pharmacological Activities

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Abstract

N-arachidonoyl dopamine (NADA) is a member of the family of endocannabinoids to which several other N-acyldopamines belong as well. Their activity is mediated through various targets that include cannabinoid receptors or transient receptor potential vanilloid (TRPV)1. Synthesis and degradation of NADA are not yet fully understood. Nonetheless, there is evidence that NADA plays an important role in nociception and inflammation in the central and peripheral nervous system. The TRPV1 receptor, for which NADA is a potent agonist, was shown to be an endogenous transducer of noxious heat. Moreover, it has been demonstrated that NADA exerts protective and antioxidative properties in microglial cell cultures, cortical neurons, and organotypical hippocampal slice cultures. NADA is present in very low concentrations in the brain and is seemingly not involved in activation of the classical pathways. We believe that treatment with exogenous NADA during and after injury might be beneficial. This review summarizes the recent findings on biochemical properties of NADA and other N-acyldopamines and their role in physiological and pathological processes. These findings provide strong evidence that NADA is an effective agent to manage neuroinflammatory diseases or pain and can be useful in designing novel therapeutic strategies.

Keywords: cannabinoid receptor 1; endocannabinoid; endovanilloid; N-arachidonoyl dopamine; transient receptor potential vanilloid 1

Introduction

The endocannabinoid (EC) system consists of cannabinoid receptors, mediators, and enzymes responsible for the synthesis and degradation of endogenous ligands, namely ECs. ECs are lipid signaling molecules, which are involved in a diverse range of physiological and pathological processes.1–3 N-acyldopamines consist of a hydrocarbon tail and a polar head group capable of interacting with cell membranes, membrane proteins, or ion channels function. The best examined member of this group is N-arachidonoyl dopamine (NADA) next to endogenous N-oleoyl dopamine (OLDA), N-palmitoyl dopamine (PALDA), and N-stearoyl dopamine (STERDA) and synthetic N-octanoyl dopamine (NOD).4 The formation and inactivation of N-acyldopamines as well as their significance under physiological and pathological conditions are not fully understood yet. NADA was first synthesized as a pharmacological tool to study the EC system.5 Later NADA and other N-acyldopamines were identified as endogenous cannabinoids in the mammalian nervous tissue.6,7 Several lines of evidence identified NADA, next to anandamide (AEA), as a member of the endovanilloid family acting as an agonist with similar potency as capsaicin.6 NADA and OLDA act on transient receptor potential vanilloid (TRPV)1 and play an important role in nociception. It was postulated that endovanilloids such as AEA or NADA participate in the
development of neuropathic pain and inflammatory hyperalgesia. Despite similarity in the structures of NADA and AEA, these two ECs vary in their functional activity: some of them will be mentioned later.

This review is divided in four sections. (1) The first section describes distribution and the current status of our understanding on the synthesis, transport, and degradation of N-acyldopamines. (2) The second section summarizes our current knowledge on the pharmacology of N-acyldopamines and their receptors, such as cannabinoid (CB), CB-like, and TRP receptors, and coupled signal transduction pathways under physiological and pathological circumstances will be reported. (3) The third section deals with N-acyldopamines mediated modulation of neuropathic pain and inflammatory hyperalgesia. (4) Finally, other effects of N-acyldopamines, protective versus toxic, with actions on immune and those in vascular system are mentioned.

**NADA: Chemistry, Distribution, Synthesis, Transport, and Degradation**

NADA is an arachidonic acid derivative with a dopamine moiety in its structure (Fig. 1). Using quadrupole time-of-flight analysis, the presence of NADA has been reported in the striatum, hippocampus, cerebellum, thalamus, midbrain, and dorsal root ganglia (DRGs). However, Bradshaw et al. detected NADA exclusively in striatum and hippocampus. Other N-acyldopamines such as OLDA, PALDA, and STEARDA were found in bovine brain. A recent study reported NADA at a concentration of 0.74±0.20 pg mg⁻¹ and OLDA at 0.15±0.08 pg mg⁻¹ in the murine striatum. A basal level of 2.6±1.2 pmol g⁻¹ wet tissue weight NADA was found in rat substantia nigra pars compacta. Human plasma and human postmortem brain were devoid of NADA as analyzed by different chromatographical methods.

The synthesis of N-acyldopamines is not yet fully understood. The biosynthesis of NADA has been examined by using both in vivo and in vitro assays. In striatum, a region with high dopamine concentrations, NADA biosynthesis primarily occurred through an enzyme-mediated conjugation of arachidonic acid with dopamine requiring tyrosine hydroxylase (TH).

NADA synthesis was observed almost exclusively in dopaminergic terminals, indicating that the dopamine level seemed to be the limiting factor. Fatty acid amide hydrolase (FAAH), a membrane-bound enzyme involved in AEA degradation, seemed also to be a rate-limiting enzyme in NADA biosynthesis, as the lack of FAAH led to a decrease of the striatal NADA concentration. Concomitant NADA was shown to be a weak substrate and a competitive inhibitor (IC₅₀ = 19–100 μM) of FAAH. As a competitive inhibitor, NADA binds to the active site of the enzyme and reduces the proportion of enzyme molecules available for binding the main substrate, namely AEA. In this case, NADA inhibits the inactivation of AEA, suggesting a NADA-dependent potentiation of the AEA effects, or a control mechanism to prevent overloading with NADA.

Nevertheless, incubation of dopamine with arachidonic acid in the presence of FAAH led to the production of detectable amounts of NADA in vitro. Dopamine receptors and FAAH were found in the same brain regions, such as hippocampus, striatum, and parts of cortex. NADA can, therefore, be produced in brain regions with a meaningful expression of FAAH and TH and released into the extracellular space to act on cells expressing target receptors.

ECs are degraded through a three-step mechanism namely cellular uptake, enzymatic hydrolysis, and re-esterification into membrane phospholipids. These pathways are also involved in the degradation of NADA (Fig. 1). The cellular uptake of lipids such as AEA occurs through diffusion or/and transporter proteins. So far, there is no direct evidence of a membrane transporter for NADA, although numerous studies assumed its presence. Pharmacological studies have revealed a rapid uptake of NADA by anandamide membrane transporter (AMT) in C6 glioma cells, leading to inhibition of AMT (IC₅₀ = 21.5 ± 9.1 μM). At high concentration OLDA (IC₅₀ = 17.5 μM), PALDA (IC₅₀ > 25 μM), and STEARDA (IC₅₀ > 25 μM) inhibit AMT in the [¹⁴C] AEA uptake test in RBL-2H3 cells. Regulation of an intracellular transport might have an important protective function. An increase in intracellular NADA concentration can deactivate AMT, preventing the receptor or/and intracellular signal cascades from overstimulation. In C6 glioma cells, NADA was hydrolyzed slower than AEA, probably by FAAH to arachidonic acid and dopamine. As originally postulated and later confirmed, NADA acts as a substrate for catechol-O-methyl-transferase (COMT). COMT is involved in the inactivation of catecholamines including dopamine and is distributed in the rat cerebral cortex, neostriatum, and cerebellar cortex. This enzyme transforms NADA to O-methyl-NADA, which is less active at TRPV1 than NADA.

Like other ECs, NADA was shown to be metabolized through the cytochrome P450 pathway in rat liver.

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Furthermore, oxidation of the arachidonoyl moiety played a minor role in endovanilloid inactivation. More immunohistological and colocalization studies are needed to verify the subcellular distribution of FAAH, TH, or COMT, major metabolizing enzymes for NADA (Fig. 1).

Receptors and the Signaling Pathways

CB1 receptor

So far, only NADA and OLDA have been described as agonists of CB1, which is mainly present on neurons (Table 1). The CB1 receptor is expressed presynaptically on neurons among others in forebrain, hindbrain, and in the spinal cord. Not only neurons but also glial cells have been reported to express CB1 receptors. The EC signaling plays a crucial modulating role in hippocampal formation, basal ganglia, cerebellum, and neocortex. In the peripheral nervous system, the activity of nerve fibers innervating smooth muscles is modulated by the CB1 receptor. Knockout of the CB1 receptor in mice is not lethal, but significantly leads to affected behavior and learning processes.

