Cannabis and Cannabinoid Research
Volume 2.1, 2017
DOI: 10.1089/can.2017.0015

Cannabis and Cannabinoid Research
Mary Ann Liebert, Inc. publishers

REVIEW Open Access

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

Urszula Grabiec and Faramarz Dehghani*

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

Department of Anatomy and Cell Biology, Martin Luther University Halle-Wittenberg, Halle (Saale), Germany.

*Address correspondence to: Prof. Dr. med. Faramarz Dehghani, Department of Anatomy and Cell Biology, Martin Luther University Halle-Wittenberg, Grosse Steinstrasse 52, Halle (Saale) 06108, Germany, E-mail: faramarz.dehghani@medizin.uni-halle.de

development of neuropathic pain and inflammatory hyperalgesia.[8] 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 theirs 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).[6] However, Bradshaw et al. detected NADA exclusively in striatum and hippocampus.[9,10] Other *N*-acyldopamines such as OLDA, PALDA, and STEARDA were found in bovine brain.[6,7] 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.[11] A basal level of 2.6 ± 1.2 pmol g^{-1} wet tissue weight NADA was found in rat substantia nigra pars compacta.[11] Human plasma and human postmortem brain were devoid of NADA as analyzed by different chromatographical methods.[12–14] 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.[15] 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.[15] Concomitant NADA was shown to be a weak substrate and a competitive inhibitor ($IC_{50} = 19–100\ \mu M$) of FAAH.[5] 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*.[15] Dopamine receptors and FAAH were found in the same brain regions, such as hippocampus, striatum, and parts of cortex.[16,17] 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.[18,19] 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.[19] So far, there is no direct evidence of a membrane transporter for NADA, although numerous studies assumed its presence.[19–23] Pharmacological studies have revealed a rapid uptake of NADA by anandamide membrane transporter (AMT) in C6 glioma cells, leading to inhibition of AMT ($IC_{50} = 21.5 \pm 9.1\ \mu M$).[5] At high concentration OLDA ($IC_{50} = 17.5\ \mu M$), PALDA ($IC_{50} > 25\ \mu M$), and STERDA ($IC_{50} > 25\ \mu M$) inhibit AMT in the [^{14}C] AEA uptake test in RBL-2H3 cells.[7] 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).[6,24] COMT is involved in the inactivation of catecholamines including dopamine and is distributed in the rat cerebral cortex, neostriatum, and cerebellar cortex.[25] This enzyme transforms NADA to *O*-methyl-NADA, which is less active at TRPV1 than NADA.[6]

