Arachidonyl dopamine as a ligand for the vanilloid receptor VR1 of the rat

Attila Tóth, Noémi Kedei, Yun Wang, Peter M. Blumberg*

Molecular Mechanisms of Tumor Promotion Section, Laboratory of Cellular Carcinogenesis and Tumor Promotion, National Cancer Institute, National Institutes of Health, Bldg. 37, Rm. 4048, 37 Convent Dr., MSC 4255, Bethesda, MD 20892-4255, USA

Received 25 September 2002; accepted 12 February 2003

Abstract

The vanilloid receptor VR1 is a nonspecific Ca\(^{2+}\) channel, expressed in sensory neurons in the peripheral nervous system and in various brain regions, which is believed to be an important molecular integrator of several chemical (acid, vanilloids) and physical stimuli (heat) that cause pain. Recently, several endogenous ligands for VR1 have been identified such as arachidonyl ethanolamide (anandamide) and the more potent arachidonyl dopamine (AA-DO). Here, we further characterize AA-DO as a ligand for rat VR1, heterologously expressed in CHO and HEK293 cells. AA-DO inhibited the binding of \([^{3}H]\)RTX to VR1 with a \(K_d\) value of 5.49 ± 0.68 \(\mu\)M and with positive cooperativity (\(p = 1.89 ± 0.27\)), indicating that AA-DO was about 5-fold more potent than anandamide in this system. The \(K_d\) (9.7 ± 3.3 \(\mu\)M), and \(p\) values (1.54 ± 0.04) for the binding of AA-DO to spinal cord membranes are in good correlation with the CHO-VR1 data. AA-DO stimulated \(^{45}\)Ca\(^{2+}\) uptake on CHO-VR1 and HEK-VR1 cells with EC\(_{50}\) values of 4.76 ± 1.43 and 7.17 ± 1.64 \(\mu\)M and Hill coefficients of 1.28 ± 0.11 and 1.13 ± 0.13, respectively, consistent with the binding measurements. In contrast to anandamide, AA-DO induced virtually the same level of \(^{45}\)Ca\(^{2+}\) uptake as did capsaicin (90 ± 6.6% in the CHO cells expressing VR1 and 89.3 ± 9.4% in HEK293 cells expressing VR1). In a time dependent fashion following activation, AA-DO partially desensitized VR1 both in \(^{45}\)Ca\(^{2+}\) uptake assays (IC\(_{50}\) = 3.24 ± 0.84 \(\mu\)M, inhibition is 68.5 ± 6.85%) as well as in Ca\(^{2+}\) imaging experiments (35.8 ± 5.1% inhibition) using the CHO-VR1 system. The extent of desensitization was similar to that caused by capsaicin itself. We conclude that AA-DO is a full
agonist for VR1 with a potency in the low micromolar range and is able to significantly desensitize the cells in a
time and dose dependent manner.
Published by Elsevier Science Inc.

Keywords: Vanilloid receptor subtype 1 (VR1); Capsaicin; Anandamide; Arachidonyl dopamine; Resiniferatoxin (RTX)
binding; $^{45}$Ca$^{2+}$ uptake; Intracellular Ca$^{2+}$ imaging; VR1-transfected CHO cells (CHO-VR1); Desensitization; Antagonism;
Agonism; Calcium

Introduction

The vanilloid receptor subtype 1 (VR1) is a cation channel (Caterina et al., 1997) which functions as a
molecular integrator of chemical and physical stimuli that elicit pain (Tominaga et al., 1998). Consistent
with this function, VR1 is expressed in nociceptive primary afferents of the C-fiber ‘pain’ pathway
(Caterina et al., 2000) and in various brain regions (Mezey et al., 2000). VR1 represents the specific site of
action for vanilloids like capsaicin, the pungent ingredient in hot peppers, and for resiniferatoxin, an
ultrapotent analog. Recently, VR1 has attracted much attention as a novel, potential therapeutic target for
the treatment of pain and other conditions involving C fiber sensory neurons including neurogenic
bladder, benign prostatic hypertrophy, interstitial cystitis, urinary tract infection, pharyngitis, mucositis,
pancreatitis, enteritis, cellulitis, postherpetic neuralgia, peripheral neuropathy, arthritis, and bony fractures
(Robbins, 2000).

