Effects of Oleamide on the Vasomotor Responses in the Rat

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Abstract

Introduction: Cardiovascular effects of endocannabinoids (eCBs) have generated considerable interest since it has been suggested that the eCB system could become the new pharmacological target, either by blocking its activity or by promoting its effects on several cardiovascular diseases such as hypovolemic and septic shock or hypertension. The purpose of this study was to examine the effects of oleamide on several vasomotor responses in adult rats.

Materials and Methods: Blood pressure (BP) was measured both directly and indirectly. Coronary flow was quantified with Langendorf preparation, and the vasomotor responses induced by oleamide were analyzed in the aortic rings.

Results: Oleamide induced a decrease in BP, by both direct and indirect methods, which were dose dependent. An increase in coronary flow was observed with Langendorf preparation depending on the dose. Oleamide produced a vasodilator response in aortic rings pre-contracted with phenylephrine (10^{-5} M), which was concentration and endothelium dependent. This relaxing effect was of minor magnitude than that induced with the same dose on BP. L-NAME did not modify these effects. However, indomethacin induced a shift to the left of the concentration-response curve to oleamide and an increase in the magnitude of maximum vasodilation in rings with endothelium. Oleamide produced the maximal relaxant response at 10^{-5} M concentration.

Conclusions: Oleamide has both *in vivo* and *in vitro* vasodilator effects. Vasodilator effects could be mediated by compounds synthesized/released by the endothelium (hyperpolarizing factor) or acting directly on vascular smooth muscle in aortic rings. The TRPV1 and CB1R receptors could mediate these effects. Finally, the results suggest that oleamide probably induces the synthesis/release of a vasoconstrictor prostanoid.

Keywords: vascular reactivity; prostanoids; endothelium; eCBs

Introduction

In recent years, abundant evidence has accumulated, suggesting that lipid mediators may be involved in various physiological mechanisms. These studies have shown that these lipid mediators are involved in intracellular signaling, in inflammatory processes, as well as in various diseases, including those of the cardiovascular system.^{1,2} These lipid mediators include amides derived from fatty acids, which are considered a type of

signal lipids. Endocannabinoids (eCBs) are synthesized from cell membrane phospholipids, among others, in cardiac cells and other tissues of the cardiovascular system.³

eCBs are endogenous neuromodulators whose dysfunction can cause disorders with different clinical manifestations: drug addiction, eating disorders such as anorexia or bulimia, and certain cardiovascular diseases such as hypertension. The most studied are

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anandamide, 2-arachidonoylglycerol (2-AG), and oleamide [(Z)-9-octadecenamide].¹ Their effects are mainly due to binding to specific receptors (CB1 and CB2) in the nervous, immune, and cardiovascular systems (Fig. 1).

eCBs have protective effects on the cardiovascular system, in addition to the psychoactive ones. For example, they decrease blood pressure (BP) and heart rate in humans, as well as in experimental animals, reduce tissue damage, delay the progression of the lesion, reduce the likelihood of presenting arrhythmias due to acute myocardial infarction,^{4,5} and delay the development of atherosclerosis.^{6–8}

On the other hand, it has been shown that activation of CB1 receptors by anandamide produced by macrophages⁹ or 2-AG by platelets¹⁰ significantly contributes to the hypotension that occurs in different types of shock.^{8,11} However, the mechanisms by which eCBs produce these physiological effects are not yet elucidated.¹²

Oleamide, an amide derived from oleic acid, is considered an eCB that is naturally produced in some animals.^{13–15} The biological effects of oleamide appear to be mainly mediated by CB1 receptors, whose presence has already been demonstrated in the mesenteric arteries.^{16,17}

Other receptors acting as mediators of the reactions induced by oleamide are the vanilloid receptors, and the non-cannabinoid receptors type 1 and 2, whose presence has already been demonstrated in the mesenteric and pulmonary arteries of rats.^{17–19} Oleamide also has implications for other receptors, such as γ -aminobutyric acid type A receptors and serotonin receptors of the 5-HT_{1A}, 5-HT_{2A/2C}, and 5-HT₇ type.^{20–22}

Recent studies have shown that the vasodilator effects of oleamide depend mainly on endothelium.¹⁷ However, it has also been shown that part of the vasodilator effect persists even after endothelium elimination, meaning that receptors can also mediate

the effect of oleamide at the vascular smooth muscle level or in the nerve endings existing in the vessel wall.¹³

The purpose of this study was to analyze the effects of oleamide on some parameters of cardiovascular function such as vascular tone, BP, and coronary flow, using an animal model and preparations both *in vitro* and *in vivo*.

