Effect of fatty acid anilides on the generation of arachidonic acid by human polymorphonuclear leukocytes

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The addition of oleoylanilide or linoleylanilide to human polymorphonuclear leukocytes induces a time- and dose-dependent generation of arachidonic acid. Half-maximal effect is caused by a dose of 0.2 mg linoleylanilide/ml. Fatty acid anilides also produce a time- and dose-dependent inhibition of the synthesis of triacylglycerol. Half-maximal effect is caused by 1 μg linoleylanilide/ml. These results indicate that fatty acid anilides, which have been found in the illegal cooking oil which intoxicated thousands of Spaniards, alter lipid metabolism in human polymorphonuclear leukocytes.

Oleoylanilide *Linoleylanilide* *Triacylglycerol* *Phospholipid* *Arachidonic acid* *Human neutrophil*

1. INTRODUCTION

Polymorphonuclear leukocytes (PMNs) generate arachidonic acid during phagocytosis [1,2] and in response to chemoattractants [3]. Arachidonic acid is then mainly used for the synthesis of leukotrienes, potent chemoattractants and initiators of smooth muscle contraction, which are powerful mediators of the inflammatory response of the PMNs [4,5]. Although arachidonic acid and its derivatives may play an essential beneficial role under most conditions, its generation in vivo must be carefully controlled to avoid life-threatening reactions. We have studied the effects of fatty acid anilides on the generation of arachidonic acid by human PMNs. This is of interest since fatty acid anilides have been found in the cooking oil which is thought to have intoxicated thousands of Spaniards and caused hundreds of deaths and disabilities [6,7]. The present results indicate that fatty acid anilides induce the generation of arachidonic acid from PMNs and that this effect is additive with that induced by the addition of phagozitizable particles or the cationophore A23187. This report also shows that fatty acid anilides are potent inhibitors of the synthesis of triacylglycerol by PMNs. These data indicate that fatty acid anilides alter the lipid metabolism of human PMNs.

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2. MATERIALS AND METHODS

Human polymorphonuclear leukocytes (PMNs) were obtained from venous blood of normal volunteers as in [8]. The incorporation of [*methyl*-^{3}H]choline into phosphatidylcholine, [*methyl*-^{3}H]methionine into phospholipids, [1-^{3}H]-ethanolamine into phosphatidylethanolamine and [2-^{3}H]myoinositol into phosphatidylinositol were done as in [2,9,10]. PMNs were prelabeled with [5,6,7,8,9,11,12,14,15-^{3}H]arachidonic acid for 30 min as in [2]. After preincubation, the cell suspension was washed 3 times in 6 mM Hepes (4-(2-hydroxyethyl)-1-piperazine-ethanesulphonic acid) buffer (pH 7.4), containing 136.8 mM NaCl, 2.61 mM KCl, 1.3 mM $CaCl_2$, 1 mM $MgCl_2$, 0.1%

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glucose and 0.25% delipidated bovine serum albumin and resuspended in the same buffer at 10^7 cells/ml. Cells were then incubated at 37°C in the presence or absence of fatty acid anilides and at various times the generation of arachidonic acid or oleic acid was determined after extraction of the lipids and their separation by thin layer chromatography as in [2]. The synthesis of triacylglycerol was measured by incubating cells with [^{3}H]glycerol (1 μCi/ml). At various times after the addition of [^{3}H]glycerol the lipids were extracted and triacylglycerol separated by thin-layer chromatography under the same conditions as those described for arachidonic acid [2].

Platelet activating factor (PAF), β-glucuronidase and phagocytosis of opsonized zymosan particles were assayed as in [8]. Fatty acid anilides were prepared as in [11]. Before use, the fatty acid anilides were sonicated at 4°C, about 30 times for 30 s in Hepes buffer at 2 mg/ml. [*methyl*-^{3}H]Choline (70 mCi/mmol), [*methyl*-^{3}H]-methionine (70 mCi/mmol), [1-^{3}H]ethanolamine (5 Ci/mmol), [^{3}H]arachidonic acid (100 Ci/mmol), [1-^{14}C]oleic acid (50 mCi/mmol) and [1-^{3}H]glycerol (2.5 Ci/mmol) were from Amersham (Bucks).

