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# Analysis of anandamide- and lysophosphatidylinositol-induced inhibition of the vasopressor responses produced by sympathetic stimulation or noradrenaline in pithed rats

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ABSTRACT

The endocannabinoid system exhibits multiple functions in cardiovascular regulation mainly by cannabinoid ($CB_1$ and $CB_2$) receptors, vanilloid TRPV1 receptors and, probably, by the orphan G protein-coupled receptor 55 (GPR55). Hence, the role of these receptors was investigated in Wistar pithed rats on anandamide- and lysophosphatidylinositol (LPI)-induced inhibition of the vasopressor responses induced by preganglionic ($T_7$-$T_9$) stimulation of the vasopressor sympathetic outflow or i.v. bolus injections of noradrenaline. The corresponding frequency- and dose-dependent vasopressor responses were analyzed before and during i.v. continuous infusions of anandamide ($CB_1$, $CB_2$, TRPV1 and GPR55), JWH-015 ($CB_2$) and LPI (GPR55) in animals receiving (i.v.) the antagonists NIDA41020 ($CB_1$), AM630 ($CB_2$), capsazepine (TRPV1) and/or cannabidiol (GPR55). Anandamide (0.1-3.1 μg/kg min) inhibited the vasopressor responses by electrical stimulation, but not those by noradrenaline; while LPI (5.6–10 μg/kg min) inhibited both responses. In contrast, JWH-015 (5.6–10 μg/kg min) failed to induce sympatho-inhibition. Anandamide-induced sympatho-inhibition was: (i) dose-dependently blocked by 31 and 100 μg/kg NIDA41020; (ii) slightly blocked by 310 μg/kg AM630 or 31 μg/kg cannabidiol; and (iii) unaffected by 310 μg/kg capsazepine. Moreover, LPI-induced inhibition of both vasopressor responses was blocked and abolished by 10 and 31 μg/kg cannabidiol, respectively, and weakly blocked by 100 μg/kg NIDA41020. Thus, the sympatho-inhibition by anandamide is primarily mediated by cannabinoid $CB_1$ and, minimally, by cannabidiol-sensitive receptors. In contrast, LPI-induced inhibition of both responses seems to be mainly mediated by postjunctional cannabidiol-sensitive (presumably endothelial GPR55) receptors.



## 1. Introduction

The endocannabinoid system exhibits multiple biological functions including, among others, pain modulation, cell proliferation, cardiovascular homoeostasis as well as appetite and body weight control (Di Marzo, 2011; Kirkham, 2005; Walker and Huang, 2002). These functions involve the activation of cannabinoid and non-cannabinoid receptors. With the conjunction of structural, transductional and operational criteria, cannabinoid (CB) receptors have been classified into $CB_1$ and $CB_2$ types (Matsuda et al., 1990; Munro et al., 1993). These receptors are coupled to $G_{i/o}$-proteins and their signal transduction mechanisms include inhibition of adenylyl cyclase, activation of mitogen-activated protein kinase and, in the case of the cannabinoid $CB_1$ type receptor, regulation of $Ca^{2+}$ and $K^+$ channels (Pertwee, 1997). While cannabinoid $CB_1$ receptors modulate neurotransmitter release, cannabinoid $CB_2$ receptors are mainly present on immune cells (Pertwee and Ross, 2002; Sugiura and Waku, 2002).