Materials and Methods

All the experimental protocols used in this study were carried out following the regulations for the protection of laboratory animals (NOM-062-ZOO, Mexico) established by the Ethics Committee of the Faculty of Medicine of Universidad Nacional Autónoma de México (UNAM), under project no. 001/2011.

Animals

The experiments were carried out on male rats of the Wistar strain weighing between 250 and 300 g, from the vivarium of the Faculty of Medicine of the UNAM. All animals were housed in individual cages with free access to food and water, and they were exposed to a 12-h light-dark cycle.

Measurement of BP

To analyze the effects of oleamide on BP, two groups of animals were formed: The first group was used to quantify BP by the direct method, and the second group was quantified through the indirect method.

Direct method. The effects of oleamide on mean arterial pressure were analyzed with the direct method.

Rats were anesthetized with chloralose (40 mg/kg) and intraperitoneal urethane (1200 mg/kg); this combination of anesthetics results in a reasonable level of anesthesia for a prolonged period without compromising cardiovascular function or altering the reflexes that

FIG. 1. Chemical structure of endocannabinoids (A) anandamide, (B) 2-arachidonoylglycerol, and (C) oleamide.

regulate it.²³ To ensure adequate ventilation of the animal during the procedure, a tracheotomy was previously performed. The body temperature of the animal was maintained at 37°C with a thermostatically controlled heating pad. BP was measured through a cannula inserted into the right femoral artery connected in parallel with a mercury manometer and a pressure transducer (Model PT-300, Grass). Drugs and solutions were administered through a cannula in the left femoral vein. To avoid the formation of clots within the cannula, heparin was administered (500,000 U/kg IV).

Two groups of animals were formed. The first was used as a control, to which only the vehicle was applied, to check whether the volume of the vehicle administered in each dose exerts, by itself, some effect on BP; the second group of animals received this vehicle plus oleamide. Cumulative dose-response curves were performed by using increasing concentrations of oleamide (10^{-7} – 10^{-5} M). The hypotensive effect was not immediate; a period of latency was observed between 4 and 5 min after the administration of the compound. The effect was transient and concentration dependent, which was accompanied by an almost total recovery (around 90%) after 5 to 10 min, possibly due to the activation of BP compensation mechanisms at the cardiovascular level.

Indirect method. The animals were conditioned for a week to remain immobilized in a chamber and with a cuff at the base of the tail for half an hour, always at the same time of day. At the end of this period, the animals were anesthetized, and a surgical procedure was performed to place a catheter in the jugular vein, which was used to administer drugs and solutions. Overall, 48 h was allowed for their recovery, with free access to food and water. Animals were divided into two groups: a control group, to which only the vehicle was administered; and the experimental group, to which the vehicle plus oleamide was administered.

The measurement of BP was performed with a "tail cuff" method (pressure gauge LE 5001; Panlab/Harvard Apparatus), which consists of a cuff where the tail of the animal is introduced, and a sensor detects its volume pressure. The included software can continuously read the data in real time. BP measurements were always made at the same time to avoid fluctuations due to the circadian rhythm. These measurements were obtained after the animals were kept at a temperature of 29–30°C for half an hour before, and throughout the measurement cycle, to promote vasodilation of the blood vessels of the tail. This was achieved by plac-

ing the animals between two moderate sources of heat. The data were collected and analyzed by a computer.

Determination of total coronary flow in the isolated heart

Oleamide effects on coronary flow (mL/min) were determined by using the Langendorff preparation. For the removal of the heart, the rat was euthanized by cervical dislocation. Immediately after that, a thoracotomy was performed; the heart was identified and carefully extracted by severing the large vessels and attempting to obtain the most significant possible portion of the ascending aorta. The heart was immediately attached with 2-0 silk to a glass cannula connected to the perfusion system. Perfusion was started to avoid secondary damage to ischemia. The heart held in a humid chamber was continuously perfused at 37°C.

