Stress-induced generation of *N*-acylethanolamines in mouse epidermal JB6 P⁺ cells

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It has long been known that *N*-acylethanolamine phospholipids [*N*-acylphosphatidylethanolamine (*N*-acyl PE)] and *N*-acylethanolamines (NAEs) accumulate in mammalian tissues undergoing degenerative membrane changes associated with necrosis. Here we studied the effects of stress factors (UVB irradiation and serum deprivation) on the endogenous levels of *N*-acyl PE and NAE in mouse epidermal JB6 P⁺ cells. We found that 16:0, 18:0, 18:1,*n*–9 and 18:1,*n*–7 are the predominant amide-linked fatty acids in both *N*-acyl PE and NAE in these cells. UVB irradiation and serum deprivation resulted in significantly increased levels of *N*-acyl PE and NAE, especially 18:1,*n*–9 *N*-acyl PE and NAE. UVB challenge increased the cellular

content of anandamide (20:4,*n*–6 NAE), but this increase was the lowest of all NAEs measured. Serum deprivation resulted in a decreased cellular anandamide level, as well as a decrease in 20:4,*n*–6 *N*-acyl PE. Interestingly, the replacement of serum-free medium with medium containing 5% (v/v) fetal calf serum after 36 h of serum deprivation restored *N*-acyl PE and NAE levels almost completely within 4–8 h. These data suggest the involvement of *N*-acyl PE and NAE in cellular responses to stress.

Key words: *N*-acylphosphatidylethanolamine, starvation, UV irradiation.

INTRODUCTION

It is now well established that long-chain *N*-acylethanolamines (NAEs) and their precursors, *N*-acylethanolamine phospholipids [*N*-acylphosphatidylethanolamine (*N*-acyl PE)], can accumulate in mammalian tissues as the result of injury (reviewed in [1]). Under conditions of necrosis, such as in myocardial infarction where extensive membrane degradation occurs, this accumulation can be massive [2,3]. However, pronounced increases in these lipids are also seen in post-decapitative cerebral ischaemia [4], glutamate-induced neuronal cytotoxicity [5,6] and cadmium-induced testicular inflammation [7]. In fact, maintenance *post mortem* of various mammalian tissues, especially brain [8], can lead to the generation and accumulation of both NAE and *N*-acyl PE.

Because both *N*-acyl PE [9–11] and NAE [12] exhibit membrane-stabilizing effects *in vitro*, and NAE was shown to inhibit the injury-induced mitochondrial permeability transition [13], the ‘transacylation/phosphodiesterase pathway’ leading to the formation of *N*-acyl PE and NAE [14–16] was proposed to represent a sequence of reactions designed to minimize injury and promote cell survival [1]. This concept was supported by the observation that 16:0 NAE actually protected cerebellar granule neurons against glutamate-induced excitotoxic death [17]. However, the levels of *N*-acyl PE and NAE in intact cells and tissues are extremely low and, hence, it is difficult to interpret their function in terms of their physical interaction with biological membranes.

With the discovery of ‘anandamide’ (*N*-arachidonylethanolamine; 20:4,*n*–6 NAE) as a potential endogenous cannabinoid-receptor agonist [18,19], NAE production and

degradation became of great interest in terms of receptor-mediated signal transduction, especially when it was shown [20–22] that the transacylation/phosphodiesterase pathway may be involved in anandamide biosynthesis (reviewed in [23]). However, this reaction sequence begins with a Ca²⁺-dependent energy-independent transacylation of *O*-acyl groups from the *sn*-1 position of various glycerophospholipids to the amino group of PE [14], followed by phosphodiesterase cleavage of *N*-acyl PE to NAE and phosphatidic acid catalysed by a membrane-bound phospholipase D [16]. Because arachidonic acid and other polyunsaturated fatty acids are esterified primarily at the *sn*-2 position of glycerophospholipids, and because neither the transacylase nor the phospholipase D appear to exhibit any substrate specificity with respect to *O*-acyl- or *N*-acyl-linked fatty acids, anandamide and other polyunsaturated NAEs usually represent only a very small proportion of total NAE [8,21,22,24,25].