Natural and recombinant CB1 receptors can be coupled to Gs, G11/o, and Gq proteins even in the same system. Several receptors that preferentially couple to G11/o are able to interact with Go, particularly when receptors and/or G proteins are expressed at high densities and high concentrations of agonists are present. Signaling downstream of cannabinoid receptors is linked to regulator molecules and intracellular signaling networks that control basic cell functions. The precise characterization signaling effects are different because of strong differences in experimental design.
In addition, homo- and heterodimerization of CB1 with other receptors such as dopamine receptors, expression and coupling of CB1 to channels/signaling cascades, or basal activity of those receptors are still a matter of controversial debate.38

Typical actions mediated by Gi/o are direct inhibition of adenylyl cyclase, ensuing inhibition of protein kinase A, direct modulation of p44/42 mitogen-activated protein kinase (MAPK), activation of G protein coupled inwardly rectifying potassium channels, and inhibition of calcium channels. Modulation of p38 MAPK and c-Jun N-terminal kinases (JNKs) was also observed after Gi coupling. In contrast, Gq was shown to increase the intracellular calcium concentration.33,34,39–42

After activation of CB1 receptor, a transient Ca2+ elevation is evoked in a phospholipase C-dependent manner through either Gi/o or Gq proteins.43,44 Activation of Gq leads to receptor-mediated Ca2+ influx and to continued activation of different phospholipases.38 In the absence of extracellular calcium, NADA stimulated an intracellular Ca2+ mobilization in undifferentiated N18TG2 neuroblastoma cells. This effect was counteracted by the CB1 antagonist SR141716A and mimicked by CB1 agonist, HU-210.5 Redmond et al. were unable to reproduce the NADA-mediated elevation of [Ca]i under different assay and cell handling conditions.45 In the study by Bisogno et al., cells were resuspended in a continuously stirred cuvette, and in Redmonds experiment, monolayer of the cells was plated.5,45

In human breast MCF-7 cancer cells, NADA potently inhibited (IC50 = 0.25 μM) the proliferation in a CB1-dependent and D2-independent manner.5 In radioligand binding assay, NADA was shown to bind to hCB1 receptors and displaced both [3H]-CP55940 (Ki = 780 ± 240 nM) and [3H]-SR141716A (Ki = 230 ± 36 nM) with a similar affinity.5,45 NADA did not modulate p44/42 phosphorylation, adenylate cyclase, and potassium channels in cells expressing CB1. The authors concluded that NADA did not activate Gi/o or Gs coupled signaling. Interestingly, NADA (10–30 μM) mediated an activation of Gq/11 subunit of CB1, which led to an elevation in [Ca]i and induced an internalization of CB1.45

NADA (Ki = 250 nM) has an affinity to CB1 in the [3H]SR141716A binding inhibition assay, even stronger than AEA (Ki = 0.8 μM).5 Other compounds such as PALDA and STEARDA were inactive at concentrations smaller than 5 μM. OLDA exhibited some activity on CB1 receptor (Ki = 1.6 μM) in the mentioned assay7 (Table 1). In autaptic hippocampal neurons, NADA did not inhibit the excitatory postsynaptic current (EPSC) through CB1 in comparison with 2-AG.46,47

### Table 1. N-Arachidonoyl Dopamine Effects on Receptors and Enzymes

| Enzyme, receptor | Effect | Concentration | Experiment | Reference |
|------------------|--------|---------------|------------|-----------|
| abn-CBD | Antagonized by O1918 | | | 114 |
| CB1 | Agonist | Ki = 0.25 ± 0.13 (0.8 μM, brain) | Rat brain membranes, binding assay with [3H]SR141716A | 5,6 |
| CB2 | Agonist | pEC50 = 6.15 ± 0.09, Ki = 12.0 ± 4.0 (spleen) | The proliferation of human breast MCF-7 cancer cells were not inhibited by haloperidol a D2 antagonist | 6 |
| D2 | — | | | |
| FAAH | Inhibitor | IC50 = 19–100 μM | N18TG2 cells | 5 |
| MAGL | Inhibitor | pIC50 = 6.11 ± 0.08 (NPA) | Two in vitro assays | 116 |
| PPARγ1 | Agonist | 1–20 μM | GW9662 (1 μM), vasorelaxant response | 72 |
| 12-LOX | Inhibitor | IC50 = 150 ± 5 nM | Activity assay | 105 |
| TRPV1 | Activator | Kd = 5.49 ± 0.68 μM; EC50 = 40–6 mM (human); EC50 = 48 ± 7 nM (rat) | Binding of [3H]RTX, calcium imaging | 6 |
| TRPM8 | Antagonist | Submicromolar | TRPM8-HEK-293 cells overexpressing the human CB1 receptor | 117 |
| Voltage-gated sodium channel | Inhibitor | EC50 = 21 μM | Binding assay, mouse brain | 89 |

CB, cannabinoid; EC, endocannabinoid; FAAH, fatty acid amide hydrolase; HEK, human embryonic kidney; PPARγ1, peroxisome proliferator-activated receptor-γ1; TRPV, transient receptor potential vanilloid.

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Effects on TRP channels

NADA and OLDA increase intracellular calcium/induced calcium influx through activation of TRP receptors, especially the nonselective cation channel TRPV1.\(^6\)\(^,\)\(^7\) Not only NADA and AEA but also lipooxygenase products of arachidonic acid and NADA metabolites activated the TRPV1 receptors.\(^26\),\(^48\),\(^49\) Nevertheless, distribution studies have revealed inconsistent results, beginning with the presence of this receptor in the brain tissue. Data about the localization in dentate gyrus, hippocampal pyramidial neurons, are still a matter of debate. Therefore, it is difficult to assess the results describing the action of NADA on TRPV1. The use of unspecific antibodies or the lack of suitable, reliable controls in immunohistological and Western blot analysis may result in such findings. Even in electrophysiological experiments, it is possible to trigger unspecific effects by using nonphysiological high ligand concentrations. Over the years, Trpv1 mRNA was shown to be widely expressed in primary sensory fibers and in diverse areas of the central nervous system, most abundant in the limbic system, striatum, hypothalamus, centromedian and paraventricular thalamic nuclei, substantia nigra, reticular formation, and cerebellum.\(^48\),\(^50\)–\(^55\) However, later studies provide strong evidence for the presence of TRPV1 in low levels in the brain.\(^56\)

Although the expression of trpv1 mRNA in the brain was confirmed by many authors, the functionally active TRPV1 proteins seem to be missing in some cells. The issues of distribution of functionally active TRPV1 remain open, more studies are needed to confirm the role of TRPV1 in the brain.

To study the pharmacology of TRPV1 receptor and NADA-mediated effects, heterologous expression systems, such as transfected Chinese hamster ovary and HEK-293 cells with the TRPV1 (HEK-293-TRPV1) receptor, were used. Activation of the TRPV1 receptor led to nonselective cation influx, calcium influx, membrane depolarization, and glutamate release and cell death.\(^48\),\(^52\) The phosphorylation of the TRPV1 receptor induced also a sensitization, whereas desensitization was caused by dephosphorylation.\(^52\) NADA has been shown to activate both human and rat TRPV1 overexpressed in HEK-293 cells (EC\(_{50}\) \(\approx\) 50 nM).\(^6\),\(^46\),\(^57\) OLDA activated TRPV1 in human embryonic kidney (HEK) cells overexpressing human TRPV1 (EC\(_{50}\) \(\approx\) 36 nM) because PALDA and STERDA were inactive.\(^7\) In TRPV1 knockout murine trigeminal ganglion (TG) cells, NADA did not induce a current contrary to AEA, indicating the agonistic nature of NADA on TRPV1.\(^58\) Other ECs such as PALDA and STERDA, mediated an entourage effect on NADA-mediated actions, indicating an enhanced calcium mobilization through TRPV1 when coapplied with NADA.\(^59\)

NADA binds to the intracellular domain of TRPV1 and requires a transport across the cell membrane.\(^54\),\(^56\),\(^61\) It has been reported that NADA and capsaicin were equipotent in rat neonatal DRG neurons.\(^6\) However, we demonstrated that the response of NADA (10 \(\mu\)M) application was relatively small in comparison with capsaicin (10 \(\mu\)M) in patch clamp experiments on HEK-293-TRPV1.\(^46\) Our results were supported by electrophysiological approaches in DRG neurons and in isolated guinea pig bronchi and urinary bladder\(^62\),\(^63\) (Table 2).