Although the presence in plants of potent activators of VR1 such as capsaicin or resiniferatoxin has
suggested that there may be corresponding endogenous ligands, the physiological regulation of VR1 is
only beginning to be understood. Coordinate mechanisms of regulation of VR1 suggested so far include
activation by heat (Caterina et al., 1997), acid (Tominaga et al., 1998) and phosphorylation by protein
kinase C (Premkumar and Ahern, 2000), as well as activation by the endogenous ligands arachidonyl
ethanolamide (anandamide) (Zygmunt et al., 1999), lipoxygenase products (Hwang et al., 2000), and,
most recently, arachidonyl dopamine (Huang et al., 2002).

Anandamide was isolated and characterized as an endogenous eicosanoid with moderate affinity for the
cannabinoid receptors CB1 and CB2 (Devane et al., 1992). The CB1 receptor, heterogeneously distributed
in the brain, PNS and peripheral organs, is a G-protein-coupled receptor which is currently thought to be a
specific receptor for anandamide. The finding that three cannabimimetic effects elicited by anandamide in
div (i.e. antinociception on a hot plate, immobility on a ring and suppression of spontaneous activity)
persisted in mice in which the gene encoding the CB1 receptor had been genetically disrupted (Di Marzo et
al., 2000) suggested that anandamide mediated these effects via another target. Since the expression of CB2
receptors is restricted to the immune system (Di Marzo et al., 2001) and the overall potency and efficacy of
anandamide to activate CB2 receptors is weak (Howlett and Mukhopadhyay, 2000), the vanilloid receptor
1 (VR1) was suggested to be responsible for some of the cannabimimetic effects of anandamide (Zygmunt
et al., 1999). However, the overall contribution of anandamide to endogenous regulation of VR1 remains
uncertain (Di Marzo et al., 2002a,b; Szolcsanyi, 2000; Zygmunt et al., 2000). When VR1 is heterologously
expressed in cell systems, the potency of anandamide for inducing typical VR1-mediated effects (e.g.
cation currents, Ca$^{2+}$ influx and cell depolarization) is low (1–10 μM) (Smart et al., 2000; Zygmunt et al.,
1999). Its behavior also depends on the species. Although anandamide is a partial agonist on rat VR1
(maximal calcium uptake is about 50% of that induced by capsaicin) (Zygmunt et al., 1999), it is a full
agonist on human VR1 (Smart et al., 2000).
Recently, arachidonyl dopamine (AA-DO) has been proposed as another endogenous agonist of VR1 structurally related to anandamide (Huang et al., 2002). The reported potency of AA-DO showed substantial differences, however, depending on the system. With VR1 expressed in HEK293 cells, its EC$_{50}$ was reported to be $\sim$ 40 nM whereas it was $\sim$ 800 nM in isolated DRG cells. Like anandamide, AA-DO was described as a partial agonist (Huang et al., 2002). The binding potency of AA-DO was reported as higher than 25 $\mu$M (Bisogno et al., 2000) using rat spinal cord membranes or 5.3 $\mu$M using human VR1 expressing HEK293 cells (Di Marzo et al., 2002b).

Here we characterize the effects of AA-DO on CHO cells overexpressing rat VR1 (CHO-VR1). Although AA-DO was several-fold more potent than anandamide under our conditions, the potency of AA-DO in functional assays was substantially lower than that of capsaicin. Like capsaicin, it caused partial desensitization of VR1. In contrast to anandamide, it was a full agonist.

**Methods**

**Materials**

Arachidonyl dopamine was from Cayman Chemicals (Ann Arbor, MI, USA), anandamide and RTX were obtained from Biomol (Plymouth Meeting, PA, USA). Capsaicin and other chemicals were from Sigma (St. Louis, MO, USA).