Saturated, together with mono- and di-unsaturated, NAEs not only represent the overwhelming majority of cellular NAEs, but they are ubiquitous constituents of mammalian cells and tissues. Since they do not bind to and activate cannabinoid receptors [23], it is of great interest to determine whether or not their intracellular levels are regulated and whether they mediate cellular signalling.

In order to address these questions, we have chosen cultured mouse epidermal JB6 P⁺ cells as a model. These cells are well characterized in terms of their cellular signalling machinery. They can be subjected to neoplastic transformation, and they have served as models for the study of responses to various stress conditions, including UV irradiation [26–28]. Exposure of these and other mammalian cells to UV irradiation not only causes DNA damage, resulting in either cell death or somatic mutation,

Abbreviations used: NAE, *N*-acylethanolamine; *N*-acyl PE, *N*-acylphosphatidylethanolamine (natural *N*-acyl PE may consist of diacyl- as well as alkylacyl- and alk-1-enylacylglycerol subclasses); EMEM, Eagle’s minimal essential medium; FBS, fetal bovine serum.

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but also induces specific cellular reactions, such as transcriptional activation of AP-1 (activator protein-1) and NF- κ B (nuclear factor- κ B) [29–31].

Here we report for the first time that the levels of *N*-acyl PE and NAE in intact viable cells respond to stress and may be subject to regulation as part of an intracellular signalling system.

MATERIALS AND METHODS

Reagents

Eagle's minimal essential medium (EMEM) was from Life Technologies Inc. Fetal bovine serum (FBS) was from BioWhittaker, Life Technologies Inc. and Gemini Bio-products Inc. *N*-Heptadecanoyl PE and deuterated ($^2\text{H}_4$) derivatives of 16:0, 18:0, 18:1,*n*–9, 18:2,*n*–6 and 20:4,*n*–6 NAE were prepared as described previously [8,24].

Table 1 *N*-Acyl PE and NAE composition of JB6 P⁺ cells

Cells were grown in EMEM containing 5% (v/v) FBS. The medium was removed before lipid extraction, and lipids were extracted and processed as described in the Materials and methods section. Values (pmol/ μ mol of lipid phosphorus) represent means $\pm$ S.E.M. of ten samples taken from three different experiments.

<i>N</i> -Acyl	<i>N</i> -Acyl PE		NAE	
	(pmol/ μ mol)	(%)	(pmol/ μ mol)	(%)
16:0	85.62 $\pm$ 2.75	47.6	18.32 $\pm$ 0.91	30.3
18:0	42.84 $\pm$ 1.59	23.8	22.89 $\pm$ 0.95	37.9
18:1, <i>n</i> –9	21.02 $\pm$ 0.98	11.7	7.36 $\pm$ 0.81	12.2
18:1, <i>n</i> –7	27.01 $\pm$ 0.55	15.0	8.06 $\pm$ 0.67	13.3
18:2, <i>n</i> –6	1.71 $\pm$ 0.06	1.0	1.06 $\pm$ 0.11	1.8
20:4, <i>n</i> –6	1.54 $\pm$ 0.12	0.9	2.71 $\pm$ 0.44	4.5
Total	178.42 $\pm$ 5.98		57.92 $\pm$ 2.52	

Table 2 *N*-Acyl PE and NAE composition of commercial FBS

Serum samples (1 ml) were extracted [32] and lipids were processed as described in the Materials and methods section. Values represent means of two determinations. N.D., not detected.