3. RESULTS AND DISCUSSION

The addition of linoleylanilide or oleoylanilide at 1 mg/ml to a suspension of PMNs induces a time-dependent generation of arachidonic acid (fig.1). This effect is specific for arachidonic acid and the addition of fatty acid anilides had no effect on the generation of oleic acid in cells prelabeled with [1-^{14}C]oleic acid (not shown). The effect of fatty acid anilides on the generation of arachidonic acid by PMNs is dose-dependent. Half-maximal effect on arachidonate generation is observed at ~0.2 mg linoleylanilide/ml (fig.2). The incubation of PMNs with linoleic acid had no effect on the generation of arachidonic acid. This indicates that the effects observed with the fatty acid anilides are not due to their possible hydrolysis to fatty acids and aniline. The addition of fatty acid anilides to PMNs did not stimulate the generation of PAF or β-glucuronidase (not shown). These results indicate that fatty acid anilides are incomplete secretagogues for the PMNs. Cells treated with fatty acid anilides for 30 min still responded to the addition of opsonized zymosan with normal ingestion of the particles and normal secretion of β-glucuronidase and PAF. The increased generation of arachidonic acid in response to zymosan and fatty acid anilides are additive. Similarly, the generation of arachidonic acid in response to the cationophore A23187 and to the fatty acid anilides are additive also (table 1). Thus, the addition of

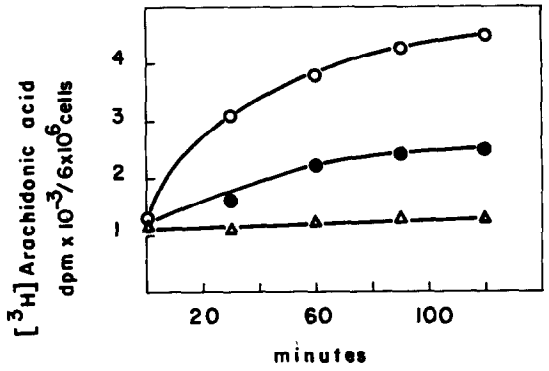

Fig.1. Effect of fatty acid anilides on the generation of arachidonic acid by human polymorphonuclear leukocytes. At zero time cells were treated with oleoylanilide (●, 1 mg/ml), linoleylanilide (○, 1 mg/ml) or Hepes buffer (△). At various times samples were taken and the generation of arachidonate measured as in section 2. Results are the average of 3 independent experiments in duplicate.

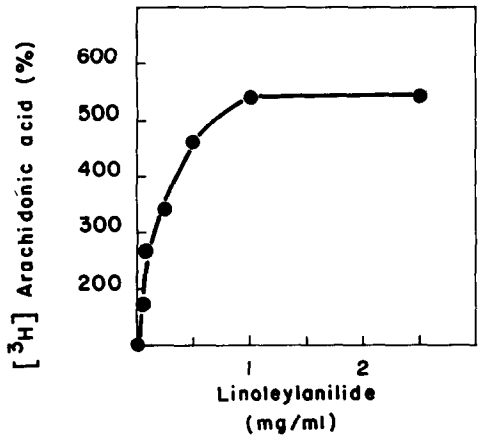

Fig.2. Effect of different doses of linoleylanilide on the generation of arachidonic acid by human polymorphonuclear leukocytes. The generation of arachidonic acid was measured as in section 2. Results are the average of 3 independent experiments in duplicate.