**Cell culturing**

CHO-VR1 cells were cultured as previously described (Toth et al., 2002). Briefly, Tet-Off induced CHO-VR1 cells were cultured in maintaining media-F12 supplemented with 10% FBS (USA sourced), 25 mM HEPES pH 7.5, 250 $\mu$g/ml geneticin (all from Life Technologies Inc., Rockville, MD, USA) and 1 mg/L tetracycline (Calbiochem, La Jolla, CA, USA). HEK293 cells were cultured in DMEM (from Life Technologies Inc., Rockville, MD, USA) containing 10% FBS. The cells were subcultured every 2–3 days depending on the level of confluency (80–90%) and the cultures were split 1 to 8 in T75 flasks.

**HEK293 transfection**

HEK293 cells were subcloned into 24 well plates. On the second day when the confluency was about 50%, cells were transfected using the Lipofectamine plus reagent kit (Invitrogen, Carlsbad, CA, USA) with 4 $\mu$g EGFP-VR1 plasmid (Olah et al., 2001) to each plate according to the manufacturer’s instructions. The media was changed to 10% FBS containing DMEM after 4 hours of transfection. The experiments were performed on the second day, as described below.

$^{45}$Ca$^{2+}$ uptake experiments

Experiments were performed as described (Toth et al., 2002). Briefly, cells (CHO-VR1 Tet-off cells) were plated in maintaining media into 24-well plates. The second day, the medium was changed to
remove the tetracycline and induce VR1 expression (maintaining media without tetracycline but containing 1 mM sodium butyrate). Experiments were performed approximately 24 h after induction. For assay of $^{45}$Ca$^{2+}$ uptake, cells (CHO-VR1 Tet-off and VR1 transfected HEK293 cells) were incubated for 5 min at 37 °C in a total volume of 400 μl of serum-free DMEM (Life Technologies Inc., Rockville, MD, USA) containing 1.8 mM CaCl$_2$ in the presence of 0.25 mg/ml bovine serum albumin (BSA, Sigma, St. Louis, MO, USA), 1 μCi/ml $^{45}$Ca$^{2+}$ (ICN, Costa Mesa, CA, USA), and increasing concentrations of AA-DO. Immediately after the incubation, extracellular $^{45}$Ca$^{2+}$ was removed by washing the cells (three times for CHO-VR1 or once for HEK-VR1 cells, respectively) with cold DPBS (Life Technologies Inc., Rockville, MD, USA) containing 1.8 mM CaCl$_2$. Then, 400 μl RIPA buffer (50 mM Tris–Cl, pH 7.4; 150 mM NaCl; 1% Triton X-100; 0.1% SDS; 1% sodium deoxycholate) was added to each well to lyse the cells. Plates were shaken slowly for 20 min; then 300 μl of cell lysate was transferred from each well into a scintillation vial and radioactivity was determined by scintillation counting. In each experiment, four wells were assayed under each assay condition. Data from these experiments were analyzed by computer fit to the Hill equation. For agonism and antagonism four independent experiments were performed, while 3 experiments were done for the kinetic measurements.

$[^3]$H]RTX binding experiments

Preparation of membranes: CHO-VR1 cells were subcultured into T75 flasks. On the second day, when the confluency was about 50–70%, the medium was changed to inducing media (maintaining media without tetracycline but containing 1 mM sodium butyrate). After two days of induction, the cells were collected and stored at −20 °C. For spinal cords, adult female Sprague–Dawley rats were euthanized using CO$_2$. The spinal cords were dissected and were homogenized in a glass tissue homogenizer using 10 ml per spinal cord of ice-cold DPBS buffer without Ca$^{2+}$ or Mg$^{2+}$. The homogenate was centrifuged (5 000 × g, 15 min) and the pellet was stored at −20 °C.