Cross-talk between CB\(_1\) and TRPV1 receptors

The interaction between CB\(_1\) and TRPV1 has been postulated in several studies.\(^6\),\(^46\)–\(^66\) There is an evidence of a high degree of colocalization of CB\(_1\) and TRPV1 in DRG, and in neuron-enriched mesencephalic cultures, hippocampus, and cerebellum.\(^65\),\(^67\)–\(^70\) To our knowledge, none of the studies have demonstrated the colocalization of both receptors at synaptic levels. It was suggested that the activation of CB\(_1\) receptor may inhibit TRPV1-mediated toxic events.\(^66\)

In adult rat DRG neurons, NADA evoked significantly CB\(_1\)- and TRPV1-dependent increases in intracellular calcium.\(^6\),\(^63\) It was postulated that blocking of CB\(_1\) receptor by the selective antagonist SR141716A alters NADA uptake into neurons and, thereby, reduces the ability of NADA to activate TRPV1.\(^63\) In the presence of an antagonist, CB\(_1\) may block AMT and prevent thereby the NADA binding to the active site of TRPV1.

Application of capsaicin or NADA (1 \(\mu\)M) evoked increases in intracellular calcium concentration in DRG neurons through activation of TRPV1. This response was attenuated by both FAAH inhibitor (URB597) and AMT inhibitor (UCM707). Reduction in synthesis or uptake of NADA may explain this effect.\(^71\) In substantia nigra pars compacta, NADA in the presence of one of the antagonists activated CB\(_1\) and TRPV1 in a concentration-dependent manner.\(^65\)

In patch clamp experiments, NADA led to an increase in glutamatergic transmission through TRPV1 but decreased the GABAergic transmission through CB\(_1\) in dopaminergic neurons measured as sEPSC (spontaneous excitatory postsynaptic currents), resulting in an excitatory effect. In contrast, NADA (1 \(\mu\)M)-mediated
## Table 2. N-Arachidonoyl Dopamine Effects on Different Organ Systems and Processes

| Pain                                      | ±       | Concentration | Model system | Proposed mechanism                                                                 | Reference |
|-------------------------------------------|---------|---------------|--------------|-------------------------------------------------------------------------------------|-----------|
| Allodynia                                 | Induction | 0.0013–0.004 M |              | Unanesthetized rhesus monkey                                                        |           |
| Thermal hyperalgesia                      | Reduction | 1.5–50 µg; ED_{50}=22.5 µg |              | In vivo: rat with unilateral hind paw carrageenan-induced inflammation                   | 82        |
| Analgesia                                 | Inhibition | 1–10 mg kg^{-1} |              | Awake sats injected intraplanar                                                        | 5         |
| Innocuous and noxious mechanically         | Inhibition | 1.5 or 5 µg in 50 µL |              | C_{B}, TRPV1                                                                         | 63        |
| Prolonged elevation of presynaptic (Ca^{2+}) | Induction | 5 µM          |              | Rat sensory neurons/cell cultures                                                     | 1.18      |
| Mechanically evoked responses of dorsal horn neurons | Reduction | 5 µg mL^{-1}   |              | C_{B} (SR141716A); TRPV1 (capsazepine)                                               | 63        |
| CGRP                                      | Release/increase | 4 mg/kg; 1, 10 µM |              | In vivo, isolated mesenteric artery                                                   | 1.15      |

### Calcium mobilization from intracellular stores

| Ca^{2+} influx                          | Increase | EC_{50}=6.15±0.09 | HEK-293-TRPV1, CHO-VR1, TRPV1-Xenopus oocytes | Neuroblastoma N18TG2 cells | 7.2, 6, 6, 3, 11 |
|-----------------------------------------|----------|-------------------|---------------------------------------------|---------------------------|-----------------|
| [Ca^{2+}]                                | Increase | 1 µM; 3, 10 µM | DRG                                           | TRPV1                    | 1.25            |
| Calcium mobilization from intracellular stores | Increase | 3 µM; EC_{50}=1.6–794 nM | |                              | 88               |
| Calcium mobilization from intracellular stores | Increase | 1–100 µM; EC_{50}=2.4±M | |                              | 88               |

### Outward currents

| ^{3}H GABA release                      | Reduction | 10 µM | Rat hippocampal nerve terminals/synaptosomes | TASK-3                  |           |
|-----------------------------------------|-----------|-------|---------------------------------------------|-------------------------|-----------|
| ^{3}H GABA release                      | Induction | 30 µM | Rat hippocampal nerve terminals              | TRPV1^{-1} TG           | 1.25      |
| Glutamatergic transmission sIPSC        | Decrease  | 10 µM | Substantia nigra pars compacta rat           | C_{B}                   | 65        |
| Glutamate release                       | Induction | 30 µM | Rat hippocampal nerve terminals              | C_{B}                   | 65        |
| Glutamatergic transmission sEPSC       | Increase  | 5 µM  | Rat sensory neurons/sensory synapses         | TRPV1                   | 1.18      |
| Glutamate release                       | Induction | 5 µM  | Substantia nigra pars compacta, rat          | TRPV1                   | 65        |
| Glutamatergic transmission sEPSC       | Decrease  | 3–10 µM | Substantia nigra pars compacta, rat         | C_{B}                   | 65        |

### Spontaneous and heat-evoked activity

| Enhancement Spontaneous and heat-evoked activity | EC_{50}=1.55 µg | SPG nociceptive neurons | TRPV1 | 8 |
| Discharge of spinal nociceptive neurons | Increase | 0.1% | Hindpaw injection | TRPV1 | 8 |

### Toxic

| Vascular system | Increase | 3.4–100 µM; EC_{50}=30 µM | Peripheral blood mononuclear preparation | TRPV1, C_{B} | 1.22 |
| Vascular system | Induction | 10 nM–100 µM; pEC_{50} = 6.39±0.12 | Small mesenteric vessel | TRPV1 | 1.22 |
| Vascular system | Induction | 10 nM–100 µM; pEC_{50} = 5.45±0.15 | Rat aorta, in vitro | TRPV1 | 1.14 |
| Vascular system | Induction | 10 nM–100 µM; pEC_{50} = 5.99±0.17 | Superior mesenteric artery | TRPV1 | 1.14 |

### Urinary system

| Contraction Urinary system | Induction | EC_{50}=3.7±0.3 µM; E_{max}=12.0±0.1% of carbachol E_{max} | Guinea pig urinary bladder | TRPV1, CB_{1} | 62 |
| Contraction Urinary system | Induction | EC_{50}=19.9±0.1 µM; E_{max}=20.7±0.7% of carbachol E_{max} | Rat urinary bladder | TRPV1, CB_{1} | 62 |

### Respiratory system

| Contraction Respiratory system | Induction | EC_{50}=12.6±1.7 µM; E_{max}=69.2±2.4% of carbachol E_{max} | Guinea pig bronchi | TRPV1 | 1.24 |

### Sensitization

| Sensitization          | Induction | 400 µg kg^{-1} min^{-1} | 0.5 mL; 2 min | In vivo: lung vagal afferents | TRPV1 | 62 |

