Anandamide (AEA) is a lipid molecule belonging to the family of endocannabinoids. Various studies report neuroprotective activity of AEA against toxic insults, such as ischemic conditions and excitotoxicity, whereas some show that AEA has pro-apoptotic effects. Here we have shown that AEA confers a protective activity in N18TG2 murine neuroblastoma cells subjected to low serum-induced apoptosis. We have demonstrated that the protection from apoptosis by AEA is not mediated via the CB1 receptor, the CB2 receptor, or the vanilloid receptor 1. Interestingly, breakdown of AEA by fatty acid amide hydrolase is required for the protective effect of AEA. Furthermore, the ethanolamine (EA) generated in this reaction is the metabolite responsible for the protective response. The elevation in the levels of reactive oxygen species during low serum-induced apoptosis is not affected by AEA or EA. On the other hand, AEA and EA reduce caspase 3/7 activity, and AEA attenuates the cleavage of PARP-1. Taken together, our results demonstrate a role for AEA and EA in the protection against low serum-induced apoptosis.

Arachidonoyl ethanolamide (also named anandamide or AEA) is a well-known member of a family of endogenous lipid mediators termed endocannabinoids. A wide range of physiological functions of endocannabinoids was discovered in recent years. It appears that the endocannabinoids play crucial roles in the central and autonomous nervous system, the reproductive system, the cardiovascular system, the gastrointestinal tract, and in microcirculation (1).

The endocannabinoids, as well as plant-derived and synthetic cannabinoids, were shown to activate two distinct G protein-coupled cannabinoid receptors, the CB1 receptor, which is present at high concentrations in the nervous system, and the CB2 receptor, which is located primarily in immune cells (2). The endocannabinoid AEA is also capable of activating the vanilloid receptor 1 (VR1) (3). However, both cannabinoids and endocannabinoids were found to affect various physiological functions by mechanisms that are not dependent upon the receptor subtypes with which they are known to interact.

Evidence has accumulated in recent years in support of involvement of cannabinoids in the modulation of cell fate-related processes, such as proliferation, death, and differentiation (4–6). Some studies report protective activity of the endocannabinoid AEA against apoptosis, whereas others describe AEA as an apoptosis inducer (4). AEA was shown to induce apoptosis in cell lines from different origins, including neuronal cells (7). Pro-apoptotic activity of AEA was documented in primary neurons, in PC-12 pheochromocytoma cells, and in CHP-100 neuroblastoma cells (8–10). On the other hand, neuroprotective activities of AEA have been implicated in various pathological conditions. For example, AEA protects cultured cerebral cortical neurons from the effects of a combination of hypoxia and glucose deprivation, conditions serving as an in vitro model of ischemia (11), and Milton has demonstrated inhibition of β-amyloid toxicity by AEA, claiming a protective role in Alzheimer disease (12).

Interestingly, elevated levels of AEA were reported to accompany both neuronal apoptotic and necrotic cell death (13). Moreover, AEA levels were up-regulated in the hippocampus following glutamate excitotoxicity (14), in models of Alzheimer and Parkinson disease (1), and in focal cerebral ischemia (15). At this stage, it is not clear whether the increase in AEA levels under these conditions could point to a protective effect of AEA or whether it might be part of the mechanism causing the pathological manifestation and/or its consequences.

The mechanisms underlying the involvement of AEA in apoptosis or neuroprotection are not yet understood. The VR1 receptor was reported as a regulator of AEA-induced apoptosis in human cervix cancer cells as well as in human glioma and endothelial cells (16–18). Interestingly, in human cervix cancer and glioma cells, the pro-apoptotic effect of AEA via VR1 was coupled with a protective effect of AEA via the cannabinoid receptors CB1 and CB2 (16, 17). Neuroprotection by AEA has been mainly attributed to CB1 receptor-mediated signaling. However, in some studies, the neuroprotective response to AEA was neither CB1 nor CB2 receptor-dependent (19).