Binding assay mixtures were set up on ice and contained about 0.1 mg/assay CHO-VR1 cells or about 0.5 mg/assay spinal cord membranes, 0.25 mg/ml BSA (Cohn fraction V, Sigma, St. Louis, MO, USA), $[^3]$H]resiniferatoxin ($[^3]$H]RTX; 37 mCi/mol; Perkin Elmer, Boston, MA, USA) and non-radioactive AA-DO. The final volume was adjusted to 400 μl with the buffer described above. Non-specific binding was defined as binding occurring in the presence of 100 nM non-radioactive RTX. Binding was analyzed in the presence of a fixed concentration of $[^3]$H]RTX (≈ 40 pM) and various concentrations of competing AA-DO. The binding was initiated by transferring the assay tubes into a 37 °C water bath and then was terminated following a 60 min incubation period by cooling the assay mixtures on ice. Non-specific binding was reduced by adding 200 μg of bovine glycoprotein fraction VI (α-glycoprotein, ICN, Costa Mesa, CA, USA) to each tube. Membrane-bound RTX was separated from the free and the glycoprotein-bound RTX by pelleting the membranes in a Beckman 12 benchtop centrifuge (15 min; maximal velocity), and the radioactivity was determined by scintillation counting (Toth et al., 2002).

Ca$^{2+}$ imaging

Experiments were performed as described (Toth et al., 2002). Briefly, CHO-VR1 cells were plated on 25 mm round glass coverslips in maintaining media. The next day the media was changed to inducing
media (maintaining media without tetracycline but containing 1 mM sodium butyrate) to induce VR1 expression. Experiments were done approximately 24 hours after induction. For the fura2 loading, the cells were transferred to DPBS containing 0.25% BSA and 5 μM fura2-AM (Molecular Probes, Eugene, OR, USA) for 2 hours at 20 °C. The cells were kept in maintaining media at room temperature until the measurements, which were carried out in DPBS. The fluorescence of individual cells was measured with an IntCyt Im2 fluorescence imaging system (Intracellular Imaging Inc., Cincinnati, OH, USA). The cells within a field were illuminated alternately at 340 and 380 nm. Emitted light >510 nm was measured. Data were analyzed with the Incyt 4.5 software and further processed with Excel (Microsoft) and GraphPad Prism 2.0 (Graphpad Software Inc.) software.

Results and discussion

Two arachidonic acid derivatives, arachidonyl ethanolamide (anandamide, Fig. 1) (Zygmunt et al., 1999) and arachidonyl dopamine (AA-DO, Fig. 1) (Huang et al., 2002), are endogenous compounds

---

![Chemical structures](image)

Fig. 1. Structures of arachidonyl dopamine, anandamide, capsaicin and resiniferatoxin.
with affinity for VR1. Anandamide has a potency in the micromolar range (Smart et al., 2000; Zygmunt et al., 1999), whereas AA-DO was reported to be at least one order of magnitude more potent (Huang et al., 2002). Its effect has already been detected on (i) intracellular calcium concentration in transfected cells as well as in DRG neurons, (ii) substance P and CGRP release from spinal cord slices, and (iii) hippocampal paired-pulse depression (Huang et al., 2002). Furthermore the capability to desensitize VR1 (Huang et al., 2002) and its effects on cannabinoid receptors were also reported (Bisogno et al., 2000). Here, we further investigated the pharmacological properties of AA-DO.