The endogenously-produced cannabinoids include the eicosanoids anandamide, 2-arachidonoylglycerol, 2-arachidonylglyceryl ether, virhodamine and N-arachidonoyl-dopamine (De Petrocellis et al., 2004). Interestingly, anandamide induces local and systemic effects on the cardiovascular system through a wide variety of receptors including, among others: cannabinoid $CB_1$/$CB_2$ receptors (Pacher et al., 2008), non-cannabinoid $CB_1$/$CB_2$ receptors (Herradón et al., 2007) and vanilloid TRPV1 receptors (Poblete et al., 2005). Likewise, anandamide seems to interact with GPR55 (see Table 1) and causes vasorelaxation of the pulmonary artery (Baranowska-Kuczko et al., 2012); this vasorelaxing mechanism

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**Table 1**
Binding affinity constants ($pK_i$) of several ligands for $CB_1$, $CB_2$, TRPV1 and GPR55 receptors, except for α, β and γ, which stand for $pEC_{50}$, $PK_B$ and $pIC_{50}$, respectively, determined in transfected cells.

| Compound | $CB_1$ | $CB_2$ | TRPV1 | GPR55 |
|---|---|---|---|---|
| **Agonist** | | | | |
| Anandamide | ~7.05[a] | ~6.4[a] | 5.78[b] (r) | 7.7[α,c] |
| JWH-015 | ~6.4[a] | ~7.8[a] | N.D. | <4.5[α,d] |
| Lysophosphatidylinositol | <4.5[α,d] | N.D. | N.D. | 7.31[α,e] |
| **Antagonist** | | | | |
| NIDA42020 | ~8.4[f] | 6.0[β,g] | N.D. | N.D. |
| AM630 | ~5.2[h] | ~7.5[h] | N.D. | N.D. |
| Capsazepine | N.D. | N.D | ~5.8[b] (r) | N.D. |
| Cannabidiol | ~5.3[i] | ~5.4[i] | N.D. | 6.3[γ,c] |

(r) Interaction of anandamide with vanilloid receptors in CHO cells transfected with rVR1 (TRPV1) receptors; N.D. not determined; ~ stands for "approximately".
Data taken from.

[a] Showalter et al. (1996).
[b] Ross et al. (2001).
[c] Ryberg et al. (2007).
[d] Kapur et al. (2009).
[e] Oka et al. (2007).
[f] Katoch-Rouse et al. (2003).
[g] Donohue et al. (2006).
[h] Ross et al. (1999).
[i] Thomas et al. (2007).

could be related with endothelial GPR55 (Waldeck-Weiermair et al., 2008). Although the GPR55 may correspond to the novel cannabinoid $CB_3$ receptor, the high activation of these receptors by lysophosphatidylinositol (LPI; Oka et al., 2007, 2009) raises controversy as to whether it may be considered a cannabinoid receptor.

A rapid i.v. bolus injection of anandamide produces a triphasic blood pressure response in anaesthetised rats consisting of: (i) an initial transient bradycardic and hypotensive phase (abolished in pithed rats) due to stimulation of vagal afferents via vanilloid TRPV1 receptors; (ii) a rapid pressor response produced by an increase in cardiac contractility and systemic vasoconstriction; and (iii) a late, long-lasting hypotensive response mainly mediated by negative cardiac inotropy, systemic vasodilatation and sympatho-inhibition (Bonz et al., 2003; Malinowska et al., 2012; Zakrzeska et al., 2010). This sympatho-inhibition induced by anandamide and related agonists has been reported to involve activation of cannabinoid $CB_1$ receptors (Ishac et al., 1996; Malinowska et al., 1997), a response absent in cannabinoid $CB_1$-knockout mice (Ledent et al., 1999). However, the possible role of cannabinoid $CB_2$, GPR55 and vanilloid TRPV1 receptors in this sympatho-inhibition has not been reported. Hence, this study sets out to investigate by pharmacological means in pithed rats the specific role of cannabinoid ($CB_1$ and $CB_2$), GPR55 and vanilloid TRPV1 receptors in anandamide- and LPI-induced inhibition of the vasopressor responses induced by: (i) preganglionic ($T_7$-$T_9$) stimulation of the sympathetic vasopressor outflow; and (ii) i.v. bolus injections of exogenous noradrenaline.