In this report, we have examined the effect of AEA on low serum-induced apoptosis. Using N18TG2 murine neuroblastoma, a cell line known to contain high levels of the CB1 recep-
tor (2, 20, 21), we have shown that AEA reverses the low serum-induced apoptosis and that this effect is not mediated via the CB1 receptor. AEA is degraded in the cells by fatty acid amid hydrolase (FAAH) to arachidonic acid (AA) and ethanolamine (EA) (22). We found that the degradation of AEA by FAAH is a step required for AEA-protective activity and that EA is the degradation product that mediates the protection from low serum-induced apoptosis.

**EXPERIMENTAL PROCEDURES**

**Cell Culture**—The N18TG2 cell line was grown at 37 °C in a humidified atmosphere of 95% air and 5% CO₂ in Dulbecco’s modified Eagle’s medium containing 10% fetal calf serum (FCS). The cells were transferred to Dulbecco's modified Eagle’s medium containing 0.2% FCS for 24–48 h as detailed below.

**Chemicals and Antibodies**—Dulbecco’s modified Eagle’s medium was obtained from Invitrogen. Trypsin/EDTA was purchased from Biological Industries Ltd. (Kibbutz Beit Haemek, Israel). AEA was a kind gift from Prof. R. Mechoulam (The Hebrew University of Jerusalem, Jerusalem, Israel). SR141716, a CB1 receptor antagonist, and SR144528, a CB2 receptor antagonist, were from RTI International (Research Triangle Park, NC). Propidium iodide (PI), AA, EA, N-mono methylethanolamine (MEA), N,N’-dimethylethanolamine (DEA), DNase-free RNase A, fatty acid-free bovine serum albumin, 2′,7′-dichlorodihydrofluorescein diacetate (DCFH-DA), Hoechst 33342, phosphate-buffered saline (PBS), and Hanks’ balanced salt solution (HBSS) were from Sigma. Capsazepine, a VRI receptor antagonist, was from Tocris Cookson (Bristol, UK). URB597, an inhibitor of FAAH, was a generous gift from Dr. D. Piomelli (University of California, Irvine, CA). Stock solutions of AEA, SR141716, SR144528, AA, EA, MEA, and DEA were prepared in ethanol, and aliquots were stored at −80 °C. For experiments, alcoholic solutions were diluted in fatty acid-free bovine serum albumin as described previously (23). Capsazepine and URB597 were prepared in Me₃SO and diluted for experiments in PBS. The mouse monoclonal anti-poly(ADP-ribose) polymerase-1 (PARP-1) antibody and the mouse monoclonal anti-GAPDH antibody were gifts from Profs. Y. Zick and I. Ginzburg (Weizmann Institute of Science, Rehovot, Israel), respectively.

**Hoechst Staining**—Cultured cells were washed twice in PBS and then fixed with 4% paraformaldehyde for 20 min at 37 °C. The cells were washed twice with PBS, incubated with PBS containing 6 μg/ml Hoechst 33342 and 0.1% Triton X-100 for 15 min at room temperature. Cell nuclei were observed by fluorescence microscopy and photographed using an OLYMPUS IX81 microscope.

**Detection of Apoptotic DNA Fragmentation**—DNA was extracted using the Apoptotic DNA Ladder Detection Kit (Chemicon International, Temecula, CA) using their recommended protocol. Briefly, equal numbers of cells were seeded in culture dishes, and apoptosis was induced by low serum treatment for 48 h. The cells were counted, pelleted by centrifugation, washed with PBS, and lysed with Tris-EDTA lysis buffer by gentle pipetting. Lysates were then incubated with DNase-free RNase A at 37 °C for 10 min and with proteinase K at 50 °C for 30 min. Intact and fragmented DNA was precipitated by the addition of ammonium acetate solution and isopropyl alcohol at −20 °C. The DNA pellet was washed with ice-cold 70% ethanol, air-dried, and resuspended in DNA loading Buffer. DNA was then subjected to electrophoresis in a 2% agarose gel containing 0.5 μg/ml ethidium bromide and visualized by transillumination with UV light.