One issue is the binding potency of AA-DO for VR1. AA-DO (0.5–25 μM) was reported (Bisogno et al., 2000) not to displace [3H]RTX significantly from rat spinal cord membranes. In contrast, a K_i value of 5.3 μM was reported using higher receptor concentrations and the human VR1 (hVR1-HEK293) (Di Marzo et al., 2002b). These binding affinity results are in marked contrast with the reported potency of AA-DO in 45Ca^{2+} uptake experiments (EC_{50} = 48 nM) or in intracellular Ca^{2+} imaging data using isolated DRG neurons (EC_{50} = 794 nM) (Huang et al., 2002). Under our conditions, we found that AA-DO inhibited [3H]RTX binding to rat VR1 (Fig. 2A for CHO-VR1, spinal cord is not shown). Its potency was similar using CHO-VR1 and spinal cord membranes (K_d = 5.49 ± 0.68 μM, n = 3 experiments for CHO-VR1 and K_d = 9.7 ± 3.3 μM, n = 3 experiments for spinal cord) and was ∼5 fold stronger than that of anandamide. The binding was cooperative (p = 1.89 ± 0.27, n = 3 experiments, for CHO-VR1 and p = 1.54 ± 0.04, n = 3 experiments for spinal cord), as found for other vanilloids (Kedei et al., 2001). A methodological difference that might account for the difference between our findings and those reported previously (Bisogno et al., 2000) was that we included α-glycoprotein in the wash procedure to reduce the nonspecific binding. As we have described previously, inclusion of α-glycoprotein enhances the efficiency of the wash and reduces the non-specific binding (Szallasi and Blumberg, 1999). Another possibility could be that the AA-DO binds to the relatively high concentration of BSA added into our assay mixture. Therefore, we also carried out experiments in which the BSA was replaced with the same concentration of IgG. No difference was observed (data not shown).

Using the CHO-VR1 and HEK-VR1 cells, we determined the ability of AA-DO to stimulate 45Ca^{2+} uptake (Fig. 2B for CHO-VR1, HEK-VR1 is not shown). The measured potency (EC_{50} = 4.76 ± 1.43 μM, n = 4 experiments for CHO-VR1 and 7.17 ± 1.64 μM, n = 3 experiments for HEK-VR1) was consistent with that observed for binding of AA-DO (K_d = 5.49 ± 0.68 and 9.7 ± 3.3 μM) but was in contrast with the previously published value (EC_{50} = 48 ± 7 nM for HEK-VR1) (Huang et al., 2002). For capsaicin, unlike AA-DO, the EC_{50} for 45Ca^{2+} uptake that we obtained under our conditions (44.8 ± 3.8 nM, n = 7 experiments) was similar to that reported by Huang and coworkers (33 ± 7 nM). It is also noteworthy that we did not find a significant difference in the efficacy of AA-DO compared to that of a maximal dose (300 nM) of capsaicin (90 ± 6.6%, n = 4 experiments for CHO-VR1 and 89.3 ± 9.4%, n = 3 experiments for HEK-VR1, respectively), whereas anandamide is a partial agonist (about 50% activation) (Zygmunt et al., 1999).

An endogenous ligand for VR1 could function either to stimulate this receptor, or, through its continued presence, to desensitize it. Indeed, it is this latter behavior that forms the basis for the therapeutic application of VR1 agonists like RTX or capsaicin (Robbins, 2000). We therefore examined the ability of AA-DO to reduce the subsequent stimulation of 45Ca^{2+} uptake in response to capsaicin challenge on CHO-VR1 cells (Fig. 2B). AA-DO blocked response to capsaicin (50 nM) in a concentration dependent fashion (Fig. 2B). The IC_{50} value (3.24 ± 0.84 μM, n = 4 experiments) is consistent with the EC_{50} value for agonism (4.76 ± 1.43 μM) or for inhibition of [3H]RTX binding (K_d = 5.49 ± 0.68 μM) in the same system. The observed maximal inhibition of 45Ca^{2+} uptake (68.5 ± 6.85%, n = 4 experiments)
Fig. 2. Pharmacological characterization of arachidonyl dopamine. The binding of 70 pM $[^{3}H]$RTX to CHO-VR1 cell membranes was inhibited by AA-DO (1–100 µM) in a dose dependent manner (panel A). Values are expressed as average ± S.E.M. from a single experiment. The experiment was repeated an additional two times with similar results. In the $^{45}$Ca$^{2+}$ uptake experiments the AA-DO was tested first as an agonist (panel B, ) on CHO-VR1 cells. The $^{45}$Ca$^{2+}$ uptake was initiated by adding the AA-DO (0.3–100 µM). The extent of stimulation over background (buffer alone) was compared to the stimulation by 300 nM capsaicin. Values are expressed as average ± S.E.M. from a single experiment. The experiment was repeated an additional three times with similar results. To study the desensitization (panel B, ), CHO-VR1 cells were incubated with AA-DO (15 min, 0.3–100 µM), then 50 nM capsaicin and $^{45}$Ca$^{2+}$ were added. After 5 min the $^{45}$Ca$^{2+}$ uptake was measured and plotted as % of control (preincubation with buffer alone). Values are expressed as average ± S.E.M. from a single experiment. The experiment was repeated an additional three times with similar results.
experiments) is somewhat similar to that published previously (inhibition from 51% to 8%) (Huang et al., 2002) but the potency was less in our system.