(continued)
Table 2. (Continued)

| Concentration | Model system | Proposed mechanism | Reference |
|---------------|--------------|--------------------|-----------|
| ±             |              |                    |           |
| Immunological processes | | | |
| p112-Lipooxygenase | Inhibition | IC\textsubscript{50} = 150 ± 5 nM | LO inhibition assay | 105 |
| PGE2 release | Inhibition | 1–2.5 μM | b.end5 cell line | 107 |
| PGD2 release | Increase | 1–2.5 μM | b.end5 cell line | 107 |
| COX-2 mRNA | Stabilization | 1–2.5 μM | | 107 |
| IL-2 and TNFα gene transcription | Inhibition | 2.5–5 μM | Stimulated Jurkat T cells | 108 |
| Transcription factor NF-κB, NFAT, AP-1 | Inhibition | 2.5 μM | Stimulated Jurkat T cells | 111 |
| HIV replication | Inhibition | 1–10 μM | Staphylococcal enterotoxin B-activated peripheral T cells | 111 |
| Neutrophil migration and chemotaxis | Inhibition | IC\textsubscript{50} = 8.80 nM (4.7–16.2); EC\textsubscript{50} = 64 (56.6–71.5)% | Boyden chamber, induced by fMLP | 113 |
| PGE2 synthesis | Inhibitor | 1–2.5 μM | Primary glial (microglia, astrocytes) cells | 104 |
| Free radical formation | Prevent | 1–2.5 μM | Primary glial (microglia, astrocytes) cells | 106 |
| Inflammatory responses | Reduction | 10 μM | Human lung microvascular endothelial cells CB\textsubscript{1} partially | 125 |
| Protection | | | |
| Neuroprotection | Induction | 100 pM–10 μM | Excitotoxically lesioned OHSC | CB\textsubscript{1} partially | 46 |
| Protection of cortical neurons | Induction | 10 μM | Hypoxia-induced cytotoxicity in SK-N-SH cell line | | 100 |
| Protection in hypoxia model | Induction | 10 μM | Exctotoxity of D-glucaric monoesters | | 98 |
| Mean arterial pressure (MAP) | Decrease | 1, 4, 10 mg kg\textsuperscript{-1} | In vivo: high sodium/normal treated rats | CB\textsubscript{1} partially | 99 |
| Neuroprotective and angiogenesis genes | Induction | 10 μM | Human primary astrocytes, SK-N-SH cells, HUVECs, HBMECs cells | | 113 |
| Antioxidative properties | + | 0.1–10 μM | Cerebellar granule neurons, H\textsubscript{2}O\textsubscript{2} | | 98 |
| Neuroprotection | | | |
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| Antioxidative properties | + | 0.1–10 μM | Cerebellar granule neurons, H\textsubscript{2}O\textsubscript{2} | | 98 |
| Others | | | |
| Teratogenic actions | Antagonist | 2 mg kg\textsuperscript{-1} | Strongly proconvulsant, Lytechinus variegatus | | 126 |
| Neuroprotection | Induction | 100 pM–10 μM | Excitotoxically lesioned OHSC | CB\textsubscript{1} partially | 46 |
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AMT, anandamide membrane transporter; CGRP, calcitonin gene-related peptide; CHO, Chinese hamster ovary; DRGs, dorsal root ganglia; GABA, gamma-aminobutyric acid; HUVECs, human umbilical vein endothelial cells; sEPSCs, spontaneous excitatory postsynaptic currents; sIPSC, spontaneous inhibitory postsynaptic current; TG, trigeminal ganglion.
CB₁ activation had an inhibitory effect measured as spontaneous inhibitory postsynaptic current on dopamine neurons after blockade of TRPV1. Furthermore, tonic inhibition of GABAergic transmission was mediated by NADA (1 μM) in a CB₁-dependent way without the involvement of TRPV1.10

Peroxisome proliferator-activated receptor-γ and NADA

Peroxisome proliferator-activated receptor-γ (PPARγ) is a nuclear receptor and transcription factor in the steroid superfamily. An increase in its transcriptional activity was induced by NADA among other cannabinoids.72 NADA caused a time-dependent PPARγ-mediated, NO-dependent vasorelaxation of rat aorta. NADA’s mentioned activity was inhibited by PPARγ antagonist (GW9662), CB₁ receptor antagonist (AM251), and FAAH inhibitor (URB597). One possible explanation might be the involvement of FAAH in the synthesis of NADA. The inhibition of FAAH decreases NADA concentration and the dependent receptors remain inactivated. In addition, it results in an increased AEA concentration. Binding of NADA to CB₁ receptor initiates also different intracellular pathways, like MAPK that presumably activates PPARγ.73,74

The Role of NADA in Tissue Function and Diseases

NADA and pain

Nociception is defined as a process by which thermal, mechanical, or chemical stimuli are detected by nociceptors. The cell bodies of the nociceptors are localized in DRG and TG. There are two major classes of nociceptors. One class is regulated by TRPV1 depending on changes in the local tissue and thermal chemical signals and activation of CB receptors. The other class of nociceptors, the peptidergic C nociceptors, release neuuropeptides, such as substance P and calcitonin gene-related peptide (CGRP), and express TrkA receptor that binds nerve growth factor. It was demonstrated that the activation of antinociceptive CB₁ by NADA reduces the pronociceptive actions evoked by TRPV1.81

NADA displays antinociceptive and antihyperalgesic effects in models of inflammatory pain after intrathecal administration. These effects were reversed by antagonists of both CB₁ (all NADA concentrations) and TRPV1 (high NADA concentrations).84 NADA (5 μg) mimicked the action of TRPV1 antagonist and inhibited the neuronal responses to mechanical stimulation in electrophysiological recordings from the dorsal horn in anesthetized rats.83 In contrast to previous findings, NADA evoked CGRP release from TG neurons and from slices of rat dorsal horn spinal cord in a TRPV1-dependent manner, resulting in neurogenic inflammation. NADA injected in TG or administrated intraocularly was excitatory, pungent, and evoked nociceptive responses.85 It could also modulate different cation channels involved in pain sensation, like T-type calcium channels (Ca(V)3) (Tables 1 and 2). NADA strongly inhibits human recombinant T-type
calcium channels (Ca(V)3 channels) expressed in HEK-293 cells and native mouse T-type, which was shown to play an important role in modulating peripheral and central pain processing in a variety of pain models. The role of calcium channels in NADA-mediated processes provides an explanation for the lack of complete blockade with CB1 as well as TRPV1 antagonists.

In the hippocampus as well, NADA produces opposite effects on Ca\(^{2+}\) entry. For example, NADA induced a rise of resting presynaptic Ca\(^{2+}\) and enhanced the release of gamma-aminobutyric acid (GABA) and glutamate. However, in low micromolar range, NADA inhibited the K\(^{+}\)-evoked Ca\(^{2+}\) entry and K\(^{+}\)-evoked Ca\(^{2+}\)-dependent release of GABA and glutamate. These effects were not counteracted by JWH133 (CB2 antagonist), AM251 (CB1 antagonist), ruthenium red (TRPV1 antagonist), and sulpride (D2, D3, and D4 antagonist). Only TASK-3 inhibitors triggered the rise of resting intracellular Ca\(^{2+}\). NADA was shown to inhibit voltage-gated sodium channel (veratridine-dependent), release of \(\lambda\)-glutamate and GABA in the low micromolar range from synaptosomes isolated from murine brain. This EC may modulate neuronal excitation and depression in a CB1-independent way. NADA had no effect on N-type Ca\(^{2+}\) channels (Cav2.2) in rat sympathetic neurons in comparison with other cannabinoids. Furthermore, NADA controls striatal input terminals through novel ligand-gated cation channels and triggered the release of dopamine and glutamate in synaptosomes. These effects were not observed with capsaicin. Köfalvi et al. postulated that NADA affects TASK-3 channels.