**FACS Analysis of Apoptosis**—Cells were fixed in 70% ethanol-30% HBSS and stored at −20 °C. Cells were then washed once with HBSS, resuspended in HBSS containing 0.2 mg/ml DNase-free RNase A, and incubated at 37 °C for 1 h. This RNase treatment was used to prevent PI binding to double-stranded RNA. Cells were then washed with HBSS and resuspended in HBSS containing 50 μg/ml PI. Cells were analyzed by FACS using the FACSort flow cytometer (excitation at a wavelength of 488 nm and emission at 600 nm) and the CellQuest list mode analysis software (BD Biosciences).

**Detection of Intracellular Reactive Oxygen Species**—Production of reactive oxygen species (ROS) was measured by flow cytometry using the cell-permeable ROS-specific dye DCFH-DA. This compound diffuses into the cell where it is cleaved by intracellular esterases to yieldDCFH, which is trapped within the cell. This nonfluorescent molecule is then oxidized by low molecular weight peroxides to the fluorescent compound dichlorofluorescein. Therefore, the fluorescence intensity measured reflects the amount of ROS present in the cells (24). Following the different treatments, cells were detached with trypsin/EDTA and incubated at 37 °C for 30 min with 5 μM DCFH-DA in the appropriate medium for each treatment (10 or 0.2% FCS). The cells were then washed once with PBS and analyzed by FACS using the FACSort flow cytometer (excitation at a wavelength of 488 nm and emission at 525 nm) and the CellQuest list mode analysis software.

**Caspase 3/7 Assay**—Caspase 3/7 activity was determined by the Apo-ONE™ homogeneous caspase-3/7 assay kit according to the manufacturer’s protocol (Promega, Madison, WI). This assay is based on the cleavage of the substrate benzoyloxy carbonyl-DEVD-rhodamine 110 by caspases 3 or 7 and the analysis of the amount of fluorescent rhodamine liberated. Briefly, following treatment, the cells were harvested, and equal numbers of cells (1 × 10⁵) were resuspended in 50 μl of medium containing either 10 or 0.2% FCS, according to the treatment before harvesting and loaded into each well of a black 96-well plate. Lysis buffer and the substrate (benzoyloxy carbonyl-DEVD-rhodamine 110) were added to the cells and incubated at room temperature for 2 h. The amount of rhodamine 110-leaving group was determined by excitation at 499 nm and measuring the emission at 521 nm. The amount of fluorescent product generated is proportional to the amount of caspase-3/7 cleavage activity present in the samples.

**Western Blot Analysis**—Cells were lysed in lysis buffer (50 mM Tris–HCl, pH 7.5, 100 mM NaCl, 1% Triton X-100, 0.5% sodium-deoxycholate, 0.1% SDS), and the amount of protein in each sample was normalized by the Bradford assay (Bio-Rad). Laemml sample buffer was then added, and the samples were boiled for 10 min and loaded on SDS-10% polyacrylamide gels. Following gel electrophoresis, the proteins were transferred to nitrocellulose membranes using a semidy transfer cell (Bio-
Rad) and the blots incubated with anti-PARP-1 and anti-
GAPDH antibodies. The protein-antibody complexes were
detected using a goat anti-mouse horseradish peroxidase-con-
jugated secondary antibody (Jackson ImmunoResearch Labo-
ratories, West Grove, PA) and the Super-signal-enhanced
chemiluminescence assay system (Pierce).

Data Analysis—Data are presented as means ± S.D. of three
independent experiments, each performed in duplicate. Results
were analyzed using Student’s t test (Figs. 2–6 and 8; ▲▲, p < 0.01 compared with nontreated control (NT); ●●, p < 0.05;
▲▲▲, p < 0.01 compared with vehicle).