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

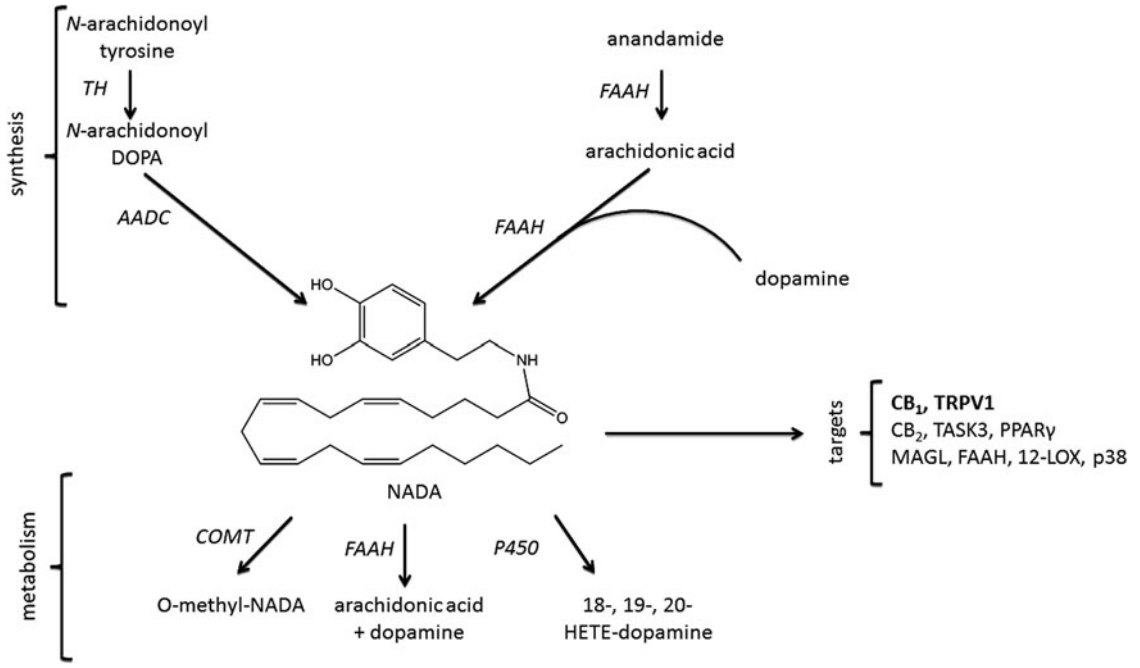

FIG. 1. Potential synthesis and degradation pathways of NADA, including its targets. The first proposed pathway involves *N*-arachidonoyl tyrosine, which can be metabolized by tyrosine hydroxylase (TH) to *N*-arachidonoyl DOPA and by L-amino acid decarboxylase (AADC) to NADA. The second pathway describes the formation of NADA from arachidonic acid and dopamine by FAAH. Three different ways of NADA inactivation were postulated: catechol-*O*-methyl-transferase (COMT) mediated its transformation to an *O*-methyl derivate, fatty acid amide hydrolase (FAAH) hydrolases NADA to arachidonic acid, and dopamine and cytochrome P450 (CYP450) pathway metabolizes NADA to omega hydroxylated metabolites (HETE-dopamine). Main targets of NADA, among others, are CB_1 and TRPV1 receptors mainly located on central neurons and DRGs, intracellular enzymes, and transcription factors. CB, cannabinoid; NADA, *N*-arachidonoyl dopamine; TRPV, transient receptor potential vanilloid.

microsomes.[26] Furthermore, oxidation of the arachidonoyl moiety played a minor role in endovanilloid inactivation.[6] 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

CB_1 receptor

So far, only NADA and OLDA have been described as agonists of CB_1, which is mainly present on neurons[5,7] (Table 1). The CB_1 receptor is expressed presynaptically on neurons among others in forebrain, hindbrain, and in the spinal cord.[27,28] Not only neurons but also glial cells have been reported to express CB_1 receptors.[29] The EC signaling plays a crucial modulating role in hippocampal formation, basal ganglia, cerebellum, and neocortex.[30] In the peripheral nervous system, the activity of nerve fibers innervating smooth muscles is modulated by the CB_1 receptor.[31] Knockout of the CB_1 receptor in mice is not lethal, but significantly leads to affected behavior and learning processes.[32]

Natural and recombinant CB_1 receptors can be coupled to G_s, $G_{i/o}$, and G_q proteins even in the same system.[33–36] Several receptors that preferentially couple to $G_{i/o}$ are able to interact with G_s, particularly when receptors and/or G proteins are expressed at high densities and high concentrations of agonists are present.[37] 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.

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

Enzyme, receptor	Effect	Concentration	Experiment	Reference
abn-CBD	Antagonized by O1918			114
CB_1	Agonist	$K_i = 0.25 \pm 0.13$ (0.8 μM, brain) nM	Rat brain membranes, binding assay with [^{3}H]SR141716A	5,6
CB_2	Agonist	$pEC_{50} = 6.15 \pm 0.09$, $K_i = 12.0 \pm 4.0$ (spleen)	[^{3}H]WIN55,212-2 binding (rat)	5
D2	—		The proliferation of human breast MCF-7 cancer cells were not inhibited by haloperidol a D2 antagonist	6
FAAH	Inhibitor	$IC_{50} = 19$–100 μM	N18TG2 cells	5
MAGL	Inhibitor	$pIC_{50} = 6.11 \pm 0.08$ (NPA) $pIC_{50} = 5.66 \pm 0.03$ (2-OG) $pIC_{50} = 4.70 \pm 0.04$ (2-OG, cytosol)	Two *in vitro* assays	116
PPARγ	Agonist	1–20 μM	GW9662 (1 μM), vasorelaxant response	72
12-LOX	Inhibitor	$IC_{50} = 150 \pm 5$ nM	Activity assay	105
TRPV1	Activator	$K_d = 5.49 \pm 0.68$ μM; $EC_{50} = 40 \pm 6$ nM (human) $EC_{50} = 48 \pm 7$ nM (rat)	Binding of [^{3}H]RTX, calcium imaging	6
TRPM8	Antagonist	Submicromolar	TRPM8-HEK-293 cells overexpressing the human CB1 receptor	117
Voltage-gated sodium channel	Inhibitor	$EC_{50} = 21$ μM	Binding assay, mouse brain	89

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

In addition, homo- and heterodimerization of CB_1 with other receptors such as dopamine receptors, expression and coupling of CB_1 to channels/signaling cascades, or basal activity of those receptors are still a matter of controversial debate.[38]

Typical actions mediated by $G_{i/o}$ 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 G_i coupling. In contrast, G_q was shown to increase the intracellular calcium concentration.[33,34,39–42]

After activation of CB_1 receptor, a transient Ca^{2+} elevation is evoked in a phospholipase C-dependent manner through either $G_{i/o}$ or G_q proteins.[43,44] Activation of G_s leads to receptor-mediated Ca^{2+} influx and to continued activation of different phospholipases.[38] In the absence of extracellular calcium, NADA stimulated an intracellular Ca^{2+} mobilization in undifferentiated N18TG2 neuroblastoma cells. This effect was counteracted by the CB_1 antagonist SR141716A and mimicked by CB_1 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 ($IC_{50} = 0.25$ μM) the proliferation in a CB_1-dependent and D2-independent manner.[5] In radioligand binding assay, NADA was shown to bind to hCB_1 receptors and displaced both [^{3}H]-CP55940 ($K_i = 780 \pm 240$ nM) and [3H]-SR141716A ($K_i = 230 \pm 36$ nM) with a similar affinity.[5,45] NADA did not modulate p44/42 phosphorylation, adenylate cyclase, and potassium channels in cells expressing CB_1. The authors concluded that NADA did not activate $G_{i/o}$ or G_s coupled signaling. Interestingly, NADA (10–30 μM) mediated an activation of $G_{q/11}$ subunit of CB_1, which led to an elevation in $[Ca]_i$ and induced an internalization of CB_1.[45]