The behavior of AA-DO was further evaluated by intracellular calcium imaging using CHO-VR1 cells. Data in each experiment represent the averaged response of 34–49 cells measured simultaneously. First, we verified that AA-DO displayed similar potency (2.79 μM, a single experiment) and efficacy (98% compared to 300 nM capsaicin, a single experiment, data not shown) to that found by us in the 45Ca2+ uptake assays. The antagonists capsazepine and ruthenium red inhibited the AA-DO mediated elevation of [Ca2+], arguing that the detected response was mediated by VR1 rather than non-specific (results of duplicate experiments, data not shown). Moreover, AA-DO did not stimulate calcium uptake in control CHO cells without VR1, again arguing against a non-specific action (results of a single experiment, data not shown). In further analysis, we studied the kinetics of the response of the CHO-

![Diagram](image)

Fig. 3. Stimulation of intracellular calcium as a function of time after addition of AA-DO or capsaicin. The experiments were carried out in low extracellular Ca2+ concentrations (DPBS without Ca2+, open bars) or in normal Ca2+ concentrations (DPBS containing 2 mM Ca2+, filled bars). The AA-DO and capsaicin were added as indicated. The fluorescence signals (340/380 nm) from individual cells were recorded (34–49 cells) and were plotted as the average of the fluorescence ratios (340/380 nm). The results from representative experiments are shown. Each experiment was repeated 2 additional times with similar results.
VR1 cells to AA-DO and the inhibition by AA-DO of the response to subsequent challenge with capsaicin (Fig. 3). Addition of AA-DO (50 µM, Fig. 3A) caused an increase in \( [Ca^{2+}] \), which diminished somewhat after 5–10 min but remained elevated relative to its initial level (Fig. 3A and Table 1). Capsaicin behaved similarly (Fig. 3F and Table 1). Plateau levels of \( [Ca^{2+}] \) for AA-DO and capsaicin relative to maximal levels, expressed as the fluorescent ratios, were 64.2 ± 5.1% (n = 6 experiments) and 62.8 ± 7.1% (n = 3 experiments), respectively (Table 1). Subsequent capsaicin challenge (50 nM) in the continued presence of AA-DO had little effect (Fig. 3B, fluorescence ratios changed from 0.50 ± 0.04 to 0.61 ± 0.05, n = 3 experiments). Similar behavior was observed upon initial capsaicin treatment followed by challenge with AA-DO (Fig. 3F, ratios elevated from 0.48 ± 0.05 to 0.53 ± 0.09, n = 3 experiments).