RESULTS
AEA Protects N18TG2 Cells from Low Serum-induced
Apoptosis—Studies were performed using N18TG2 neuroblas-
toma cells, a murine cell line known to contain a relatively high
amount of the CB1 receptor (2, 20, 21) and from which the CB1
receptor was cloned. Apoptosis was induced in N18TG2 neu-
roblastoma cells by replacing the normal growth medium (con-
taining 10% FCS) with low serum medium (containing 0.2%
FCS). AEA was added, unless otherwise indicated, concomi-
tantly with the transfer of the cells to the low serum conditions.
Staining with Hoechst 33342 (Fig. 1A) revealed that 36 h in low
serum-containing medium induced apoptosis of the cells, man-
ifested in an increased number of cells with condensed nuclei.
Interestingly, treatment with AEA protected the cells from low
serum-induced apoptosis. To verify that the DNA condensa-
tion observed using the Hoechst staining was indeed due to
apoptosis, we have analyzed chromosomal DNA fragmentation
by agarose gel electrophoresis. As shown in Fig. 1B, the low
serum treatment induced the formation of distinct internucleo-
somal DNA fragmentation, one of the hallmarks of apoptosis.

On the other hand, the addition of AEA to the 0.2% FCS-con-
taining medium significantly inhibited the DNA fragmenta-
tion. To quantify the extent of AEA-induced protection, we
evaluated the percentage of cell apoptosis by FACS-based stain-
ing with PI. Using this technique, we found that, under normal
growth conditions, the addition of AEA to the medium did not
affect cell cycle distribution (Fig. 2A). However, when the
N18TG2 cells were grown in low serum for 48 h, a large sub-G1
population was detected, indicating that a significant fraction of
the cells underwent apoptosis. Interestingly, the addition of
AEA to the low serum-containing medium produced a pro-
nounced protection from apoptosis. In the experiment shown
in Fig. 2A, although the percentage of cells undergoing apop-
tosis following 48 h in 0.2% FCS-containing medium was 78.2%,
the addition of 1 μM AEA to the low serum medium lowered the
apoptosis percentage to 21.4%. We then found (Fig. 2B) that
AEA protects the N18TG2 cells from apoptosis in a dose-de-
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Protection from Apoptosis by AEA Is Cannabinoid and Vanilloid Receptor-independent—As described above, CB1 receptors were previously characterized in N18TG2 cells (2, 20, 21). However, there is no documentation of CB2 or VR1 expression in these cells. Because AEA is known to interact with all of these receptor types, it was of interest to examine their possible involvement in the modulation of apoptosis by AEA. To this end, we first applied the CB1-specific antagonist, SR141716, and the CB2 receptor antagonist SR144528, before the addition of AEA. As can be seen in Fig. 3A, these antagonists had no effect on cell apoptosis by themselves. Moreover, AEA elicited similar protection against apoptosis in the presence of SR141716 or SR144528 as that observed when added alone, ruling out the possible involvement of either CB1 or CB2 receptors in this process.

We next investigated whether VR1 could be involved in the AEA-induced anti-apoptotic effect. Capsazepine, a VR1 antagonist, did not affect the protective activity of AEA against low serum-mediated apoptosis (Fig. 3B). Thus, the VR1 receptor is not involved in the AEA protective activity. Taken together, AEA protective effect in N18TG2 cells maintained in low serum conditions is cannabinoid and vanilloid receptor-independent.

Metabolism of AEA to Ethanolamine Is a Critical Step in AEA-induced Protection—FAAH is a key enzyme in the enzymatic degradation of AEA to AA and EA (22, 25). We utilized the FAAH inhibitor URB597 (26) to address the possible requirement of AEA degradation for AEA anti-apoptotic activity. We found that blockade of FAAH activity by pretreatment with URB597 resulted in loss of AEA-protective effect (Fig. 4). Thus, we concluded that the activity of FAAH is critical for AEA-mediated protection.