NADA ($K_i = 250$ nM) has an affinity to CB_1 in the [^{3}H]SR141716A binding inhibition assay, even stronger than AEA ($K_i = 0.8$ μM).[5] Other compounds such as PALDA and STEARDA were inactive at concentrations smaller than 5 μM. OLDA exhibited some activity on CB_1 receptor ($K_i = 1.6$ μM) in the mentioned assay[7] (Table 1). In autaptic hippocampal neurons, NADA did not inhibit the excitatory postsynaptic current (EPSC) through CB_1 in comparison with 2-AG.[46,47]

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 pyramidal 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 TPRV1 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.[54,60,61] 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 μM) application was relatively small in comparison with capsaicin (10 μM) 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.[64–66] 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 μM) 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 μM)-mediated

Table 2. *N*-Arachidonoyl Dopamine Effects on Different Organ Systems and Processes

	±	Concentration	Model system	Proposed mechanism	Reference
Pain	+				
Allodynia	Induction	0.0013–0.004 *M*	Unanesthetized rhesus monkey		82
Thermal hyperalgesia	Reduction	1.5–50 μg; ED_{50} = 22.5 μg	*In vivo*: rat with unilateral hind paw carrageenan-induced inflammation	15 μg (TRPV1-AMG9810) 50 μg (CB_1-AM251/TRPV1)	84
Analgesia	Inhibition	1–10 mg kg^{-1}	*In vivo*: mouse		5
Innocuous and noxious mechanically evoked responses of dorsal horn neurons	Inhibition	1.5 or 5 μg in 50 μL	Awake rats, injected intraplanar	CB_1, TRPV1	63
Prolonged elevation of presynaptic $[Ca^{2+}]_i$	Induction	5 μM	Rat sensory neurons/cell cultures	TRPV1	118
Mechanically evoked responses of dorsal horn neurons	Reduction	5 μg μL^{-1}	Rat	CB_1 (SR141716A). TRPV1 (capsazepine)	63
CGRP	Release/increase	4 mg/kg; 1, 10 μM	*In vivo*, isolated mesenteric artery	Blocked with capsazepine (10 μM)	115
Calcium mobilization from intracellular stores		pEC_{50} = 6.15 ± 0.09	Neuroblastoma N18TG2 cells		5
Ca^{2+} influx	Increase	EC_{50} = 63.0 ± 5.5; 1 μM; $EC_{50\ CHO}$ = 4.76 ± 1.43 μM; 10 μM; $EC_{50\ HEK}$ = 7.17 ± 1.64 μM	HEK-293-TRPV1, CHO-VR1, TRPV1-Xenopus oocytes		7,26,46,55,119
		10 μM	Rat striatal nerve terminals		120
$[Ca^{2+}]_i$	Increase	1 μM; 1, 3, 10 μM	DRG	FAAH (URB597) AMT (UCM707) Attenuate by preincubation with AM251 (CB_1)	6,63,71,88,119
		3 μM; EC_{50} = 1.6–794 nM	TG	TRPV1	121
		1–100 μM; EC_{50} = 2.4 μM	Rat hippocampal nerve terminals/synaptosomes	TASK-3	88
Outward currents	Reduction	10 μM	$TRPV1^{-/-}$ TG		58
$[^3H]$ GABA release	Induction	30 μM	Rat hippocampal nerve terminals		88
GABAergic transmission sIPSC	Decrease	10 μM	Substantia nigra pars compacta rat	CB_1	65
Glutamate release	Induction	30 μM	Rat hippocampal nerve terminals		88
		5 μM	Rat sensory neurons/sensory synapses	TRPV1	118
Glutamatergic transmission/sEPSC	Increase	1 μM	Substantia nigra pars compacta, rat	TRPV1	65
Glutamatergic transmission	Decrease	3–10 μM	Substantia nigra pars compacta, rat	CB_1	65
eIPSC amplitude	Reduction	1 μM	Substantia nigra pars compacta, dopaminergic neurons in midbrain slices, rat	CB_1, mGlu1	11
Spontaneous and heat-evoked activity	Enhancement	EC_{50} = 1.55 μg	Spinal nociceptive neurons	TRPV1	8
Pungent/nocifensive responses	Increase	0.1%	Eye wipe test		70
Discharge of spinal nociceptive neurons		EC_{50} = 1.55 μg	Hindpaw injection		8
Toxic					
Cell death	Increase	3.4–100 μM; EC_{50} = 30 μM	Peripheral blood mononuclear preparation	TRPV1, CB_2	122
		10, 25, 50, 100 μM	Hepatic stellate cells	Reactive oxygen species	123
Vascular system					
Vasorelaxation	Induction	10 nM–100 μM; pEC_{50} = 6.39 ± 0.12	Small mesenteric vessel	abn-CBD, TRPV1	114
		10 μM	Rat aortae, *in vitro*	PPARγ	73
		10 nM–100 μM; pEC_{50} = 5.45 ± 0.15	Suprerior mesenteric artery	CB_1, TRPV1	114
		10 nM–100 μM; pEC_{50} = 5.99 ± 0.17	Aorta		114
Urinary system					
Contraction	Induction	EC_{50} = 3.7 ± 0.3 μM; E_{max} = 12.0 ± 0.1% of carbachol E_{max}	Guinea pig urinary bladder	Blocked by pretreatment with 10 μM capsaicin; 10 μM capsazepine	62
		EC_{50} = 19.9 ± 0.1 μM; E_{max} = 20.7 ± 0.7% of carbachol E_{max}	Rat urinary bladder	Blocked by pretreatment with 10 μM capsaicin; 10 μM capsazepine	62
Respiratory system					
Contraction	Induction	EC_{50} = 12.6 ± 1.7 μM; E_{max} = 69.2 ± 2.4% of carbachol E_{max}	Guinea pig bronchi	Blocked by pretreatment with 10 μM capsaicin; 10 μM capsazepine	62
Sensitization	Induction	400 μg kg^{-1} mL^{-1}; 0.5 mL min^{-1}, 2 min	*In vivo*: lung vagal afferents	TRPV1	124