<i>N</i> -Acyl	Amount (pmol/ml)					
	Gemini Bio-products			BioWhittaker	Gibco BRL	
	Lot A1745N	Lot A1756N	Lot A1301L	Lot 5M0992	Lot 1007762	Lot 1010711
<i>N</i> -Acyl PE						
16:0	42.84	65.78	305.62	265.86	195.58	236.77
18:0	15.05	23.37	118.14	100.70	47.55	40.50
18:1, <i>n</i> –9	13.81	22.00	112.94	97.65	73.86	83.61
18:1, <i>n</i> –7	14.63	25.83	133.92	117.26	99.00	122.70
18:2, <i>n</i> –6	1.94	1.87	6.93	5.90	5.27	5.70
20:4, <i>n</i> –6	1.75	2.81	14.86	11.67	12.89	16.42
Total	90.02	141.66	692.41	599.04	434.15	505.70
NAE						
16:0	25.26	18.51	18.90	27.59	48.82	48.90
18:0	12.73	8.32	7.19	8.68	13.40	14.29
18:1, <i>n</i> –9	30.88	1.35	4.12	2.96	4.84	13.59
18:1, <i>n</i> –7	N.D.	1.09	8.22	16.00	27.53	25.04
18:2, <i>n</i> –6	0.87	0.20	1.42	0.28	0.49	2.88
20:4, <i>n</i> –6	N.D.	0.66	1.90	0.82	1.60	3.62
Total	69.66	30.13	41.75	56.33	96.68	108.32

Cell culture

Mouse epidermal JB6 P⁺ cells were cultured in EMEM containing 5% (v/v) FBS, 2 mM L-glutamine and 50 μ g of gentamicin/ml. Cells were grown to complete confluence at 37 °C in a humidified 5% CO₂ atmosphere, then trypsinized and seeded at 2×10^6 cells per 10 cm Petri dish. After the cells had reached 95% confluence, the medium was replaced with EMEM not containing FBS (serum deprivation experiments) and cells were starved for up to 48 h. For the UVB irradiation experiments, the medium was replaced with medium containing 0.1% (v/v) FBS, and cells were exposed to UVB light (290–320 nm; 4 kJ/m²; 16 min) 1 h after medium replacement and then cultured at 37 °C in a humidified 5% CO₂ atmosphere for the indicated time periods.

Lipid extraction, sample preparation and analysis

Cell lipids were extracted by the Bligh and Dyer procedure [32] after removing the media (serum deprivation experiments) or together with the media (UVB experiments). At the beginning of each extraction procedure, the internal standards (20 ng of each [$^2\text{H}_4$]NAE; 0.5 nmol of C₁₇ *N*-acyl PE) were added to the samples. Aliquots of the final extract were taken for total lipid phosphorus assay [33]. NAEs and *N*-acyl PEs were isolated using silica-gel solid-phase extraction cartridges with chloroform/methanol mixtures of 49:1 and 4:1 (v/v) respectively. *N*-Acyl PE samples were digested with phospholipase D (from *Streptomyces chromofuscus*) [34] and the resulting NAEs were separated by solid-phase extraction chromatography. NAE samples were converted into t-butyltrimethylsilyl derivatives and analysed by GC-MS in selected ion monitoring mode as described [24].

RESULTS

N-Acyl PE and NAE are present in JB6 P⁺ cells

As expected, JB6 P⁺ cells were found to contain low levels of *N*-acyl PE and NAE, the *N*-acyl compositions of which are listed in Table 1. Both total levels and *N*-acyl compositions of *N*-acyl PE and NAE were reproducible among different cell preparations.

Figure 1 Effects of UVB irradiation on the levels of total *N*-acyl PE (A) and NAE (B) in JB6 P⁺ cells

Cells were grown in EMEM containing 5% (v/v) FBS. After achieving 95% cell confluence, the medium was poured off and replaced with medium containing 0.1% (v/v) FBS. After 1 h, cells were exposed to UVB irradiation (4 kJ/m² for 16 min). After the indicated time periods, lipids were extracted from cells together with the media and processed as described in the Materials and methods section. Values represent means $\pm$ S.E.M. of three independent experiments, each carried out at least in duplicate at each time point. Significance of differences compared with control values at corresponding times: **P* < 0.05, ***P* < 0.01.