Taken together, the mode of action of NADA mediated in the central nervous system needs further clarification. A wide choice of models covers a wide spectrum of physiological and pathological activities, but a complete characterization is missing. Colocalization studies of TRPV1 and CB1 in combination with electrophysiological and \(\textit{in vivo}\) studies will help to clarify how NADA influences the function of nociceptors, ganglion, spinal cord neurons, and neuron–glia interactions.

**Protection and toxicity**

Cannabinoids have been shown to exert neuroprotective effects in different models. Neuroprotection is mainly associated with CB1 receptor activation, whereas neurotoxicity is associated with TRPV1 activation. After excitotoxic lesion \(\textit{in vivo}\) as well as \(\textit{in vitro}\), CB1 receptor was found to prolong preservation of neurons. These results are in line with our data on NADA. NADA (1 nM) was neuroprotective in organotypical hippocampal slice cultures after NMDA treatment, partly through CB1. In electrophysiological experiments, NADA (1, 10 \(\mu\)M) did not inhibit EPSCs in autaptic hippocampal neurons. We assume that CB1-dependent decrease in intracellular calcium concentration does not mediate NADA’s neuroprotective effects. High concentrations of NADA (10 \(\mu\)M) seem to activate additional mechanisms preventing the neuronal demise as the neuroprotection was independent of CB1, TRPV1, and abn-CBD receptors. In addition, NADA showed protective effects in cultured cerebellar neurons by reducing oxidative stress induced by hydrogen peroxide and in primary hippocampal neurons against hypoxia through CB1. Pretreatment with NADA protected human neuroblastoma cell line SK-N- SH from hypoxia. NADA (5 \(\mu\)M) induced cell death in human neuron-like cell line SH-SY5Y, stably expressing recombinant human TRPV1. Despite similarities to an apoptotic process, the cell demise took place independent of caspase activity and was blocked by a TRPV1 antagonist. In contrast, \(\textit{in vivo}\) studies demonstrated protective effects of TRPV1 activation on neurons against excitotoxicity or ischemia. Little is known about the signaling pathways involved in NADA-mediated protection and toxicity. Also NOD seems to have a specific protective function in endothelial cells. In human umbilical vein endothelial cells, NOD was protective against cold preservation injury measured in lactate dehydrogenase test. Furthermore, NOD improved the renal function in setting of ischemia \(\textit{in vivo}\) by downregulation of NFκ\(\beta\) and subsequent inhibition of vascular cell adhesion molecule 1 in proximal tubular epithelial cells.

**Immune cells**

At cellular level, microglia plays a critical role in brain damage. NADA has an anti-inflammatory potential acting through a mechanism that involves reduction in the synthesis of microsomal prostaglandin E synthase (mPGES-1) in lipopolysaccharide-activated microglia. NADA is a potent inhibitor of PGE2 synthesis, without modifying the expression or catalytic activity of COX-2, or the production of prostaglandin D2 that plays a central role during neuroinflammation. It had also the ability to prevent free radical formation in primary microglial cells. AEA and NADA had opposite effects on glial cells.

Furthermore, NADA specifically inhibits \(\text{IL}-2\) and \(\text{TNF-}\alpha\) gene transcription in Jurkat T cells and inhibits...
the signaling pathways mediating the activation of transcription factors NF-κB, NFAT, and AP-1 involved in the immune response. NFAT was shown to regulate the changes in microglial phenotype.108,109

NOD did not affect the early T cell activation (IL-2, TNF-α, and IFN-γ) but inhibited NFκB and AP-1 activation in phorbol 12-myristate 13-acetate/ionomycin-stimulated T cells. It decreased the proliferation of both naïve and memory lymphocytes without any toxic effects. Moreover, in the presence of NOD, the number of T cells, which did not pass beyond the G0/G1 phase, increased.110 NADA had an inhibitory activity on HIV-1 replication in Staphylococcal enterotoxin B-activated peripheral primary T cells, peripheral blood mononuclear cell, and in Jurkat T cell line.111 This effect, independent of CB1 and FAAH, was believed to result from changes at the transcriptional level by affecting both Tat and NFκB-dependent transcription. NADA, OLDA, and PALDA also prevented the degranulation and release of TNFα and decreased the tnfα-mRNA in RBL-2H3 mast cells treated with an IgE-antigen complex. PALDA was the most potent antiallergic N-acyldopamine, which downregulates allergic mediators through multiple targets such as Syk, Akt, p44/42, cPLA2, and 5-LO pathways.112

Human neutrophil migration in Boyden chamber assay was inhibited by NADA (nM), independent of CB1 and CB2.113 We observed that NADA (100 pM and 1 μM) significantly reduced the number of isolectin B-positive microglial cells after excitotoxicity.46 These observations support the anti-inflammatory effects of NADA directly on immune cells.

Effects on vascular system
Several lines of evidence indicate that the cardiovascu-
lar depressive effects of cannabinoids are mediated by CB1 receptors. Recent studies provide strong support for the existence of as-yet-undefined endothelial and cardiac receptors that mediate certain EC-induced cardiovascu-
lar effects. TRPV1 receptor was shown to be present on sensory neurons innervating smooth muscles in several organs and in arteriolar smooth muscle cells.56 Besides, capsaicin-sensitive sensory nerves participate in regulation of the vascular tone, inter alia through the release of vasodilator neuropeptides, such as CGRP. NADA has been demonstrated to induce vasorelaxant effects in human small mesenteric vessels, the superior mesenteric artery, and in the aorta114 (Table 2).

In small mesenteric vessels, NADA-mediated vasorelaxant effects were CB1, abn-CBD, and TRPV1 dependent and were probably mediated by activation of potassium channels and an intrinsic endothelial mecha-

NADA-mediated vasorelaxation in superior mesen-
teric artery was CB1, capsaicin dependent, and inde-
pendent of abn-CBD receptor. Moreover, NADA caused dose-dependent depressive effects in rats fed with a normal and high-sodium diet. These effects were reversed by the TRPV1 antagonist, capsazepine, and CGRP receptor antagonists but not the CB1 receptor antagonist, SR141716A. Interestingly, activation of TRPV1 by NADA mediated the CGRP release from mesenteric arteries.115

Conclusions and Outlook
The aim of this review was to summarize the current knowledge on N-acyldopamines with special reference to the functional role of the endocannabinoid NADA, in brain, pain modulation, and in other organ systems. NADA acts mainly through CB and TRPV1 receptors participating in several physiological activities in the body. NADA is neuroprotective, acts on immune cells, and mediates vasorelaxation. NOD was also shown to inhibit T cell activation and could be used for the treatment of inflammatory diseases and in the transplantation medicine. NADA, NOD, and OLDA inhibited aggregation of human platelets. Further investigation is needed to explore their therapeutic application. NOD implementation in transplantology has been proposed several times. NADA seems to affect the proliferation/migration and actions of immune cells especially microglia; NOD inhibits the prolife-
ration of T cells but does not impair T cell activation. NADA potentially mediates both anti- and pronoci-
ceptive responses depending on the balance between CB1 receptors and TRPV1 channel activation, and the kind of stimulus. TRPV1 desensitization might be a possible explanation for diversity of NADA-mediated actions. A better understanding of the mechanism behind NADA, NOD, and OLDA-mediated actions may lead to development of novel therapies in acute neurological disorders and in neuroinflammatory pain. However, we need to understand first how exactly their synthesis and degradation occur, in which cell type these process take place, and to learn more about the function of endogenous NADA. The major-
ity of data originate from animal studies. It is possible that the conflicting data on NADA represent species and cell-specific differences. Even if the distribution of the receptors is conserved between species, the coupling to the signaling cascades and effectors is often
different. It is still not known whether NADA concentration levels change after lesion or under pathological situations. Precise determination of NADA as a “tricky” compound seems to be difficult. Therefore, better, more reliable, and faster methods are urgently needed. Presumably, like other endocannabinoids, NADA is produced on demand and gets degraded very fast. Owing to NADA-mediated neuroprotection, two parallel directions need to be investigated. First, how exactly N-acylcarnitines influence immune cells, especially microglia. The changes in microglial and lymphocytes morphology, migration, cytokine profile, mRNA, and microRNA expression need to be screened. Second, the better understanding of EC system under pathological conditions might help to establish NADA as a potential therapeutic agent, if the problems with its instability and oxidation are solved. Would chemical modifications make NADA’s application possible? The role of NADA in the regulation of motor activity and in the Parkinson’s disease needs further investigation.