Hearts were initially perfused with Tyrode solution aerated with carbogen for 10 min, and coronary flow measurements were taken to determine their reference value. Height of the column was kept at 100 cm (constant pressure). The total flow through the coronary arteries was estimated by measuring the effluent volume every 60 sec.

Then, cumulative dose-response curves were established by using increasing concentrations of oleamide (10^{-7} – 10^{-4} M) added to the perfusion solution and coronary flow was evaluated every 60 sec for each administered dose, for 60 sec.

Measurement of vascular responses in *in vitro* experiments

To measure vascular reactivity, the animals were euthanized after 12 h of fasting by cervical dislocation and bleeding. Thoracotomy was used to carefully remove the thoracic aorta, which was immediately placed in a dissection chamber containing an oxygenated solution of Tyrode and dissected carefully under microscopic observation until it was periadventitial connective tissue-free. Once the dissection was completed, the aorta was crosscut to obtain 2-mm-wide rings. In each experiment, a pair of rings from the central portion of the same aorta (one with intact endothelium, the other without functional endothelium) was used. Each of the rings was suspended vertically between a pair of stainless-steel hooks within the same miniature chamber (volume 0.5 mL) for isolated organs and continuous perfusion.

One of the hooks of each ring was attached to the camera wall, whereas the other was attached to an

isometric force transducer (Grass, Model FT03). The rings were continuously perfused (1 mL/min) with a carbogen-aerated Tyrode solution (95% O₂/5% CO₂) whose millimolar composition was: NaCl 137, KCl₂ 7, MgCl₂ 0.69, NaHCO₃ 11.9, NaH₂PO₄ 0.4, CaCl₂ 1.8, and glucose 20; pH was adjusted to 7.2; and the temperature was maintained at 37°C. The rings were stretched to reach the optimal basal tension (2 g) and allowed to stabilize for 30 min while the tension of the baseline was continuously monitored by a polygraph (Grass, Model 79) and, if necessary, the tension was reset by additional stretching.

Before each experiment, the response of each ring pair to phenylephrine and carbachol was evaluated to check the integrity of the preparation and the endothelium in the ring, where it had not been eliminated. For this purpose, the rings were superfused for 10 min with a solution of Tyrode with phenylephrine (10⁻⁵ M) and then with a solution containing carbachol (10⁻⁵ M), in addition to phenylephrine. The relaxation induced by carbachol in phenylephrine-precontracted rings was considered as evidence that these rings had conserved the endothelium, whereas the absence of relaxation confirmed the lack of functional endothelium.

Cumulative dose-response curves were performed while adding increasing concentrations of oleamide (10⁻¹¹–10⁻⁵ M) to the superfusion solution, using aorta rings pre-contracted with phenylephrine (phenylephrine, 10⁻⁶ M), in the presence or absence of indomethacin (10⁻⁶ M) or N(ω)-nitro-L-arginine methyl ester (L-NAME) (300 μ M).

Perfusion system. Using a peristaltic pump and a polyethylene tube system, the Tyrode solution and test solutions were transported into the perfusion chamber. The perfusion solutions were continuously aerated with carbogen, penetrated through the bottom of the chamber, and drained to the outside by overflow through a slot at the top of the chamber equipped with a cellulose wick; this prevented sudden volume changes. The solutions were kept at a constant temperature of 37°C.

Registration system. The tension developed by each ring was recorded by an isometric force transducer (Grass FT03), and then the signal was sent to a polygraph (Model 79, Grass). The tension of each ring was continuously recorded on paper, simultaneously scanned (PowerLab 7200; ADI Instruments), and stored on a computer's hard drive for further analysis.

Used drugs

Phenylephrine, carbachol, and L-NAME were purchased from Sigma-Aldrich. The drugs were dissolved in distilled water. The oleamide was dissolved in Tyrode and 0.01% Tween 20. The indomethacin was dissolved in a 4% bicarbonate solution. All solutions were made on the same day.