In light of these observations, it was of interest to investigate which of the AEA metabolites generated by FAAH is required for the protection from apoptosis. Therefore, following the induction of apoptosis by low serum, we treated the cells with either, AEA, AA, or EA (each at 1 μM). Interestingly, the EA produced a comparable decrease in apoptosis to AEA (Fig. 5A). The AA (at 1 μM concentration) had no effect on low serum-induced apoptosis. To further confirm the differential effects of EA and AA, we conducted dose-response experiments with EA and AA. As can be seen in Fig. 5B, treatment with AA did not affect low serum-induced apoptosis at the concentration range of 0.5–2.5 μM, whereas 5 μM AA produced some protection (of 28% of the apoptotic control). On the contrary, treatment with EA caused a decrease in low serum-induced apoptosis of N18TG2 cells already at 0.5 μM, reaching a very prominent

FIGURE 3. The protective effect of AEA is cannabinoid and vanilloid receptor-independent. Percentage of apoptotic cells determined by flow-cytometric analysis of PI staining of N18TG2 cells treated as indicated for 48 h. A, the CB1 receptor antagonist SR141716 and the CB2 receptor antagonist SR144528 were added at the time of serum reduction, 30 min before the addition of AEA. B, the VR1 antagonist capsazepine (CAPZ) was applied 10 min prior to the addition of AEA.

FIGURE 4. Degradation of AEA by FAAH is required for an AEA-induced protective effect. Percentage of apoptotic cells (after 48 h), as measured by FACS analysis of PI staining. The specific FAAH inhibitor URB597 was added to N18TG2 cells at the time of serum reduction, 10 min prior to the addition of AEA.
percentage of protection (−70%) at the concentration range of 1–5 μM. These results show that the EA produced from AEA acts as the major mediator of AEA-induced protection from apoptosis in these cells and that the AA produced has a very modest effect.

Several studies have demonstrated protection against apoptosis by the methylated EA analogues MEA and DEA (27–29). Therefore, we examined the effect of these methyl derivatives of EA on low serum-induced apoptosis. As can be seen in Fig. 6, although 5 μM AEA or EA almost fully protected the cells from apoptosis, the methyl derivatives of EA at this concentration exerted no protection.

**AEA and EA Do Not Reduce ROS Production**—The levels of ROS are elevated during serum deprivation-induced apoptosis (30). Therefore, we tested whether AEA protection against apoptosis is mediated via modulation of ROS levels. The N18TG2 cells were transferred to low serum-containing medium, treated with AEA or EA, and assayed for ROS production using the DCFH-DA dye, known to be oxidized by H₂O₂ and low molecular weight peroxides. The amount of oxidized fluorescent dichlorofluorescein generated by this reaction was measured by flow cytometry. As can be seen in Fig. 7, ROS production was significantly increased following 6 h of incubation in low serum-containing medium. The exposure to 5 μM AEA or EA, a concentration that rescues the N18TG2 cells from apoptosis, did not affect the levels of intracellular ROS. The same result was observed when the cells were maintained in low serum for 24 h. ROS levels remained high and were not influenced by the addition of AEA or EA (data not shown). In conclusion, AEA anti-apoptotic effect is not mediated by regulation of ROS production.

**AEA Attenuates Low Serum-induced PARP-1 Cleavage and Caspase 3/7 Activity**—We next examined the effect of AEA on the modulation of the proteolytic cleavage of the nuclear enzyme PARP-1, which is one of the hallmarks of apoptosis. PARP-1 is activated at an intermediate stage of apoptosis and is

![Figure 5. EA is the AEA metabolite responsible for AEA anti-apoptotic activity. FACS analysis of apoptosis following 48 h in 0.2% FCS. A, in the presence of the indicated compounds at 1 μM. B, with increasing concentrations of EA (●) or AA (▲). The 0.2% FCS containing vehicle is marked by ●.](image)

![Figure 6. Effects of EA derivatives on low serum-induced apoptosis. Effect of AEA, EA, MEA, and DEA (at 5 μM) on 48 h of 0.2% FCS-induced apoptosis.](image)