## 2. Methods

### 2.1. Animals

Male Wistar normotensive rats (250–300 g) were maintained at a 12/12-h light-dark cycle (light beginning at 07:00 h) and kept in a special room at 22 ± 2 °C and 50% humidity, with food and water freely available in their home cages. All experimental protocols in this study were approved by our Institutional Ethics Committee (CICUAL-Cinvestav), in accordance with the guide for the Care and Use of Laboratory Animals in the U.S.A.

### 2.2. General methods

Experiments were carried out in a total of 234 rats. After quick anaesthesia with diethyl ether and cannulation of the trachea, the rats were pithed by inserting a stainless steel rod through the orbit and foramen magnum into the vertebral foramen, as previously established by Shipley and Tilden (1947). Then, the animals were artificially ventilated with room air using a model 7025 Ugo Basile pump (56 strokes/min; stroke volume: 20 ml/kg) (Kleinman and Radford, 1964). After bilateral vagotomy, catheters were placed in the left and right femoral and jugular veins, for the infusion of the agonists by a WPI model sp100i pump (World Precision Instruments Inc., Sarasota, FL, U.S.A.) and bolus injections of the antagonists or vehicles; and the left carotid artery, connected to a Grass pressure transducer (P23 XL), for the recording of blood pressure. Heart rate was measured with a tachograph (7P4, Grass Instrument Co., Quincy, MA, U.S.A.) triggered from the blood pressure signal. Both blood pressure and heart rate were recorded simultaneously by a model 7 Grass polygraph (Grass Instrument Co., Quincy, MA, U.S.A.). At this point, the 234 rats were divided into two main sets, so that the effects produced by i.v. continuous infusions of dimethyl sulfoxide 0.5% (DMSO 0.5%), LPI vehicle (0.04 ml chloroform:methanol:water 70:27:3 + 0.96 ml saline) as well as the agonists anandamide, JWH-015 and LPI could be investigated on the vasopressor responses induced by either: (i) selective preganglionic ($T_7$–$T_9$) stimulation of the vasopressor sympathetic outflow (set 1; $n=180$); or (ii) i.v. bolus injections of exogenous noradrenaline (set 2; $n=54$). The vasopressor stimulus–response curves (S–R curves) and dose–response curves (D–R curves) elicited by, respectively, sympathetic stimulation and exogenous noradrenaline were completed in about 30 min without changes in resting diastolic blood pressure or heart rate. Moreover, the vasopressor sympathetic stimuli and the noradrenaline injections were given using a sequential schedule, in 0.5 log unit increments at 3- to 5-min intervals. The body temperature of each pithed rat was maintained at 37 °C by a lamp and monitored with a rectal thermometer.

### 2.3. Experimental protocols

#### 2.3.1. Electrical stimulation of the vasopressor sympathetic outflow

In the first set of rats ($n=180$), the pithing rod was replaced by an electrode enamelled except for a 1-cm length 9 cm from the tip, so that the uncovered segment was situated at $T_7$–$T_9$ in the spinal cord to allow selective preganglionic stimulation of the thoracic sympathetic nerves supplying the systemic vasculature, i.e. the vasopressor sympathetic outflow; an indifferent electrode was placed dorsally (Gillespie et al., 1970; Villalón et al., 1995, 1998). Prior to electrical stimulation, all animals received gallamine (25 mg/kg, i.v.) to avoid electrically-induced muscular twitching. After 10 min, the animals were divided into two main sets ($n=156$ and 24). The first set of animals ($n=156$) was systematically pretreated with desipramine (50 μg/kg, i.v.) before each S–R curve. Under these conditions, the vasopressor responses to lower stimulation frequencies are greater than those elicited without desipramine (Ruiz-Salinas et al., 2013; Villalón et al., 1995, 1998). The second set of animals ($n=24$) did not receive desipramine and was performed for comparative purposes. After stable haemodynamic conditions for at least 30 min, baseline values of diastolic blood pressure and heart rate were determined in both sets of animals.

The preganglionic vasopressor sympathetic outflow was then stimulated in both sets to elicit vasopressor responses by applying