Table 1

Effect of fatty acid anilides on the generation of arachidonic acid by human polymorphonuclear leukocytes

Addition	Arachidonic acid generation (% control)
Oleoylanilide (1 mg/ml)	192 ± 15
Linoleylanilide (1 mg/ml)	483 ± 19
Zymosan (2 mg/ml)	195 ± 13
A23187 (5 μM)	718 ± 3
Oleoylanilide (1 mg/ml) + zymosan (2 mg/ml)	308 ± 16
Linoleylanilide (1 mg/ml) + zymosan (2 mg/ml)	695 ± 16
Oleoylanilide (1 mg/ml) + A23187 (5 μM)	902 ± 4
Linoleylanilide (1 mg/ml) + A23187 (5 μM)	1164 ± 4

PMNs were prelabeled with [^{3}H]arachidonic acid for 30 min at 37°C. Cells were then treated with the fatty acid anilides (1 mg/ml) and 30 min later zymosan (2 mg/ml), ionophore A23187 (5 μM) or buffer were added. After 30 min incubation the generation of arachidonic acid was determined as in section 2. Results are the mean ± SEM of at least 3 independent experiments in duplicate

fatty acid anilides does not prevent the inflammatory response of the PMNs; the main effect of fatty acid anilides is a marked increase in the generation of arachidonic acid.

The addition of fatty acid anilides to PMNs has no effect on the incorporation of labeled choline, methionine or ethanolamine into phospholipids but enhances the incorporation of labeled inositol into phosphatidylinositol (table 2). These results indicate that the fatty acid anilides have no effect on phosphatidylcholine- or phosphatidylethanolamine-turnover and that they specifically affect phosphatidylinositol metabolism. Cellular stimulation is often accompanied by an enhanced phosphatidylinositol turnover and arachidonic acid generation (review [12]). We have shown that phosphatidylinositol is an important reservoir of arachidonic acid in human PMNs and a source for the generation of this fatty acid during the inflammatory response of these cells [2]. Therefore, it is possible that the enhanced incorporation of inositol into phosphatidylinositol is, at least in part, the consequence of the stimulated generation of arachidonate in response to the addition of the fatty acid anilides.

These results are compatible with the activation by the fatty acid anilides of specific phospholipases which may result in the generation of arachidonic acid. This lipid is known to be a precursor of leukotrienes, potent chemoattractants and initiators of smooth muscle contraction, which are powerful mediators of the inflammatory response of the PMNs [4,5]. Since fatty acid anilides, used in illegal food processing operations, have been implicated in a number of deaths and disabilities in Spain, and since the victims appear to suffer inflammatory reactions as part of the general toxicity [6,7], it is possible that the fatty acid anilides are responsible for part of the inflammatory reactions observed in these patients.

In addition to these effects of the fatty acid

Table 2

Effect of fatty acid anilides on the incorporation of labeled precursors into phospholipids (% control)

	Phosphatidylcholine synthesis	Phospholipid methylation	Phosphatidylethanolamine synthesis	Phosphatidylinositol synthesis
Oleoylanilide	88 ± 13	103 ± 13	95 ± 9	149 ± 12
Linoleylanilide	92 ± 10	117 ± 11	97 ± 12	168 ± 28

PMNs were preincubated for 60 min with the fatty acid anilide (1 mg/ml) at 37°C. At the end of this period, the labeled precursors were added and 30 min later the incorporation of radioactivity into phospholipids measured as in section 2. Results are the mean ± SEM of 3 independent experiments in duplicate

anilides on arachidonic acid generation, these compounds also have a marked effect on triacylglycerol synthesis. Thus, the addition of 1 mg linoleylanilide/ml to PMNs completely inhibited the incorporation of [^{3}H]glycerol into triacylglycerol (fig.3). Linoleylanilide addition however, had no effect on diacylglycerol or phosphatidylcholine synthesis but reduced ~2-fold the incorporation of radioactivity into phosphatidic acid (not shown). The effect of linoleylanilide on triacylglycerol synthesis is dose-dependent. Half-maximal effect on triacylglycerol synthesis is observed at ~1 μg/ml (fig.4). The mechanisms by which the fatty acid anilides exert this effect on triacylglycerol synthesis is not known. However, one of the symptoms of the people intoxicated with oil containing fatty acid anilides was a marked reduction in body weight [13]. Furthermore, these results agree with an inhibition of triacylglycerol synthesis by a microsomal fraction preparation of lung and adipose tissue shown in rats fed with oleoylanilide [11].

The concentration of linoleylanilide necessary to observe half-maximal effect on arachidonic acid generation is about 200-fold higher than that necessary to see the same effect on triacylglycerol synthesis. These results indicate that the fatty acid anilides perturb lipid metabolism by acting at different levels and through different mechanisms. This may aid comprehension of the diversity of symptoms of the people intoxicated with oil containing fatty acid anilides.