![Figure 7. ROS production is not affected by AEA or EA. Levels of ROS in N18TG2 cells treated as indicated for 6 h were measured by flow cytometry with the DCFH-DA dye as described under “Experimental Procedures.” The number of cells is plotted versus the dichlorofluorescein fluorescence detected by the FL-1 channel. The percentage of cells containing peroxides appears in the center of each histogram.](image)
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We performed Western blot analysis of N18TG2 cellular extracts using a monoclonal antibody that cross-reacts with both the non-cleaved and cleaved forms of PARP-1. Utilizing this antibody, we found that low serum conditions resulted in the formation of the cleaved 85-kDa fragment of the PARP enzyme (Fig. 8A). The cleaved product was detected at 48 h of incubation in low serum, in agreement with the notion that PARP-1 cleavage is a late event in the apoptotic pathway. When the cells were treated with AEA following the transfer to low serum, the level of cleaved PARP-1 was significantly lower. Thus, the protection by AEA could be mediated via the attenuation of the cleavage and thereby the inactivation of PARP-1.

It is known that PARP-1 is cleaved during apoptosis by caspases 3 and 7, which are activated at an earlier stage in apoptosis (31). Therefore, we examined the effect of AEA and EA on the activity of these enzymes. Caspase 3/7 activity was measured by a quantitative fluorimetric assay. Because caspase 3/7 activation precedes PARP-1 cleavage, we examined the activity of these caspases at 24 h. As shown in Fig. 8B, 24 h in low serum-containing medium caused a large increase in caspase 3/7 activity. Conversely, the addition of 5 μM AEA or EA lowered the activity of caspase 3/7 almost to the level of non-treated cells. In conclusion, AEA- and EA-induced protection in N18TG2 cells seems to be mediated by the reduction of caspase 3/7 activity.

**DISCUSSION**

In this report, we have demonstrated that AEA protects N18TG2 neuroblastoma cells from low serum-mediated apoptosis. We show that the protection conferred by AEA is independent of the activation of either the CB1 or CB2 cannabinoid receptors or of VR1. We found that AEA neuroprotection is dependent on FAAH activity and that the EA generated by AEA degradation is the active compound, exerting protective effect in a dose-dependent manner. Interestingly, AEA was previously documented as a protective agent against various toxic insults (32). Our data show for the first time a role for AEA in the protection from low serum-induced apoptosis.

On the other hand, AEA was documented as an apoptotic agent in several other cell types. The apoptotic effects of AEA in cells from neuronal origin were demonstrated in primary neurons, in PC-12 pheochromocytoma cells, and in CHP-100 neuroblastoma cells (8–10). However, in these experiments, the concentrations of AEA were significantly higher than those applied in our study. Sarker et al. (10) have reported an AEA dose-dependent loss of PC12 cell viability under conditions of regular serum percentage (10% horse serum plus 5% fetal bovine serum), showing no effect on cell viability at AEA concentrations <7.5 μM but a massive apoptotic cell death at 10 μM AEA. Movsesyan et al. (9) have demonstrated that AEA exerts neurotoxic effects in rat cortical neurons and in cerebellar granular cell cultures at concentrations of 25 and 10 μM AEA, respectively. Lower AEA concentrations were found to have no effect on cell survival. It should also be noted that the effect of AEA could vary between different cell types as well as on the conditions used to induce cell apoptosis. For example, one of the parameters that may affect the outcome of AEA treatment is the activity of FAAH in the cell type investigated. High activity of FAAH may lead to AEA-induced protection from low serum-induced apoptosis, as observed using the N18TG2 cells in our study. On the other hand, under regular serum conditions and especially with cells with low FAAH activity, AEA could have neurotoxic effects, for example via the activation of CB1 or the VR1 receptors (10, 16–18).