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References
1. Howlett AC, Breivogel CS, Childers SR, et al. Cannabinoid physiology and pharmacology: 30 years of progress. Neuropsychopharmacology. 2004;47:345–358.
2. Lu HC, Mackie K. An introduction to the endogenous cannabinoid system. Biol Psychiatry. 2015;79:516–525.
3. Iannotti FA, Di Marzo V, Petrosino S. Endocannabinoids and neurodegenerative-related mediators: targets, metabolism and role in neurological disorders. Prog Lipid Res. 2016;62:107–128.
4. Connor M, Vaughan CW, Vandenberg RJ. N-Acyl amino acids and N-acyl neurotransmitter conjugates: neuromodulators and probes for new drug targets. Br J Pharmacol. 2010;160:1857–1871.
5. Bisogno T, Melck D, Bobrov M, et al. N-acyl-dopamines: novel synthetic CB(1) cannabinoid-receptor ligands and inhibitors of anandamide inactivation with cannabimimetic activity in vitro and in vivo. Biochem J. 2000;351:817–824.
6. Huang SM, Bisogno T, Trevisani M, et al. An endogenous capsaicin-like substance with high potency at recombinant and native vanilloid VR1 receptors. Proc Natl Acad Sci U S A. 2002;99:8400–8405.
7. Chu C, Huang S, De Petrocellis L, et al. N-Oleoyldopamine, a novel endogenous capsaicin-like lipid that produces hyperalgesia. J Biol Chem. 2003;278:13633–13639.
8. Huang SM, Walker JM. Enhancement of spontaneous and heat-evoked activity in spinal nociceptive neurons by the endovanilloid/endocannabinoid N-arachidonoyldopamine (NADA). J Neurophysiol. 2006;95:1207–1212.
9. Bradshaw HB, Rimmerman N, Krey JF, et al. Sex and hormonal cycle differences in rat brain levels of pain-related cannabinomimetic lipid mediators. Am J Physiol Regul Integr Comp Physiol. 2006;291:349–358.
10. Freestone PS, Guateto E, Piscitelli F, et al. Glutamate spillover drives endocannabinoid production and inhibits GABAergic transmission in the substantia nigra pars compacta. Neuropsychopharmacology. 2014;79:467–475.
11. J D, Jang C, Lee S. A sensitive and accurate quantitative method to determine N-arachidonyldopamine and N-oleoyldopamine in the mouse striatum using column-switching LC–MS–MS: use of a surrogate matrix to quantify endogenous compounds. Anal Bioanal Chem. 2014;406:4491–4499.
12. Balvers MGJ, Verhoeckx KCM, Witkamp RF. Development and validation of a quantitative method for the determination of 12 endocannabinoids and related compounds in human plasma using liquid chromatography-tandem mass spectrometry. J Chromatogr B Anal Technol Biomed Life Sci. 2009;877:1583–1590.
13. Lehtonen M, Stovrik M, Malinen H, et al. Determination of endocannabinoids in nematodes and human brain tissue by liquid chromatography electrospray ionization tandem mass spectrometry. J Chromatogr B Anal Technol Biomed Life Sci. 2011;879:677–694.
14. Tatemou A, Hopfpartner O, Vos C, et al. Quantitative and qualitative profiling of endocannabinoids in human plasma using a triple quadrupole linear ion trap mass spectrometer with liquid chromatography. Rapid Commun Mass Spectrom. 2009;23:629–638.
15. Hu SJJ, Bradshaw HB, Benton VM, et al. The biosynthesis of N-arachidonoyl dopamine (NADA), a putative endocannabinoid and endovanilloid, via conjugation of arachidonic acid with dopamine. Prostaglandins Leukot Essent Fat Acids. 2009;81:291–301.
16. Egerová M, Cravatt BF, Elphick MR. Comparative analysis of fatty acid amide hydrolase and CB1 cannabinoid receptor expression in the mouse brain: evidence of a widespread role for fatty acid amide hydrolase in regulation of endocannabinoid signaling. Neuroscience. 2009;119:481–496.
17. Jay TM. Dopamine: a potential substrate for synaptic plasticity and memory mechanisms. Prog Neurobiol. 2003;69:375–390.
18. Ortar G, Ligresti A, De Petrocellis L, et al. Novel selective and metabolically stable inhibitors of anandamide uptake. Biochem Pharmacol. 2003;65:1473–1481.
19. Glaser ST, Kaczocha M, Deutsch DG. Anandamide transport: a critical review. Life Sci. 2005;77:1584–1604.
20. Piomelli D, Beltramino M, Glasnapp S, et al. Structural determinants for recognition and translocation by the anandamide transporter. Proc Natl Acad Sci U S A. 1999;96:5802–5807.
21. Di Marzo V, Fontana A, Casad H, et al. Formation and inactivation of endogenous cannabinoid anandamide in central neurons. Nature. 1994;372:686–691.
22. Fowler CJ. Anandamide uptake explained? Trends Pharmacol Sci. 2012;33:181–185.
23. Vandevoorde S, Fowler CJ. Inhibition of fatty acid amide hydrolase and monoacylglycerol lipase by the anandamide uptake inhibitor VDM11: evidence that VDM11 acts as an FAAH substrate. Br J Pharmacol. 2005;145:885–893.
24. Cristina L, Starowicz K, De Petrocellis L, et al. Immunohistochemical localization of anabolic and catabolic enzymes for anandamide and other putative endovanilloids in the hippocampus and cerebellar cortex of the mouse brain. Neuroscience. 2008;151:955–968.
25. Harunun T, Tilgmann C, Ulmanen I, et al. Neuronal and non-neuronal cannabinoid receptor (CB1) and cannabinoid receptor (CB2) in primary cultures of rat brain cells. Int J Dev Neurosci. 1995;13:825–834.
26. Rimmerman N, Bradshaw HB, Basnet A, et al. Microsomal omega-hydroxylated metabolites of N-arachidonoyl dopamine are active at recombinant human TRPV1 receptors. Prostaglandins Other Lipid Mediat. 2009;88:10–17.
27. Tsou K, Brown S, Sahudo-Peña M, et al. Immunohistochemical distribution of cannabinoid CB1 receptors in the rat central nervous system. Neuroscience. 1997;83:393–411.
28. Hoffman AF, Riegel AC, Lupica CR. Functional localization of cannabinoid receptors and endogenous cannabinoid production in distinct neuron populations of the hippocampus. Eur J Neurosci. 2003;18:524–534.
29. Stella N. Cannabinoid and cannabinoid-like receptors in microglia, astrocytes, and astrocytomas. Glia. 2010;58:1017–1030.
30. Tantimanocon M, Ceci R, Sabatini S, et al. Physical activity and the endocannabinoid system: an overview. Cell Mol Life Sci. 2014;71:2681–2698.
31. Elphick MR, Egetova M. The neurobiology and evolution of cannabinoid signalling. Philos Trans R Soc B Biol Sci. 2001;356:381–408.
32. Marsigano G, Wotjak CT, Azad SC, et al. The endogenous cannabinoid system controls extinction of aversive memories. Nature. 2002;418:530–534.
33. Howlett AC, Barth F, Bonner TI, et al. International union of pharmacology. XXVII. Classification of cannabinoid receptors. Pharmacol Rev. 2002;54:161–202.
34. Pertwee RG, Howlett AC, Aboud ME, et al. International union of basic and clinical pharmacology. LXXIX. Cannabinoid receptors and their ligands: beyond CB 1 and CB 2. Pharmacol Rev. 2010;62:588–631.
35. Howlett AC, Blume LC, Dalton GD. CB(1) cannabinoid receptors and their associated proteins. Curr Med Chem. 2010;17:1382–1393.
36. Howlett AC, Reggio PH, Childers SR, et al. Endocannabinoid tone versus constitutive activity of cannabinoid receptors. Br J Pharmacol. 2011;163:1329–1343.
37. Weiss J. Molecular basis of receptor/G-protein-coupling selectivity. Pharmacol Ther. 1999;80:231–264.
38. Turu G, Hunyady L. Signal transduction of the CB1 cannabinoid receptor. J Mol Endocrinol. 2010;44:75–85.
39. Bouaboula M, Perrachon S, Milligan L, et al. A selective inverse agonist for central cannabinoid receptor inhibits mitogen-activated protein kinase activation stimulated by insulin or insulin-like growth factor 1. Evidence for a new model of receptor/ligand interactions. J Biol Chem. 1997;272:23330–23339.
40. Smith TH, Sim-Selley LJ, Selley DE. Cannabinoid CB 1 receptor-interacting proteins: novel targets for central nervous system drug discovery? Br J Pharmacol. 2010;160:455–466.
41. Deedwayer S, Hampson R, Mu J, et al. Cannabinoids modulate voltage sensitive potassium A-current in hippocampal neurons via a CAM-dependent process. J Pharmacol Exp Ther. 1995;273:733–743.
42. Twitchell W, Brown S, Mackie K. Cannabinoids inhibit N- and P/Q-type calcium channels in cultured rat hippocampal neurons. J Neurophysiol. 1997;78:43–50.
43. Sugiyama T, Kodaka T, Kondo S, et al. Is the cannabinoid CB1 receptor a 2-arachidonoylglycerol receptor? Structural requirements for triggering a Ca2+ transient in NG108-15 cells. J Biochem. 1997;122:890–895.
44. Lauckner JE, Jensen JB, Chen H, et al. GPR55 is a cannabinoid receptor sensitive to hot peppers. Cell. 2002;108:421–430.