Figure 2 Effects of UVB irradiation on the levels of individual *N*-acyl PEs in JB6 P⁺ cells

Conditions were as described in the legend to Figure 1. Significance of differences compared with control values at 0 h: **P* < 0.05.

N-Acyl PE represented less than 0.02% of total phospholipids, and its level was about 3-fold higher than that of NAE. In each case, amide-linked arachidonic acid was a minor component, amounting to 0.9% of all amide-linked fatty acids in *N*-acyl PE and 4.5% of those in NAE.

Figure 3 Effects of UVB irradiation on the levels of individual NAEs in JB6 P⁺ cells

Conditions were as described in the legend to Figure 1. Significance of differences compared with control values at 0 h: **P* < 0.05, ***P* < 0.01.

Experimentally relevant levels of *N*-acyl PE and NAE are present in serum-containing cell culture media

Because of the ubiquitous presence, including in rat blood plasma [35], of the lipid amides in question and because of their low intracellular levels in JB6 P⁺ cells, we were concerned about the presence and potential effects of *N*-acyl PE and NAE in serum-containing media. Indeed, analysis of commercial preparations of FBS revealed appreciable amounts of both *N*-acyl PE and NAE with compositions not unlike those observed in cells. Again, the majority of the amide-linked fatty acids were saturated and monounsaturated (Table 2).

The data of Table 2 also show widely different amounts of *N*-acyl PE (90–600 pmol/ml) and NAE (30–108 pmol/ml) in different FBS preparations. We used serum from BioWhittaker in our experiments (0.5 ml per dish). Hence incubation in serum-containing media provided about 300 pmol of *N*-acyl PE and 28 pmol of NAE per dish. A typical cellular content (0.3 μ mol of lipid P per dish) would be 54 pmol of *N*-acyl PE and 17 pmol of NAE per dish (calculated from Table 1).

In spite of their relative abundance in the incubation media, cellular metabolism of *N*-acyl PE and NAE appeared to proceed independently. We observed that the levels and compositions of *N*-acyl PE and NAE in the various media remained unchanged during incubation of cells, and appeared to have no effect on cellular *N*-acyl PE and NAE levels and compositions over a 48 h period. However, prolonged incubation (48 h) of JB6 P⁺ cells in serum-free media resulted in a measurable accumulation of *N*-acyl PE and NAE in the medium (see below). Also, the UVB irradiation experiments were carried out in medium containing only 0.1% (v/v) FBS.

Exposure of JB6 P⁺ cells to UVB irradiation results in elevated levels of *N*-acyl PE and NAE

When JB6 P⁺ cells were irradiated with UVB light (4 kJ/m²) for 16 min, severe cell damage occurred, leading to cell death through an apoptotic mechanism [28], as confirmed by DNA laddering

Figure 4 Effects of serum deprivation on total *N*-acyl PE and NAE levels in JB6 P⁺ cells

Cells were grown in EMEM containing 5% (v/v) FBS. After achieving 95% cell confluence, the medium was poured off and replaced by a medium without serum; cells were starved for up to 48 h (●, ■). Some cells were returned to medium containing 5% (v/v) FBS (○, □) after 36 h of starvation (indicated by arrow). Lipids were extracted from cells only and were processed as described in the Materials and methods section. Values represent means $\pm$ S.E.M. ($n = 4-16$). Significance of differences compared with control values at 0 h: * $P < 0.05$, *** $P < 0.001$.

Figure 5 Effects of serum deprivation on individual *N*-acyl PEs in JB6 P⁺ cells

Conditions were as described in the legend to Figure 4.