Analysis of the data

The phenylephrine-induced contractile responses are expressed as the tension increase in grams (g) above the basal tension (imposed on the vessel at the beginning of the experiment). The changes in vascular tone induced by oleamide are expressed as a percentage of the maximal tension induced by phenylephrine (10⁻⁵ M) in the absence of carbachol. EC₅₀ (Log. of the molar concentration of the agonist producing 50% of the maximal response). These data were obtained with the Graph Pad Prism program (San Diego, California) and are expressed as the mean $\pm$ SD. The analysis of variance (ANOVA) was used to compare the data, and the differences between the groups were assessed by using the Student Newman-Keuls test (SigmaStat software, St. Louis, MO). A *p* value of 0.05 or less was considered significant.

Results

Effects of oleamide on aorta rings pre-contracted with phenylephrine (10⁻⁶ M)

The oleamide produced a relaxing effect, which depended as much on the concentration as on the presence of endothelium. The relaxing effects of oleamide on rings without endothelium were not observed (Fig. 2). Maximum relaxation was observed with 10⁻⁵ M concentration (79.32 $\pm$ 6.69% in rings with endothelium vs. 102.96 $\pm$ 11.97% in rings without endothelium, *p* < 0.05).

Addition of indomethacin (10⁻⁶ M), a non-selective inhibitor of the cyclooxygenase pathway, to superfusion solution with oleamide, caused a significant increase in the relaxing response induced by oleamide (60.82 $\pm$ 11.93% vs. 79.32 $\pm$ 6.69%). This effect was observed only in rings with endothelium (Fig. 3). The concentration-response curve was shifted to the left (EC₅₀ 7.51 $\pm$ 0.34 oleamide vs. 9.84 $\pm$ 0.23 oleamide + indomethacin, *p* < 0.05).

Phenylephrine pre-contracted aortic rings in the presence of L-NAME, oleamide (10⁻¹¹ M), which induced an additional increase in the maximum tension developed, compared with that of the control

FIG. 2. Cumulative dose-response curve to oleamide (10^{-11} – 10^{-5} M) in pre-contracted aortic rings with phenylephrine (10^{-6} M) with (●) and without endothelium (○). The data are expressed as the percentage of the maximum stress induced by phenylephrine and are shown as the mean $\pm$ SD of the vessels of four rats in each group. *Denotes that the relaxation of the rings with endothelium is significantly higher ($p < 0.05$) than that of the rings without endothelium. SD, standard deviation.

($111.72 \pm 10.20\%$ vs. $98.46 \pm 2.13\%$, $p < 0.05$). At higher concentrations, oleamide induced a relaxing response of minor magnitude compared with that observed in the absence of L-NAME. However, this effect was not significant (Fig. 4).

Effects of oleamide on mean arterial pressure

Direct method. Cumulative dose-response curves were performed by using increasing concentrations of oleamide (10^{-7} – 10^{-5} M). Oleamide produced a decrease in arterial pressure, which was significant in comparison with that of the control group (Fig. 5). The maximal decrease in mean arterial pressure was observed with 10^{-5} M (73.37 ± 3.19 mmHg of the experimental group vs. 103.22 ± 1.28 mmHg of the control group, $p < 0.05$).

Indirect method. Cumulative dose-response curves were performed by using increasing concentrations of oleamide (10^{-7} – 10^{-5} M). The drugs were administered intravenously through the catheter previously placed in the jugular vein. Oleamide produced a significant decrease in BP, compared with that of the control group ($p < 0.05$). The maximal decrease in mean arterial pressure was observed with 10^{-5} M concentration (92.15 ± 3.41 mmHg for the experimental group vs. 117.62 mmHg for the control group) (Fig. 6). This hypotensive effect was dependent on the concentration and was lower than that observed with the direct method (73.37 mmHg ± 3.19 with the direct method vs. 92.15 mmHg ± 3.41 with the indirect method).