45. Mezey E, Toth ZE, Cortright DN, et al. Distribution of mRNA for vanilloid receptor type 1 (VR1) in the rodent urinary bladder. Eur J Pharmacol. 2003;475:107–114.
46. Marinelli S, Di Marzo V, Florenzano F, et al. N-arachidonoyl-dopamine opening of N-arachidonoyl-dopamine receptors. Br J Pharmacol. 2005;143:251–256.
47. Robert LA, Ross HR, Connor M. Methanandamide activation of a novel current in mouse trigeminal ganglion sensory neurons in vitro. Neuropharmacology. 2008;54:172–180.
48. Caterina MJ, Schumacher MA, Tominga M, et al. The capsaicin receptor: an androstenedione receptor with transient properties in NG108-15 cells. J Biochem. 1997;122:890–895.
49. 39. Bouaboula M, Perrachon S, Milligan L, et al. A selective inverse agonist for central cannabinoid receptor inhibits mitogen-activated protein kinase activation stimulated by insulin or insulin-like growth factor 1. Evidence for a new model of receptor/ligand interactions. J Biol Chem. 1997;272:23330–23339.
50. Malenka RC, Bear MF. LTP and LTD: an embarrassment of riches. Neuron. 2004;44:5–21.
51. Millns PJ, Chimenti M, Ali N, et al. Effects of inhibition of fatty acid amide hydrolase vs. the anandamide membrane transporter on TRPV1-mediated calcium responses in adult DRG neurons; the role of CB1 receptors. Eur J Neurosci. 2006;24:3489–3495.
52. O’Sullivan SE. Cannabinoids go nuclear: evidence for activation of peroxisome proliferator-activated receptors. Br J Pharmacol. 2007;152:576–582.
53. O’Sullivan SE, Kendall DA, Randall MD. Time-dependent vascular effects of endocannabinoids mediated by Peroxisome Proliferator-Activated Receptor Gamma (PPAR γ). PPAR Res. 2009;2009:1–9.
54. Yano M, Matsumura T, Senokuchi T, et al. Statins activate peroxisome proliferator-activated receptor gamma through extracellular signal-regulated kinase 1/2 and p38 mitogen-activated protein kinase-dependent cyclooxygenase-2 expression in macrophages. Circ Res. 2007;100:1442–1451.
55. Jordt SE, Julius D. Molecular basis for species-specific sensitivity to “hot” noxious stimuli. Neuron. 2002;37:383–394.
56. Harrisson S, De Petrocellis L, Transvahi M, et al. Capsaicin-like effects of N-arachidonoyl-dopamine in the isolated guinea pig bronchi and urinary bladder. Eur J Pharmacol. 2003;475:107–114.
57. Sagar DR, Smith PA, Mills PJ, et al. TRPV1 and CB 1 receptor-mediated effects of the endovanilloid / endocannabinoid N-arachidonoyl-dopamine on primary afferent fibre and spinal cord neuronal responses in the rat. Eur J Neurosci. 2004;20:175–184.
58. Hermann H, Petrocellis L, De, Bisogno T, et al. Dual effect of cannabinoid CB 1 receptor stimulation on a vanilloid VR1 receptor-mediated response. Cell Mol Life Sci. 2003;60:607–616.
59. Marinelli S, Di Marzo V, Florenzano F, et al. N-arachidonoyl-dopamine tunes synaptic transmission onto dopaminergic neurons by activating both cannabinoid and vanilloid receptors. Neuropsychopharmacology. 2007;32:298–308.
60. Maccarrone M, Lorenzon T, Bar M, et al. Anandamide induces apoptosis in human cells via vanilloid receptors. J Biol Chem. 2000;275:31938–31945.
61. Agarwal N, Pacher P, Tegeder I, et al. Cannabinoids mediate analgesia largely via peripheral type 1 cannabinoid receptors in nociceptors. Nat Neurosci. 2007;10:870–879.
62. Bridges D, Rice ASC, Egerrová M, et al. Localisation of cannabinoid receptor 1 in rat dorsal root ganglion using in situ hybridisation and immunohistochemistry. Neuroscience. 2003;119:803–812.
63. Hohmann A, Herkenham M. Cannabinoids undergo axonal flow in the nervous system of the rat and human. Proc Natl Acad Sci U S A. 2000;97:3655–3660.
78. Basbaum AI, Bautista DM, Scherrer G, et al. Cellular and molecular mechanisms of pain. Cell. 2010;139:267–284.
79. Chiu IM, Von Hefn CA, Woolf CJ. Neurogenic inflammation and the peripheral nervous system in host defense and immunopathology. Nat Neurosci. 2012;15:1063–1067.
80. Julius D. Molecular mechanisms of nociception. Nature. 2001;413: 203–210.
81. Sagar DR, Kelly S, Millins PJ, et al. Inhibitory effects of CB1 and CB2 receptor agonists on responses of DRG neurons and dorsal horn neurons in neuropathic rats. Eur J Neurosci. 2005;22:371–379.
82. Butelman ER, Ball JW, Harris TJ, et al. Topical capsaicin-induced alldynia in unanaesthetized primates: pharmacological modulation. J Pharmacol Exp Ther. 2003;306:1106–1114.
83. Frid E, Mechoulam R. Pharmacological activity of the cannabinoid receptor agonist, anandamide, a brain constituent. Eur J Pharmacol. 1993;231:313–314.
84. Farkas I, Tuboly G, Benedek G, et al. The antinoceptive potency of N-arachidonoyl-dopamine (NADA) and its interaction with endomorphin-1 at the spinal level. Pharmacol Biochem Behav. 2011;99:731–737.
85. Price TJ, Patwardhan A, Akopian AN, et al. Modulation of trigeminal sensory neuron activity by the dual cannabinoid CB1 receptor agonists anandamide, N-arachidonoyl-dopamine and arachidonoyl-2-chloroethylamide. Br J Pharmacol. 2004;141:1118–1130.
86. Ross HR, Gilmore AJ, Connor M. Inhibition of human recombinant T-type calcium channels by the endocannabinoid N-arachidonoyl dopamine. Br J Pharmacol. 2009;156:740–750.
87. Todorovic S, Jevtovic-Todorovic V. The role of T-type calcium channels in peripheral and central pain processing. CNS Neurosci Drug Targets. 2006;5:639–653.
88. Kofali A, Pereira MF, Rebola N, et al. Anandamide and NADA bi-directionally modulate presynaptic Ca2+ levels and transmitter release in the hippocampus. Br J Pharmacol. 2007;151:551–563.
89. Duan Y, Zheng J, Nicholson RA. Inhibition of 3H[batrachotoxinin A-20]benzoate binding to sodium channels and sodium channel function by endocannabinoids. Neurochem Int. 2008;52:438–446.
90. Guo J, Williams DJ, Ikeda SR. N-arachidonoyl L-serine, a putative endocannabinoid, alters the activation of N-type Ca2+ channels in sympathetic neurons. J Neurophysiol. 2008;100:1147–1151.
91. Eljaschewitsch E, Witting A, Mawrin C, et al. The endocannabinoid receptor agonists and modulated by TRPA1 and Ca v2.2 channels. Hippocampus. 2011;21:554–564.
92. Wedel J, Hottenrott MC, Stamellou E, et al. N-Octanoyl dopamine transiently inhibits T cell proliferation via G1 cell-cycle arrest and inhibition of redox-dependent transcription factors. J Leukoc Biol. 2014;96:453–462.
93. Sancho R, Macho A, de la Vega L, et al. Immunosuppressive activity of endovaniloids: N-arachidonoyl-dopamine inhibits activation of the NF-kappa B, NFAT, and activator protein 1 signaling pathways. J Immunol. 2004;172:2341–2351.
94. Nagamoto-Combs K, Combs CK. Microglial phenotype is regulated by activity of the transcription factor, NFAT (nuclear factor of activated T cells). J Neurosci. 2010;30:9641–9646.
95. Wedel J, Hottenrott MC, Stamellou E, et al. N-Octanoyl dopamine transiently inhibits T cell proliferation via G1 cell-cycle arrest and inhibition of redox-dependent transcription factors. J Leukoc Biol. 2014;96:453–462.
96. Sancho R, de la Vega L, Macho A, et al. Mechanisms of HIV-1 inhibition by the lipid mediator N-arachidonoyldopamine. J Immunol. 2005;175:3990–3999.
97. Yoo JM, Sok DE, Kim MR. Effect of endocannabinoids on IgE-mediated allergic response in RBL-2H3 cells. Int Immunopharmacol. 2013;17:123–131.
98. McHugh D, Tanner C, Mechoulam R, et al. Inhibition of human neutrophil chemotaxis by endogenous cannabinoids and phytocannabinoids: evidence for a site distinct from CB1 and CB2. Mol Pharmacol. 2007;74:441–450.
99. O’Sullivan SE, Kendall DA, Randall MD. Characterisation of the vasorelaxant properties of the novel endocannabinoid N-arachidonoyl-dopamine (NADA). Br J Pharmacol. 2004;141:803–812.
100. Wang Y, Wang DH. Increased depressor response to N-arachidonoyl-dopamine during high salt intake: role of the TRPV1 receptor. J Hypertens. 2007;25:2426–2433.
101. Björklund E, Nore´n E, Nilsson J, et al. Inhibition of monoacylglycerol lipase by the endocannabinoid N-arachidonoyldopamine during high salt intake: role of the TRPV1 receptor. J Hypertens. 2007;25:2426–2433.
121. McDonald HA, Neelands TR, Kort M, et al. Characterization of A-425619 at native TRPV1 receptors: a comparison between dorsal root ganglia and trigeminal ganglia. Eur J Pharmacol. 2008;596:62–69.