(results not shown). There was a slight increase in NAE levels at 1 and 6 h after irradiation, and very marked increases in both total NAE (4.8-fold) and *N*-acyl PE (2.9-fold) after 14 h (Figure 1). These increases occurred mainly among the saturated, mono-

Figure 6 Effects of serum deprivation on individual NAEs in JB6 P⁺ cells

Conditions were as described in the legend to Figure 4.

and di-unsaturated *N*-acyl groups, whereas *N*-arachidonate (20:4, $n-6$) increased only moderately (Figures 2 and 3). It therefore appears that, during the apoptotic process initiated by UVB irradiation, NAE accumulates via *N*-acyl PE through the transacylation/phosphodiesterase pathway, and that anandamide is not a major product of this reaction sequence.

Serum deprivation increases cellular NAE and *N*-acyl PE levels in a reversible manner

Because experimental conditions for studying alterations in the intracellular machinery triggered by UV irradiation or other agonists that initiate apoptosis require initial periods of serum deprivation, it was of interest to investigate the effects of serum deprivation itself. In fact, the data of Figure 1 show that increases in NAE and *N*-acyl PE levels occurred not only in UVB-exposed cells but, to some extent, in control cells as well.

In systematic studies aimed at correlating cellular *N*-acyl PE and NAE levels with the stress induced by serum deprivation, we observed increased levels of NAE after 12, 24 and 36 h, followed by a very sharp increase after 48 h of serum deprivation. *N*-Acyl PE levels decreased initially, but exhibited a sharp increase after 48 h as well (Figure 4). As observed with UVB-induced stress, these increases were mostly due to saturated, as well as mono- and di-unsaturated, *N*-acyl groups, with little change or actual decreases seen in anandamide (20:4, $n-6$ NAE) and the corresponding *N*-acyl PE (Figures 5–7).

Perhaps most importantly, the changes in NAE and *N*-acyl PE levels induced by 36 h of serum deprivation were fully reversible on continued incubation of the cells in medium supplemented

Figure 7 Effects of serum deprivation on individual *N*-acyl PEs and NAEs containing amide-linked arachidonic acid

Conditions were as described in the legend to Figure 4.

with 5% (v/v) fetal calf serum, and the sharp rise in the levels of both NAE and *N*-acyl PE seen after 48 h of serum deprivation was completely prevented when serum was added after 36 h (Figures 4–7). Again, anandamide (20:4,*n*–6 NAE) appeared to respond differently from the saturated and monounsaturated NAEs.

Serum-deprived cells release NAE and *N*-acyl PE into the media

When both cells and media were analysed after prolonged incubation under serum-free conditions, we observed increased levels of *N*-acyl PE and NAE not only in the cells but in the media as well (Table 3). At this time, the reason for and the

mechanism of *N*-acyl PE and NAE release into the medium remain unclear.

DISCUSSION

We have shown here that cultured JB6 P⁺ cells respond to UVB irradiation and serum deprivation with the production of increased levels of NAE and its precursor, *N*-acyl PE. In the case of serum deprivation this phenomenon is fully reversible. The JB6 P⁺ cells used in our experiments are sensitive to tumour promoters and have therefore been used extensively for the study of neoplastic transformation induced by UVB irradiation or treatment with other agonists [36,37]. They are also known to undergo apoptosis as a result of UVB irradiation and prolonged serum deprivation [38].

Because our assay method is based on the use of deuterated internal standards, it is well suited for comparisons between the major cannabinoid-receptor agonist anandamide (20:4,*n*–6 NAE) and the receptor-inactive saturated, as well as mono- and di-unsaturated, NAEs. Even though other polyunsaturated NAEs may be present in trace amounts [39], they would not be assayed by our method due to the absence of internal standards. However, our data show clearly that both anandamide and its precursor, *N*-arachidonoyl PE, respond differently from the other NAEs to UVB-induced stress (Figures 1–3) and starvation (Figures 4–7). Because the major changes in NAE levels are seen in the saturated and monounsaturated NAEs and not in anandamide (20:4,*n*–6 NAE), a trace NAE constituent, cannabinoid-receptor signalling is most probably not involved. Whether or not NAE-mediated signalling is involved in injury-induced apoptosis will require further work. However, our present results suggest that this may be the case. Interestingly, anandamide was shown to act as a growth factor in haematopoietic cells [40,41], but does so via a cannabinoid-receptor-independent pathway [41]. If anandamide can elicit biological effects with-