(*continued*)

Table 2. (Continued)

	±	Concentration	Model system	Proposed mechanism	Reference
Immunological processes					
p112-Lipooxygenase	Inhibition	$IC_{50} = 150 \pm 5$ 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	b.end5 cell line	p38	107
IL-2 and TNFα gene transcription	Inhibition	2.5–5 μM	Stimulated Jurkat T cells		108
Transcription factors NF-$\kappa\beta$, NFAT, AP-1	Inhibition	2.5 μM	Stimulated Jurkat T cells		108
HIV replication	Inhibition	1–10 μM	Staphylococcal enterotoxin B-activated peripheral primary T cells		111
Neutrophil migration and chemotaxis	Inhibition	$IC_{50} = 8.80$ nM (4.7–16.2); $EC_{50} = 64$ (56.6–71.5)%	Boyden chamber, induced by fMLP		113
PGE2 synthesis	Inhibitor	1–2.5 μM	Primary glial (microglia, astrocytes) cells		106
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_1 (CP945598), CB_2 (SR144528) dependent, TRPV1 (AMG9810) dependent	125
Protection					
Neuroprotection	Induction	100 pM–10 μM	Excitotoxically lesioned OHSC	CB_1 partially	46
		10 μM	Hypoxia-induced cytotoxicity in SK-N-SH cell line		100
Protection of cortical neurons	Induction	10 μM	Excitotoxicity model of cerebellar granule neurons		98
Protection in hypoxia model	Induction	10 μM	Murine primary hippocampal neurons	CB_1 (SR141716A)	99
Mean arterial pressure (MAP)	Decrease	1, 4, 10 mg kg^{-1}	*In vivo*: high sodium/normal treated rats		115
Neuroprotective and angiogenesis genes	Induction	10 μM	Human primary astrocytes, SK-N-SH cells, HUVEC, HBMECs cells		100
Antioxidative properties	+	0.1–10 μM	Cerebellar granule neurons, H_2O_2		98
Others					
Teratogenic actions	Antagonist (against AM-630, SB366791)		Strongylocentrotus purpuratus, Lytechinus variegatus		126
Antiemetic		2 mg kg^{-1}	*In vivo*: Ferret, injected	CB_1 (AM251), TRPV1 (6-iodoresiniferatoxin)	127
Grooming behaviors and licking	Reduction	2 mg kg^{-1}	*In vivo*: Ferret, injected	CB_1 (AM251)	127
Hypothermia	Induction	1–10 mg kg^{-1}	*In vivo*: mouse		5
Hypolocomotion	Induction	1–10 mg kg^{-1}	*In vivo*: mouse		5
Catalepsy	Inhibition	1–10 mg kg^{-1}	*In vivo*: mouse		5
Antidepressant-like effect	No effect	0.1, 1, 10 mg kg^{-1}	*In vivo*: mouse		128
Tension in fast skeletal muscle fibers	Decrease	5 μM	*In vitro*: frog	CB_1 (AM281), TRPV1 (capsezepine)	129
Collagen/ADP-induced platelet aggregation	Inhibition	0–100 μM	*In vitro*: human blood cells	TRPV1 independent	130
Adipocyte differentiation	Inhibition	10 μM	*In vitro*: human mesenchymal stem cells	CB_1 (rimonabant)	131
Signaling pathways					
Proliferation	Inhibition	$EC_{50} = 0.25$ μM; 0–10 μM	Human breast cancer cells MCF-7	CB_1 (SR141716A)	5
T-type calcium channel	Inhibition	10 μmol L^{-1}; 30 μmol L^{-1}	HEK 293 cells		86
Sodium channel (veratridine-dependent) release of L-glutamate and GABA	Inhibition	$IC_{50} = 20.7$ μM; 1–100 μM	Brain (mouse), synaptoneurosomes		89

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.