In the case of capsaicin, extracellular Ca\(^{2+}\) is thought to play a crucial role in the desensitization (Koplas et al., 1997; Liu and Simon, 1998), whereas other agonists such as zingerone or olvanil could desensitize in the absence of Ca\(^{2+}\) as well (Liu and Simon, 1998). To explore the role of external Ca\(^{2+}\) in the desensitization by AA-DO, we incubated the cells with a buffer containing low Ca\(^{2+}\) and 50 µM AA-DO (DPBS without Ca\(^{2+}\)), then we added Ca\(^{2+}\) (2 mM final concentration, at 16 min, Fig. 3C) or capsaicin and Ca\(^{2+}\) together (2 mM Ca\(^{2+}\) and 50 nM capsaicin, at 16 min, Fig. 3D). Treatment of the cells with AA-DO under low Ca\(^{2+}\) conditions led to lower elevation in the \([Ca^{2+}]_i\) (Fig. 3C and 3D) than in normal Ca\(^{2+}\) conditions (Fig. 3A), consistent with the well characterized role of VR1 as a Ca\(^{2+}\) channel allowing external Ca\(^{2+}\) to flow into the cell. Moreover, there was no decrease in \([Ca^{2+}]_i\) with time of incubation. The value at 15 min was 110.2 ± 9.6% (n = 9 experiments) of the initial elevated value, suggesting no desensitization under low calcium conditions. Likewise, subsequent addition of capsaicin and Ca\(^{2+}\) after a 15 min incubation in the presence of 50 µM AA-DO caused similar elevation in the \([Ca^{2+}]_i\) (ratios rose from 0.34 ± 0.07 to 0.79 ± 0.02, n = 3 experiments) as did addition of Ca\(^{2+}\) alone (from 0.30 ± 0.07 to 0.86 ± 0.05, n = 3 experiments). We conclude that the intracellular Ca\(^{2+}\) plays a role in the desensitization by AA-DO.

To measure the kinetics of desensitization, we used the \(^{45}\)Ca\(^{2+}\) uptake assay (Fig. 4A and 4B). Application of capsaicin (50 nM) and AA-DO together caused modestly higher \(^{45}\)Ca\(^{2+}\) uptake than capsaicin alone (Fig. 4A and 4B, 0 min), consistent with this concentration of capsaicin not being fully saturating for VR1. In contrast, preincubation with AA-DO (Fig. 4A and 4B, 1 min) caused a significant inhibition. Maximal inhibition was observed after a 30 min preincubation. Since the incubation with the

<table>
<thead>
<tr>
<th>Table 1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Desensitization by AA-DO and capsaicin</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Buffer</td>
</tr>
<tr>
<td>Maximal response at 0–3 min (ratio)</td>
</tr>
<tr>
<td>Response at 15 min (ratio)</td>
</tr>
<tr>
<td>Desensitization (%)</td>
</tr>
</tbody>
</table>

CHO-VR1 cells were treated with 50 µM AA-DO or 50 nM capsaicin for 15 min. The fluorescence ratios of individual cells were measured in intracellular imaging experiments (representative single experiments are shown on Fig. 3). The maximal response during the first 3 min of treatment and the response after 15 min treatment were determined (34–49 cells in each experiment, values are mean ± S.E.M., n is the number of experiments performed), and the desensitization was calculated (value at 15 min as % of maximal value at the first 3 min of treatment from each experiment) (mean value of the ratios ± S.E.M., n is the number of experiments).
Fig. 4. Kinetics of the desensitization. CHO-VR1 cells were preincubated with AA-DO (1–50 μM) or buffer alone (control) for different periods of time (indicated) prior to the capsaicin challenge (50 nM, 5 min). The $^{45}$Ca$^{2+}$ uptake (added with the capsaicin) was plotted as % of control (preincubation with buffer alone). The results from a representative experiment are shown (panel A, average ± S.E.M.). Two additional experiments gave similar results. The values for maximal inhibition determined in the separate experiments are summarized on panel B. The $^{45}$Ca$^{2+}$ uptake is plotted as a function of the time of preincubation with AA-DO (bars ± S.E.M., n = 3).
buffer alone did not affect the capsaicin mediated $^{45}$Ca$^{2+}$ uptake (data not shown), the inhibition must be specific to AA-DO.

**Conclusion**

We conclude that under our conditions AA-DO is a slightly more potent activator of rat VR1 than is anandamide (~ 5 fold) but is far less potent (~ 100 fold) than capsaicin in the functional assays. Furthermore, it is a full agonist on the rat VR1 and able to relatively effectively desensitize the CHO-VR1 cells to further agonist challenge, similar to capsaicin. Finally, the observed desensitization is Ca$^{2+}$ dependent, similar to the desensitization with capsaicin.

**References**