Table 3 Release of *N*-acyl PE and NAE from JB6 P⁺ cells into the serum-free medium during starvation

Cells were grown in EMEM containing 5% (v/v) FBS up to 95% confluence. Then the medium was replaced with serum-free medium and cells were starved for up to 48 h. Samples of medium were removed at 24 and 48 h and centrifuged at 1500 *g* for 10 min, and lipids were extracted from cells and from 7.5 ml aliquots of medium [32], and processed as described in the Materials and methods section. N.D., not detected.

<i>N</i> -Acyl	Amount (pmol/dish)					
	0 h		24 h		48 h	
	Medium	Cells	Medium	Cells	Medium	Cells
<i>N</i> -Acyl PE						
16:0	1.52	22.25	4.08	21.02	17.71	51.90
18:0	1.94	10.18	3.71	12.16	15.41	55.63
18:1, <i>n</i> –9	0.36	4.82	1.80	5.26	8.59	29.59
18:1, <i>n</i> –7	N.D.	7.37	1.57	8.27	3.64	18.28
18:2, <i>n</i> –6	0.10	0.44	0.19	0.51	0.77	2.60
20:4, <i>n</i> –6	N.D.	0.55	0.53	0.37	0.29	0.44
Total	3.92	45.61	11.88	47.59	46.41	158.44
NAE						
16:0	1.53	4.13	2.61	8.29	5.95	26.06
18:0	2.71	6.01	3.57	8.46	6.67	28.65
18:1, <i>n</i> –9	0.50	1.10	0.43	4.10	4.68	23.88
18:1, <i>n</i> –7	N.D.	1.19	0.40	4.56	2.60	17.03
18:2, <i>n</i> –6	0.07	0.20	0.09	1.01	0.67	3.14
20:4, <i>n</i> –6	N.D.	1.00	0.05	0.82	0.12	0.90
Total	4.81	13.63	7.15	27.24	20.69	99.67

out activating cannabinoid receptors, the receptor-inactive saturated/monounsaturated NAEs could potentially elicit similar effects.

It is also interesting to note that NAE has structural similarity to ceramide and could therefore have analogous signalling effects, either directly by interacting with the molecular targets of ceramide or indirectly by interacting with ceramide signalling pathways [42]. In fact, *N*-oleoylethanolamine (18:1,*n*–9 NAE) has long been known as a potent inhibitor of ceramidase [43], and more recently it was shown to inhibit glucosylation of ceramides in neuroepithelioma cells [44]. Hence NAE generation could result in higher ceramide levels. In our experiments UVB irradiation, which is known to induce apoptosis through ceramide-mediated signalling [45], had the greatest effect on the production of 18:1,*n*–9 NAE. Finally, UVB irradiation also plays a role as a tumour initiator and promoter [46,47], and this activity may also be ceramide-mediated [48].

Based on the widely accepted role of ceramides as mediators of intracellular signalling, we propose that the stress-induced production of NAEs reported here may signify the presence of additional signalling pathways. Work on the identification of the potential targets and cellular effects of NAE-mediated signals is currently under way in our laboratory.