Effects of oleamide on coronary flow

Oleamide induced an increase in coronary flow, which was dependent on concentration. Maximal increase flow was observed with 10^{-4} M concentration, which

FIG. 3. Effects of indomethacin (10^{-6} M) on the cumulative dose-response curve to oleamide (10^{-11} – 10^{-5} M) in aortic rings with endothelium (A) and without endothelium (B), pre-contracted with phenylephrine (10^{-6} M), in the presence (●) or absence of indomethacin (○). The data are expressed as the percentage of the maximum stress induced by phenylephrine and are shown as the mean $\pm$ SD of the aortic rings of four rats in each group. *Denotes that the relaxation of rings with endothelium is significantly higher ($p < 0.05$).

FIG. 4. Effects of L-NAME (300 μ M) on the cumulative dose-response curve to oleamide (10^{-11} – 10^{-5} M) in aortic rings with endothelium precontracted with phenylephrine (10^{-6} M), in the presence of L-NAME (●) or absence of L-NAME (○). The data are expressed as the percentage of the maximum stress induced by phenylephrine and are shown as the mean $\pm$ SD of the aortic rings of four rats in each group. L-NAME, N(ω)-nitro-L-arginine methyl ester.

was significantly higher compared with that of coronary flow under control conditions ($115.41 \pm 3.61\%$) (Fig. 7).

Discussion

Anandamide is the eCB most studied (arachidonylethanolamide), being the first endogenous CB1 receptor agonist discovered and seeing that it produced psychoactive and cardiovascular effects such as those produced by the active substance of cannabis, Δ^9 -tetrahydrocannabinol.²⁴ However, there is evidence that there are many other similar compounds that are produced endogenously by the body in response to various stimuli.

Oleamide is a recently discovered eCB that has aroused considerable interest, and it has been associated with a wide variety of physiological actions. It has been proposed that this compound has great potential as a signaling molecule within the cardiovascular system, as a cardiovascular function protector and modulator.¹⁵

Regarding the effects of oleamide on vascular reactivity, our results indicate that this compound has relaxing effects that depend on the concentration and the presence of the endothelium. These findings are similar to those registered by other researchers.^{1,17}

FIG. 5. Effects of oleamide (10^{-7} – 10^{-5} M) on the mean arterial pressure, determined by the direct method. The curves represent the average of the decrease in blood pressure (mmHg) $\pm$ SD. The control group (●) and the group treated with oleamide (○). *Denotes that the relaxation of rings with endothelium is significantly higher ($p < 0.05$, $n = 4$).

However, these studies were performed on rat mesenteric arteries.

The vasorelaxant effects of oleamide were moderate. One possible explanation could be that oleamide, when metabolized by the fatty acid amide hydrolase

FIG. 6. Cumulative dose-response curve of the effects of oleamide (10^{-7} – 10^{-5} M) on the mean arterial pressure, determined by the indirect method. The data represent the mean (mmHg) $\pm$ SD. The control group (●) and the group treated with oleamide (○). *Significant statistical difference with respect to the control ($p < 0.05$, $n = 4$).

FIG. 7. Effect of oleamide (10^{-7} – 10^{-4} M) on coronary flow with the Langendorf preparation. Dark bar: control group. Clear bars: experimental group. The data represent the average of the increase in coronary flow at baseline conditions (mL/min) $\pm$ SD. *Significant statistical difference with respect to baseline ($p < 0.05$, $n = 4$).

(FAAH) enzyme, produces metabolites that would modify the vascular responses induced by this compound, as is the case with other compounds of the same family. On the other hand, the fact that oleic acid, a metabolite of oleamide, does not induce relaxation in the mesenteric arteries, further demonstrates that the relaxant response induced by oleamide does not depend on the metabolism by FAAH enzyme¹⁷

Regarding the participation of the endothelium in the relaxing response induced by oleamide, our results indicate that this response in rat aortic rings is endothelium dependent. This effect has been observed by other researchers,^{1,17} who additionally found that there was also a relaxing response of minor magnitude in rings without endothelium, proposing the possible interaction between oleamide and receptors present in vascular smooth muscle or nerve terminals. The explanation for the differences between our results and those obtained in other studies could be the use of different preparations (aortic rings vs. mesenteric artery rings) or of different recording systems. In addition, our findings that oleamide-induced relaxing response is endothelium dependent are supported by previous reports showing the CB1 receptor (selective oleamide ligand) on rat endo-

thelial cells.^{25,26} This is seen in addition to the presence of non-CB1 and non-CB2 receptors (another ligand of oleamide) on endothelial cells from rats' mesenteric arteries and humans' pulmonary arteries.^{18,19}