Low serum conditions are considered an appropriate model under which to study the effect of nutrient deprivation in the tumor microenvironment. Cancer cells have adopted various anti-apoptotic mechanisms to overcome stressful conditions. Elevated levels of AEA were detected in different human tumors (38–40), suggesting that AEA up-regulation provides cancer cells with a possible protective solution. Our finding of anti-apoptotic activity of AEA under conditions of low serum further supports this hypothesis. It should also be noted that, in most tumors, the cellular level of phospho-EA (generated from EA), is higher than in normal tissues (41, 42). In light of our finding of protection by AEA, due to its degradation to EA, the
elevation in the levels of AEA and phospho-EA in tumors could be interrelated.

The degradation of AEA by FAAH is considered responsible for the inactivation of AEA signaling. However, this step may actually initiate a new cascade of events leading to neuroprotection. We have shown here that the EA generated in this reaction is the molecule required for protection. The AA generated in parallel had no significant effect on low serum-induced apoptosis at 1–2.5 μM concentration. However, at higher concentration (i.e. 5 μM), AA provided a mild protection from apoptosis. Interestingly, AA was found to have the same effect as AEA on the stimulation of cell growth in the B9 murine intercellulin-6-dependent hybridoma cell line (43).

Although the signaling pathways of AEA have been recognized and investigated (44), much less is known about the possible signaling cascade(s) mediated by EA. EA was shown to potentiate or inhibit (depending on the conditions) the synergetic effects of phosphocholine with either insulin or ATP/sphingosine 1-phosphate on DNA synthesis in NIH 3T3 cells (45–47). Malewicz et al. (46) have demonstrated that increased phosphorylation of EA decreases the potentiating effect of this molecule on insulin-induced DNA synthesis and increases its ability to promote cell survival in serum-free medium. Our present findings further support a role for EA as a signaling molecule involved in cell fate modulation.

We found that, at 5 μM (a concentration in which AEA and EA produce high protection against low serum-induced apoptosis), the N-methyl derivatives of EA, MEA, and DEA did not affect low serum-induced apoptosis. This difference between the effects of EA versus MEA and DEA suggests that EA-mediated protection from low serum is a highly specific process. On the other hand, Brand et al. (27) have reported a concentration and time-dependent neuroprotective effect of MEA and DEA in oligodendroglial cells from oxidative stress-induced apoptosis. Similarly, DEA protected hematopoietic progenitor cells and murine L1210 leukemia cells from cytotoxicity of the chemotherapeutic agent mechlorethamine (28). Moreover, DEA promoted DNA synthesis of NIH-3T3 fibroblast cells in serum-free conditions, and both DEA and MEA enhanced insulin-induced DNA synthesis (29). However, it should be noted that, in these studies, the effects of MEA and DEA were examined at very high concentrations (at the mM range) and in different cell types.

The molecular targets in AEA- and EA-mediated protection from apoptosis are not known. We first examined the effect of AEA and EA on ROS production. The induction of low serum-induced apoptosis in N18TG2 cells was accompanied by significant up-regulation of intracellular ROS levels. We found that AEA and EA rescue from low serum-induced apoptosis without decreasing ROS levels, suggesting that these compounds inhibit apoptosis via a different mechanism. Protection against apoptosis without decreasing ROS levels was previously reported for several survival factors, such as epidermal growth factor, nerve growth factor, and bcl-2 (30). Moreover, although ROS levels were not affected by AEA, the cleavage of PARP-1 by caspase-3 and caspase-7 (31). Indeed, we found that the activity of caspase 3/7, which is highly increased by the reduction in serum concentration, is decreased to almost background level by AEA and EA. These findings demonstrate that EA signaling for survival involves the regulation of PARP-1 cleavage and caspase 3/7 activation.

In summary, we have shown that AEA exerts a protective effect against low serum-induced apoptosis. The protection by AEA does not involve cannabinoid or vanilloid receptor activation. It is executed by FAAH-mediated degradation to EA, which is the molecule that confers the protective activity. This protection is mediated via a large decrease in the low serum-induced activation of caspase 3/7 and cleavage of PARP-1.

Acknowledgments—We thank Drs. R. Mechoulam (Hebrew University of Jerusalem) and D. Piomelli (University of California) for their donation of cannabinoid ligands and URB597, respectively.
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