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REFERENCES

- Schmid, H. H. O., Schmid, P. C. and Natarajan, V. (1990) *Prog. Lipid Res.* **29**, 1–43
- Epps, D. E., Schmid, P. C., Natarajan, V. and Schmid, H. H. O. (1979) *Biochem. Biophys. Res. Commun.* **90**, 628–633
- Epps, D. E., Natarajan, V., Schmid, P. C. and Schmid, H. H. O. (1980) *Biochim. Biophys. Acta* **618**, 420–430
- Natarajan, V., Schmid, P. C. and Schmid, H. H. O. (1986) *Biochim. Biophys. Acta* **878**, 32–41
- Hansen, H. S., Lauritzen, L., Strand, A. M., Moesgaard, B. and Frandsen, A. (1995) *Biochim. Biophys. Acta* **1258**, 303–308
- Hansen, H. S., Lauritzen, L., Strand, A. M., Vinggaard, A. M., Frandsen, A. and Schousboe, A. (1997) *J. Neurochem.* **69**, 753–761
- Kondo, S., Sugiura, T., Kodaka, T., Kudo, N., Waku, K. and Tokumura, A. (1998) *Arch. Biochem. Biophys.* **354**, 303–310
- Schmid, P. C., Krebsbach, R. J., Perry, S. R., Dettmer, T. M., Maassen, J. L. and Schmid, H. H. O. (1995) *FEBS Lett.* **375**, 117–120; Corrigendum (1996) **385**, 125–126
- Newmann, J. L., Stiers, D. L., Anderson, W. H. and Schmid, H. H. O. (1986) *Chem. Phys. Lipids* **42**, 249–260
- Domingo, J. C., Mora, M. and de Madariaga, M. A. (1993) *Biochim. Biophys. Acta* **1148**, 308–316
- Mercadal, M., Domingo, J. C., Bermudez, M., Mora, M. and de Madariaga, M. A. (1995) *Biochim. Biophys. Acta* **1235**, 281–288
- Ambrosini, A., Bertoli, E., Mariani, P., Tanfani, F. and Wozniak, M. (1993) *Biochim. Biophys. Acta* **1148**, 351–355
- Epps, D. E., Palmer, J. W., Schmid, H. H. O. and Pfeiffer, D. R. (1982) *J. Biol. Chem.* **257**, 1383–1391
- Natarajan, V., Reddy, P. V., Schmid, P. C. and Schmid, H. H. O. (1982) *Biochim. Biophys. Acta* **712**, 342–355
- Reddy, P. V., Schmid, P. C., Natarajan, V., Muramatsu, T. and Schmid, H. H. O. (1984) *Biochim. Biophys. Acta* **795**, 130–136
- Schmid, P. C., Reddy, P. V., Natarajan, V. and Schmid, H. H. O. (1983) *J. Biol. Chem.* **258**, 9302–9306
- Skaper, S. D., Buriani, A., Dal Toso, R., Petrelli, L., Romanello, S., Facci, L. and Leon, A. (1996) *Proc. Natl. Acad. Sci. U.S.A.* **93**, 3984–3989
- Devane, W. A., Hanus, L., Breuer, A., Pertwee, R. G., Stevenson, L. A., Gibson, D., Mandelbaum, A., Etinger, A. and Mechoulam, R. (1992) *Science* **258**, 1946–1949
- Felder, C. C., Briley, E. M., Axelrod, J., Simpson, J. T., Mackie, K. and Devane, W. A. (1993) *Proc. Natl. Acad. Sci. U.S.A.* **90**, 7656–7660
- DiMarzo, V., Fontana, A., Cadas, H., Schinelli, S., Cimino, G., Schwartz, J.-C. and Piomelli, D. (1994) *Nature (London)* **372**, 686–691
- Sugiura, T., Kondo, S., Sukagawa, A., Tonegawa, T., Nakane, S., Yamashita, A. and Waku, K. (1996) *Biochem. Biophys. Res. Commun.* **218**, 113–117