CB_1 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 CB_1-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), CB_1 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 CB_1 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[75,76] and activation of CB receptors.[67,77] The other class of nociceptors, the peptidergic C nociceptors, release neuropeptides, such as substance P and calcitonin gene-related peptide (CGRP), and express TrkA receptor that binds nerve growth factor.[78–80] It was demonstrated that the activation of antinociceptive CB_1 by NADA reduces the pronociceptive actions evoked by TRPV1.[81]

NADA combines features of ECs and endovanilloids, making it an interesting and potent therapeutic agent in development of analgesic drugs. NADA activates TRPV1 and CB_1 receptor that have a well-established role in pain modulation and are present on DRG[5,78,80] (Table 2).

Summarizing the data already mentioned, NADA induces both pro- and antinociceptive effects in the central and peripheral nervous system dependent on the concentration range. In thermal allodynia, NADA has been shown to relieve pain after topical administration in primates.[82] Injected intraperitoneally in mice, NADA induced hypothermia, hypolocomotion, and analgesia[5] similar to 20 mg kg^{-1} AEA.[83] Intraplantar injection of NADA (5 μg in 50 μL = 0.07 mM) inhibited the smaller mechanically evoked innocuous responses of dorsal horn neurons through CB_1 and for stronger mechanical stimuli through TRPV1.[63]

High concentrations of NADA were pronociceptive in different model systems of male rats. When administered peripherally and intradermally, NADA caused behavioral thermal hyperalgesia through TRPV1 and CB_1.[6] NADA (EC_{50} = 1.55 μg) administrated intradermally into the receptive fields of dorsal horn increased dose dependently both spontaneous and heat-evoked activity in spinal nociceptive neurons in laminae I-V of dorsal horn of the spinal cord in a TRPV1-dependent, CB_1-independent manner, indicating pain sensation—thermal hyperalgesia.[8] Differences in the observations made by Huang et al., Huang and Walker, and Sagar et al. can be explained by a different kind of stimuli or different neurons, which were recorded. Coadministration of STERDA potentiated the induction of thermal hyperalgesia by NADA.[59] In contrast to capsaicin, subcutaneous injections of OLDA (EC_{50} = 0.72 ± 0.36 μg) into rat hind paw induced a significant dose-dependent thermal hyperalgesia lasting for 3 h. No nocifensive behavior was observed (licking and lifting the hindpaw) till 30 min after injection. PALDA and STERDA alone had no effects in this study.[7]

NADA displayed antihyperalgesic effects in models of inflammatory pain after intrathecal administration. These effects were reversed by antagonists of both CB_1 (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.[63] In contrast to previous findings, NADA evoked CGRP release from TG neurons[85] and from slices of rat dorsal horn spinal cord in a TRPV1-dependent manner,[6] resulting in neurogenic inflammation. NADA injected in TG or administrated intraocularly was excitatory, pungent, and evoked nocifensive responses.[85] It could also modulate different cation channels involved in pain sensation, like T-type calcium channels ($Ca_{(V)}3$)[86,87] (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.[86,87] The role of calcium channels in NADA-mediated processes provides an explanation for the lack of complete blockade with CB_1 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 (CB_2 antagonist), AM251 (CB_1 antagonist), ruthenium red (TRPV antagonist), and sulpride (D2, D3, and D4 antagonist). Only TASK-3 inhibitors triggered the rise of resting intracellular Ca^{2+}.[88] NADA was shown to inhibit voltage-gated sodium channel (veratridine-dependent), release of L-glutamate and GABA in the low micromolar range from synaptosomes isolated from murine brain. This EC may modulate neuronal excitation and depression in a CB_1-independent way.[89] NADA had no effect on N-type Ca^{2+} channels (Cav2.2) in rat sympathetic neurons in comparison with other cannabinoids.[90] 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 CB_1 in combination with electrophysiological and *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 CB_1 receptor activation,[91,92] whereas neurotoxicity is associated with TRPV1 activation.[66,93–95] After excitotoxic lesion *in vivo* as well as *in vitro*, CB_1 receptor was found to prolong preservation of neurons.[96,97] These results are in line with our data on NADA.[46] NADA (1 nM) was neuroprotective in organotypical hippocampal slice cultures after NMDA treatment, partly through CB_1. In electrophysiological experiments, NADA (1, 10 μM) did not inhibit EPSCs in autaptic hippocampal neurons.[46] We assume that CB_1-dependent decrease in intracellular calcium concentration does not mediate NADA's neuroprotective effects. High concentrations of NADA (10 μM) seem to activate additional mechanisms preventing the neuronal demise as the neuroprotection was independent of CB_1, TRPV1, and abn-CBD receptors.[46] In addition, NADA showed protective effects in cultured cerebellar neurons by reducing oxidative stress induced by hydrogen peroxide[98] and in primary hippocampal neurons against hypoxia through CB_1.[99] Pretreatment with NADA protected human neuroblastoma cell line SK-N-SH from hypoxia.[100] NADA (5 μM) induced cell death in human neuron-like cell line SH-SY5Y, stably expressing recombinant human TRPV1.[93] Despite similarities to an apoptotic process, the cell demise took place independent of caspase activity and was blocked by a TRPV1 antagonist.[93] In contrast, *in vivo* studies demonstrated protective effects of TRPV1 activation on neurons against excitotoxicity[101] or ischemia.[102] 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.[103] Furthermore, NOD improved the renal function in setting of ischemia *in vivo* by downregulation of NFκß and subsequent inhibition of vascular cell adhesion molecule 1 in proximal tubular epithelial cells.[104]