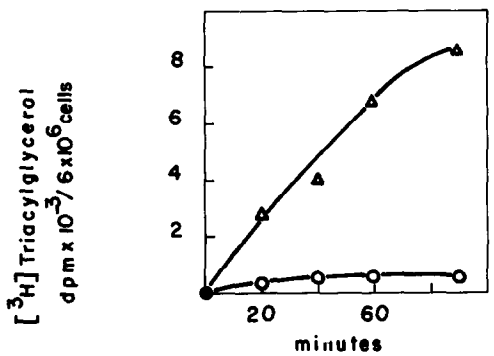

Fig.3. Effect of linoleylanilide on the synthesis of triacylglycerol by human polymorphonuclear leukocytes. Cells were preincubated for 30 min in the presence (○) or absence (△) of 1 mg/ml linoleylanilide. At the end of this period, [^{3}H]glycerol was added and the incorporation of radioactivity into triacylglycerol measured as in section 2. Results are the average of 3 independent experiments in duplicate.

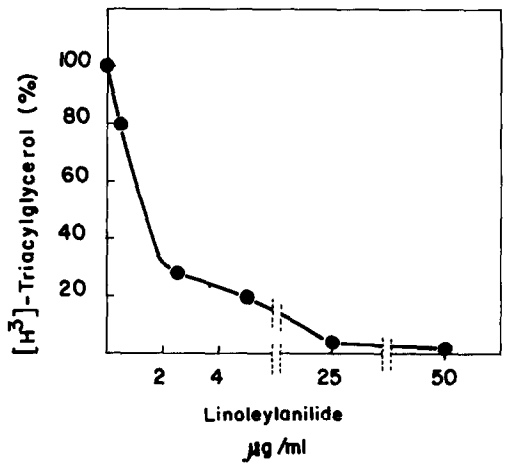

Fig.4. Effect of different doses of linoleylanilide on the synthesis of triacylglycerol by human polymorphonuclear leukocytes. The synthesis of triacylglycerol was measured as in section 2. Results are the average of 3 independent experiments in duplicate.

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REFERENCES

[1] Walsh, C.E., Waite, M.B., Thomas, I.J. and DeChatelet, L.R. (1981) J. Biol. Chem. 256, 7228–7234.

[2] García Gil, M., Alonso, F., Alvarez Chiva, V., Sanchez Crespo, M. and Mato, J.M. (1982) Biochem. J. 206, 67–72.

[3] Hirata, F., Corcoran, B.A., Venkatasubramanian, K., Schiffman, E. and Axelrod, J. (1979) Proc. Natl. Acad. Sci. USA 76, 2640–2643.

[4] Snyderman, R. and Goetzl, E.J. (1981) Science 213, 830–833.
[5] Vanderhoeh, J.Y., Tare, N.S., Bailey, J.M., Goldstein, A.L. and Pluznitz, D.H. (1982) J. Biol. Chem. 257, 12191–12196.
[6] Programa del CSIC para el Estudio del Sindrome Toxico (1983) vol.1 and 2, Consejo Superior de Investigaciones Cientificas, Madrid.
[7] Simposium Nacional del Sindrome Toxico (1982) Ministerio de Sanidad y Consumo, Madrid.
[8] Alonso, F., Sanchez Crespo, M. and Mato, J.M. (1981) Immunology 45, 493–500.
[9] García Gil, M., Alonso, F., Sanchez Crespo, M. and Mato, J.M. (1981) Biochem. Biophys. Res. Commun. 101, 740–748.
[10] Mato, J.M., Pencev, D., Vasathakumar, G., Schiffman, E. and Pastan, I. (1983) Proc. Natl. Acad. Sci. USA 80, 1929–1932.
[11] Casals, C., García-Barreno, P. and Municio, A.M. (1983) Biochem. J. 212, 339–344.
[12] Irvine, R.F. (1982) Biochem. J. 204, 3–16.
[13] Noriega, A.R. (1982) Lancet ii, 697–702.