- Sugiura, T., Kondo, S., Sukagawa, A., Tonegawa, T., Nakane, S., Yamashita, A., Ishima, Y. and Waku, K. (1996) *Eur. J. Biochem.* **240**, 53–62
- DiMarzo, V. (1998) *Biochim. Biophys. Acta* **1392**, 153–175
- Schmid, P. C., Kuwae, T., Krebsbach, R. J. and Schmid, H. H. O. (1997) *Chem. Phys. Lipids* **87**, 103–110
- Schmid, P. C., Schwindenhammer, D., Krebsbach, R. J. and Schmid, H. H. O. (1998) *Chem. Phys. Lipids* **92**, 27–35
- Ding, M., Li, J. J., Leonard, S. S., Ye, J. P., Shi, X., Colburn, N. H., Castranova, V. and Vallyathan, V. (1999) *Carcinogenesis* **20**, 663–668
- Huang, C., Ma, W. Y., Li, J. and Dong, Z. (1999) *Cancer Res.* **59**, 3053–3058
- Huang, C., Ma, W. Y. and Dong, Z. (1999) *Oncogene* **18**, 2828–2835
- Stein, B., Rahmsdorf, H. J., Steffen, A., Litfin, M. and Herrlich, P. (1989) *Mol. Cell. Biol.* **9**, 5169–5181
- Devary, Y., Gottlieb, R. A., Lau, L. F. and Karin, M. (1991) *Mol. Cell. Biol.* **11**, 2804–2811
- Derijard, B., Hibi, M., Wu, I. H., Barrett, T., Su, B., Deng, T., Karin, M. and Davis, R. J. (1994) *Cell* **76**, 1025–1037
- Bligh, E. G. and Dyer, W. J. (1959) *Can. J. Biochem. Physiol.* **37**, 911–917
- Bartlett, G. R. (1959) *J. Biol. Chem.* **234**, 466–468
- Schmid, P. C., Natarajan, V., Weis, B. K. and Schmid, H. H. O. (1986) *Chem. Phys. Lipids* **41**, 195–207
- Giuffrida, A. and Piomelli, D. (1998) *FEBS Lett.* **422**, 373–376
- Huang, C., Schmid, P. C., Ma, W. Y., Schmid, H. H. O. and Dong, Z. (1997) *J. Biol. Chem.* **272**, 4187–4194
- Dong, Z., Ma, W. Y., Huang, C. and Yang, C. S. (1997) *Cancer Res.* **57**, 4414–4419
- Chen, N., Ma, W. Y., Huang, C. and Dong, Z. (1999) *J. Biol. Chem.* **274**, 15389–15394
- Hanus, L., Gopher, A., Almog, S. and Mechoulam, R. (1993) *J. Med. Chem.* **36**, 3032–3034
- Valk, P., Verbakel, S., Vankan, Y., Hol, S., Mancham, S., Ploemacher, R., Mayen, A., Lowenberg, B. and Delwel, R. (1997) *Blood* **90**, 1448–1457
- Derocq, J. M., Bouaboula, M., Marchand, J., Rinaldi-Carmona, M., Segui, M. and Casellas, P. (1998) *FEBS Lett.* **425**, 419–425
- Hannun, Y. (1996) *Science* **274**, 1855–1859
- Sugita, M., Williams, M., Dulaney, J. T. and Moser, H. W. (1975) *Biochim. Biophys. Acta* **398**, 125–131
- Spinedi, A., Di Bartolomeo, S. and Piacentini, M. (1999) *Biochem. Biophys. Res. Commun.* **255**, 456–459
- Verheij, M., Bose, R., Lin, X. H., Yao, B., Jarvis, W. D., Grant, S., Birrer, M. J., Szabo, E., Zon, L. I., Kyriakis, J. M. et al. (1996) *Nature (London)* **380**, 75–79
- Strickland, P. T. (1986) *J. Invest. Dermatol.* **87**, 272–275
- Staberg, B., Wolf, G. C., Klemp, P., Poulsen, T. and Bradhagen, H. (1983) *J. Invest. Dermatol.* **81**, 517–519
- Huang, C., Ma, W. Y., Ding, M., Bowden, G. T. and Dong, Z. (1997) *J. Biol. Chem.* **272**, 27753–27757

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