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.[105–107] It had also the ability to prevent free radical formation in primary microglial cells. AEA and NADA had opposite effects on glial cells.[106]

Furthermore, NADA specifically inhibits *IL*-2 and *TNF*-α 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 naive 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 CB_1 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 *tnf*α-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 CB_1 and CB_2.[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 cardiovascular depressive effects of cannabinoids are mediated by CB_1 receptors. Recent studies provide strong support for the existence of as-yet-undefined endothelial and cardiac receptors that mediate certain EC-induced cardiovascular 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 aorta[114] (Table 2).

In small mesenteric vessels, NADA-mediated vasorelaxant effects were CB_1, abn-CBD, and TRPV1 dependent and were probably mediated by activation of potassium channels and an intrinsic endothelial mechanism independent of dopamine (D1) receptors.[114]

NADA-mediated vasorelaxation in superior mesenteric artery was CB_1, capsaicin dependent, and independent 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 CB_1 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 proliferation of T cells but does not impair T cell activation. NADA potentially mediates both anti- and pronociceptive responses depending on the balance between CB_1 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 majority 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*-acyldopamines 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.

Acknowledgments

The authors would like to deeply thank P. Chandra (Goethe-University, Frankfurt) for critical reading of the article and T. Hohmann (Martin Luther University, Halle-Wittenberg) for helpful discussions. Urszula Grabiec was supported by the Roux Program FKZ 26/27 and FKZ 29/18.

Author Disclosure Statement

No competing financial interests exist.

References

1. Howlett AC, Breivogel CS, Childers SR, et al. Cannabinoid physiology and pharmacology: 30 years of progress. Neuropharmacology. 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 endocannabinoid-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 cannabimimetic lipid mediators. Am J Physiol Regul Integr Comp Physiol. 2006;291:349–358.
10. Freestone PS, Guatteo E, Piscitelli F, et al. Glutamate spillover drives endocannabinoid production and inhibits GABAergic transmission in the Substantia Nigra pars compacta. Neuropharmacology. 2014;79: 467–475.
11. Ji D, Jang C, Lee S. A sensitive and accurate quantitative method to determine N-arachidonoyldopamine 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, Storvik 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. Thomas A, Hopfgartner G, Giroud 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 SSJ, 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. Egertová 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. 2003;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 cellular 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, Beltramo 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, Cadas 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. Cristino 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. Karhunen T, Tilgmann C, Ulmanen I, et al. Neuronal and non-neuronal catechol-O-methyltransferase 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, Sañudo-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. Tantimonaco 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, Egertova M. The neurobiology and evolution of cannabinoid signalling. Philos Trans R Soc L B Biol Sci. 2001;356:381–408.
32. Marsicano 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, Abood 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. Wess J. Molecular basis of receptor/G-protein-coupling selectivity. Pharmacol Ther. 1998;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;272:22330–22339.
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;160:454–466.
41. Deadwyler S, Hampson R, Mu J, et al. Cannabinoids modulate voltage sensitive potassium A-current in hippocampal neurons via a cAMP-dependent process. J Pharmacol Exp Ther. 1995;273:734–743.
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. Sugiura 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 that increases intracellular calcium and inhibits M current. PNAS. 2007;77030:2699–2704.