122. Saunders CI, Fassett RG, Geraghty DP. Up-regulation of TRPV1 in mononuclear cells of end-stage kidney disease patients increases susceptibility to N-arachidonoyl-dopamine (NADA)-induced cell death. Biochim Biophys Acta. 2009;1792:1019–1026.

123. Wojtalla A, Herweck F, Granzow M, et al. The endocannabinoid N-arachidonoyl dopamine (NADA) selectively induces oxidative stress-mediated cell death in hepatic stellate cells but not in hepatocytes. Am J Physiol Gastrointest Liver Physiol. 2012;302:873–887.

124. Hsu CC, Bien MY, Huang YT, et al. N-Arachidonyl dopamine sensitizes rat capsaicin-sensitive lung vagal afferents via activation of TRPV1 receptors. Respir Physiol Neurobiol. 2009;167:323–332.

125. Wilhelmsen K, Khakpour S, Tran A, et al. The endocannabinoid/endovanilloid N-arachidonoyl dopamine (NADA) and synthetic cannabinoid WIN55, 212-2 abate the inflammatory activation of human endothelial. J Biol Chem. 2014;289:13079–13100.

126. Buznikov GA, Nikitina LA, Bezuglov VV, et al. A putative “pre-nervous” endocannabinoid system in early echinoderm development. Dev Neurosci. 2010;32:1–18.

127. Sharkey KA, Cristino L, Oland LD, et al. Arvanil, anandamide and N-arachidonoyl-dopamine (NADA) inhibit emesis through cannabinoid CB1 and vanilloid TRPV1 receptors in the ferret. Eur J Neurosci. 2007;25:2773–2782.

128. Hayase T. Differential effects of TRPV1 receptor ligands against nicotine-induced depression-like behaviors. BMC Pharmacol. 2011;11:1–11.

129. Trujillo X, Ortiz-Mesina M, Uribe T, et al. Capsaicin and N-arachidonoyl-dopamine (NADA) decrease tension by activating both cannabinoid and vanilloid receptors in fast skeletal muscle fibers of the frog. J Memb Biol. 2015;248:31–38.

130. Almaghrabi SY, Geragthy DP, Ahuja KDK, et al. Vanilloid-like agents inhibit aggregation of human platelets. Thromb Res. 2014;134:412–417.

131. Ahn S, Yi S, Seo WJ, et al. A cannabinoid receptor agonist N-arachidonoyl dopamine inhibits adipocyte differentiation in human mesenchymal stem cells. Biomol Ther (Seoul). 2015;23:218–224.

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Abbreviations Used

| Abbreviation | Definition |
|--------------|------------|
| AEA | anandamide |
| AMT | anandamide membrane transporter |
| CB | cannabinoid |
| CGRP | calcitonin gene-related peptide |
| CHO | Chinese hamster ovary |
| COMT | catechol-O-methyl-transferase |
| D | dopamine receptor |
| DRGs | dorsal root ganglia |
| ECs | endocannabinoids |
| FAAH | fatty acid amide hydrolase |
| HEK | human embryonic kidney |
| HUVECs | human umbilical vein endothelial cells |
| MAPK | mitogen-activated protein kinase |
| mPGES-1 | microsomal prostaglandin E synthase |
| NADA | N-arachidonoyl dopamine |
| NOD | N-octanoyl dopamine |
| OLDA | N-oleoyl dopamine |
| PALDA | N-palmitoyl dopamine |
| PPAR | peroxisome proliferator-activated receptor-γ |
| sEPSCs | spontaneous excitatory postsynaptic currents |
| sIPSC | spontaneous inhibitory postsynaptic current |
| STERDA | N-stearoyl dopamine |
| TG | trigeminal ganglion |
| TH | tyrosine hydroxylase |
| TRPV1 | transient receptor potential vanilloid 1 |

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