Regarding the participation of vasoactive prostanoids, on the relaxant effects of oleamide, our results indicate that the relaxant effect of oleamide is significantly higher in the presence of indomethacin compared with those where only oleamide was administered. These findings suggest the possibility that oleamide causes the release of some prostanoid with vasoconstrictor effects, whose synthesis/release is blocked by adding a cyclooxygenase inhibitor such as indomethacin or that the effects of oleamide are attenuated due to its metabolism by several enzyme systems, among them being cyclooxygenase. This could be compatible with some studies, where it has been found that this family of compounds can be metabolized by enzyme mechanisms other than FAAH, among them being the cyclooxygenase (COX) pathway and/or the monoacylglycerol lipase (MGL) pathway.^{27,28}

Further, in some studies with rat mesenteric arteries, rat aortic rings, and sheep ophthalmic arteries, nitric oxide has been implicated in the relaxant effects of eCBs.^{29–31} It has been proposed that cannabinoid

receptor agonists induce phosphorylation of endothelial nitric oxide synthase and, therefore, increase the synthesis of nitric oxide, so that addition of L-NAME (nitric oxide synthesis inhibitor) significantly decreases the relaxing response induced by these compounds. In our study, we found that the oleamide relaxing effect in the presence of L-NAME was lower. However, this effect was not significant. This finding suggests that the oleamide relaxant effects in the rat aortic rings could be mediated, at least partially, through the release of a hyperpolarizing factor derived from the endothelium.

Regarding the effect of oleamide on arterial pressure, we found that this compound caused a significant decrease in arterial pressure, which was concentration dependent. Hypotension was greater in anesthetized rats using the direct method than in rats non-anesthetized rats using the indirect method.

Up to now, no study has reported the effects of oleamide on BP by using conscious or anesthetized animals. Our study provides evidence that oleamide causes a significant decrease in BP in both contexts, compared with control animals. However, further research is needed to study the systemic effects of oleamide and to determine whether its hypotensive effect is due to synergistic effects, specifically to a decrease in cardiac inotropy and a decrease in peripheral vascular resistance.

eCBs have cardioprotective effects. It has been found that 2-AG limits the size of damage in isolated rat hearts exposed to ischemia periods, and the same effects are observed by using synthetic receptor agonists CB1 and CB2.³²

Oleamide caused a significant increase in coronary flow in rat isolated hearts by using the Langendorff preparation; this effect was concentration dependent. With these results, we provide evidence that, similar to other eCBs, oleamide has direct effects on coronary blood flow.³³ However, future studies are needed to evaluate the effects of oleamide on the heart, specifically on cardiac automatism, cardiac inotropy, and other electrophysiological parameters of myocardial cells since, as noted earlier, it has been found that the hypotensive effect induced by oleamide could be due, at least in part, to the decrease in cardiac output.

Conclusions

Oleamide, similar to other compounds of the same family, has both *in vivo* and *in vitro* vasodilator effects on blood vessels, both by compounds synthesized/

released by endothelium (probably through a vasodilator hyperpolarizing factor) and directly on vascular smooth muscle. The TRPV1 and CB1 receptors could mediate these effects. It is also suggested that oleamide is likely to induce the synthesis/release of a vasoconstrictor prostanoid.

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Author Disclosure Statement

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Abbreviations Used

2-AG = 2-arachidonoylglycerol
 ANOVA = analysis of variance
 BP = blood pressure
 COX = cyclooxygenase
 eCB = endocannabinoid
 FAAH = fatty acid amide hydrolase
 L-NAME = N(ω)-nitro-L-arginine methyl ester
 MGL = monoacylglycerol lipase
 SD = standard deviation
 UNAM = Universidad Nacional Autónoma de México