45. Redmond WJ, Cawston EE, Grimsey NL, et al. Identification of N-arachidonoyl dopamine as a highly biased ligand at cannabinoid CB1 receptors. Br J Pharmacol. 2015;173:115–127.
46. Grabiec U, Koch M, Kallendrusch S, et al. The endocannabinoid N-arachidonoyldopamine (NADA) exerts neuroprotective effects after excitotoxic neuronal damage via cannabinoid receptor 1 (CB 1). Neuropharmacology. 2012;62:1797–1807.
47. Katona I, Freund TF. Endocannabinoid signaling as a synaptic circuit breaker in neurological disease. Nat Med. 2008;14:923–930.
48. Caterina MJ, Schumacher MA, Tominaga M, et al. The capsaicin receptor: a heat-activated ion channel in the pain pathway. Nature. 1997;389: 816–824.
49. van der Stelt M, Veldhuis WB, Bär PR, et al. Neuroprotection by Delta9-tetrahydrocannabinol, the main active compound in marijuana, against ouabain-induced in vivo excitotoxicity. J Neurosci. 2001;21:6475–6479.
50. Malenka RC, Bear MF. LTP and LTD: an embarrassment of riches. Neuron. 2004;44:5–21.
51. Steenland HW, Ko SW, Wu L-J, et al. Hot receptors in the brain. Mol Pain. 2006;2:34.
52. Szallasi A, Cortright DN, Blum CA, et al. The vanilloid receptor TRPV1: 10 years from channel cloning to antagonist proof-of-concept. Nat Rev Drug Discov. 2007;6:357–372.
53. Mezey E, Tóth ZE, Cortright DN, et al. Distribution of mRNA for vanilloid receptor subtype 1 (VR1), and VR1-like immunoreactivity, in the central nervous system of the rat and human. Proc Natl Acad Sci U S A. 2000;97:3655–3660.
54. Gavva NR, Klionsky L, Qu Y, et al. Molecular determinants of vanilloid sensitivity in TRPV1. J Biol Chem. 2004;279:20283–20295.
55. Toth A, Bocza J, Kedei N, et al. Expression and distribution of vanilloid receptor 1(TRPV1) in the adult rat brain. Brain Res Mol Brain Res. 2005;135:162–168.
56. Cavanaugh DJ, Chesler AT, Jackson AC, et al. Trpv1 reporter mice reveal highly restricted brain distribution and functional expression in arteriolar smooth muscle cells. J Neurosci. 2011;31:5067–5077.
57. Tóth A, Kedei N, Wang Y, et al. Arachidonyl dopamine as a ligand for the vanilloid receptor VR1 of the rat. Life Sci. 2003;73:487–498.
58. Roberts LA, Ross HR, Connor M. Methanandamide activation of a novel current in mouse trigeminal ganglion sensory neurons in vitro. Neuropharmacology. 2008;54:172–180.
59. De Petrocellis L, Chu CJ, Moriello AS, et al. Actions of two naturally occurring saturated N-acyldopamines on transient receptor potential vanilloid 1 (TRPV1) channels. Br J Pharmacol. 2004;143:251–256.
60. Petrocellis L De, Bisogno T, Maccarrone M, et al. The activity of anandamide at vanilloid VR1 receptors requires facilitated transport across the cell membrane and is limited by intracellular metabolism. J Biol Chem. 2001;276:12856–12863.
61. Jordt SE, Julius D. Molecular basis for species-specific sensitivity to "hot" chili peppers. Cell. 2002;108:421–430.
62. Harrison S, De Petrocellis L, Trevisani 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.
63. Sagar DR, Smith PA, Millns 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.
64. 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.
65. 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.
66. Maccarrone M, Lorenzon T, Bari M, et al. Anandamide induces apoptosis in human cells via vanilloid receptors. J Biol Chem. 2000;275: 31938–31945.
67. 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.
68. Bridges D, Rice ASC, Egertová M, et al. Localisation of cannabinoid receptor 1 in rat dorsal root ganglion using in situ hybridisation and immunohistochemistry. Neuroscience. 2003;119:803–812.
69. Hohmann A, Herkenham M. Cannabinoid receptors undergo axonal flow in sensory nerves. Neuroscience. 1999;92:1171–1175.
70. Price TJ, Helesic G, Parghi D, et al. The neuronal distribution of cannabinoid receptor type 1 in the trigeminal ganglion of the rat. Neuroscience. 2003;120:155–162.
71. 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.
72. O'Sullivan SE. Cannabinoids go nuclear: evidence for activation of peroxisome proliferator-activated receptors. Br J Pharmacol. 2007;152:576–582.
73. 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.
74. 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.
75. Julius D. TRP channels and pain. Annu Rev Cell Dev Biol. 2013;29: 355–384.
76. Patapoutian A, Tate S, Woolf CJ. Transient receptor potential channels: targeting pain at the source. Nat Rev Drug Discov. 2009;8:55–68.
77. Cravatt BF, Lichtman AH. The endogenous cannabinoid system and its role in nociceptive behavior. J Neurobiol. 2004;61:149–160.

78. Basbaum AI, Bautista DM, Scherrer G, et al. Cellular and molecular mechanisms of pain. Cell. 2010;139:267–284.
79. Chiu IM, Von Hehn 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, Millns 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 allodynia in unanesthetized primates: pharmacological modulation. J Pharmacol Exp Ther. 2003;306:1106–1114.
83. Fride 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 antinociceptive 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-vanilloid agonists anandamide, N-arachidonoyl-dopamine and arachidonyl-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 Neurol Disord Drug Targets. 2006;5:639–653.
88. Köfalvi A, Pereira MF, Rebola N, et al. Anandamide and NADA bidirectionally 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-20α-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 anandamide protects neurons during CNS inflammation by induction of MKP-1 in microglial cells. Neuron. 2006;49:67–79.
92. Koch M, Kreutz S, Böttger C, et al. The cannabinoid WIN 55,212-2-mediated protection of dentate gyrus granule cells is driven by CB1 receptors and modulated by TRPA1 and Ca v2.2 channels. Hippocampus. 2011;21:554–564.
93. Davies JW, Hainsworth AH, Guerin CJ, et al. Pharmacology of capsaicin-, anandamide-, and N-arachidonoyl-dopamine-evoked cell death in a homogeneous transient receptor potential vanilloid subtype 1 receptor population. Br J Anaesth. 2010;104:596–602.
94. Kim SR, Lee DY, Chung ES, et al. Transient receptor potential vanilloid subtype 1 mediates cell death of mesencephalic dopaminergic neurons in vivo and in vitro. J Neurosci. 2005;25:662–671.
95. Chard P, Bleakman D, Savidge J, et al. Capsaicin-induced neurotoxicity in cultured dorsal root ganglion neurons: involvement of calcium-activated proteases. Neuroscience. 1995;65:1099–1108.
96. Khaspekov LG, Verca MSB, Frumkina LE, et al. Involvement of brain-derived neurotrophic factor in cannabinoid receptor-dependent protection against excitotoxicity. Eur J Neurosci. 2004;19:1691–1698.
97. Marsicano G, Goodenough S, Monory K, et al. CB1 cannabinoid receptors and on-demand defense against excitotoxicity. Science (80). 2003;302:84–88.
98. Bobrov MY, Lizhin AA, Andrianova EL, et al. Antioxidant and neuroprotective properties of N-arachidonoyldopamine. Neurosci Lett. 2008;431:6–11.
99. Vedunova MV, Mitroshina EV, Sakharnova TA, et al. Effect of N-Arachidonoyl dopamine on activity of neuronal network in primary hippocampus culture upon hypoxia modelling. Bull Exp Biol Med. 2014;156:461–464.
100. Soler-Torronteras R, Lara-Chica M, García V, et al. Hypoximimetic activity of N -acyl-dopamines. N -arachidonoyl-dopamine stabilizes HIF-1 α protein through a SIAH2-dependent pathway. Biochim Biophys Acta. 2014;1843:2730–2743.
101. Veldhuis WB, van der Stelt M, Wadman MW, et al. Neuroprotection by the endogenous cannabinoid anandamide and arvanil against in vivo excitotoxicity in the rat: role of vanilloid receptors and lipoxygenases. J Neurosci. 2003;23:4127–4133.
102. Pegorini S, Braida D, Verzoni C, et al. Capsaicin exhibits neuroprotective effects in a model of transient global cerebral ischemia in Mongolian gerbils. Br J Pharmacol. 2005;144:727–735.
103. Losel RM, Schnetzke U, Brinkkoetter PT, et al. N-octanoyl dopamine, a non-hemodyanic dopamine derivative, for cell protection during hypothermic organ preservation. PLoS One. 2010;5:e9713.
104. Tsagogiorgas C, Wedel J, Hottenrott M, et al. N-octanoyl-dopamine is an agonist at the capsaicin receptor TRPV1 and mitigates is chemia-induced acute kidney injury in rat. PLoS One. 2012; 7:e43525.
105. Prusakiewicz JJ, Turman MV, Vila A, et al. Oxidative metabolism of lipoamino acids and vanilloids by lipoxygenases and cyclooxygenases. Arch Biochem Biophys. 2007;464:260–268.
106. Navarrete CM, Fiebich BL, De Vinuesa AG, et al. Opposite effects of anandamide and N-arachidonoyl dopamine in the regulation of prostaglandin E2 and 8-iso-PGF2α formation in primary glial cells. J Neurochem. 2009;109:452–464.
107. Navarrete CM, Pérez M, de Vinuesa AG, et al. Endogenous N-acyl-dopamines induce COX-2 expression in brain endothelial cells by stabilizing mRNA through a p38 dependent pathway. Biochem Pharmacol. 2010;79:1805–1814.
108. Sancho R, Macho A, de La Vega L, et al. Immunosuppressive activity of endovanilloids: N-arachidonoyl-dopamine inhibits activation of the NF-kappa B, NFAT, and activator protein 1 signaling pathways. J Immunol. 2004;172:2341–2351.
109. 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.
110. 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.
111. 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.
112. Yoo JM, Sok DE, Kim MR. Effect of endocannabinoids on IgE-mediated allergic response in RBL-2H3 cells. Int Immunopharmacol. 2013;17: 123–131.
113. 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. 2008;73:441–450.
114. 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.
115. 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.
116. Björklund E, Norén E, Nilsson J, et al. Inhibition of monoacylglycerol lipase by troglitazone, N -arachidonoyl dopamine and the irreversible inhibitor JZL184: comparison of two different assays. Br J Pharmacol. 2010;161:1512–1526.
117. Petrocellis L De, Starowicz K, Schiano A, et al. Regulation of transient receptor potential channels of melastatin type 8 (TRPM8): effect of cAMP, cannabinoid CB 1 receptors and endovanilloids. 2007;3:1–10.
118. Medvedeva YV, Kim M, Usachev YM. Mechanisms of prolonged presynaptic Ca2+ signaling and glutamate release induced by TRPV1 activation in rat sensory neurons. J Neurosci. 2008;28:5295–5311.
119. Premkumar LS, Qi Z-H, Van Buren J, et al. Enhancement of potency and efficacy of NADA by PKC-mediated phosphorylation of vanilloid receptor. J Neurophysiol. 2004;91:1442–1449.
120. Ferreira SG, Lomaglio T, Avelino A, et al. N-acyldopamines control striatal input terminals via novel ligand-gated cation channels. Neuropharmacology. 2009;56:676–683.

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 Membr Biol. 2015;248:31–38.
130. Almaghrabi SY, Geraghty 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.

Cite this article as: Grabiec U, Dehghani F (2017) *N*-arachidonoyl-dopamine: a novel endocannabinoid and endovanilloid with widespread physiological and pharmacological activities, *Cannabis and Cannabinoid Research* 2:1, 183–196, DOI: 10.1089/can.2017.0015.

Abbreviations Used

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 γ = 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