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Augmented production of platelet-activating factor in human polymorphonuclear leukocytes by ketone bodies

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The production of platelet-activating factor (PAF) in A23187-stimulated human polymorphonuclear leukocytes was markedly increased in the presence of 5 mM acetoacetate and β-hydroxybutyrate Such an augmentation was observed even at 500 μM but not at 50 μM The augmented production of PAF by acetoacetate was also observed in the presence of autologous serum and was most prominent in the case of opsonized zymosan-stimulation rather than A23187-stimulation These observations suggest that increased levels of acetoacetate and β-hydroxybutyrate in blood may lead to the augmented production of PAF, which would amplify the various PAF-mediated biological reactions

Platelet-activating factor, Polymorphonuclear leukocyte, Ketone body, Acetoacetate, β-Hydroxybutyrate, Acetyl-CoA

1. INTRODUCTION

Platelet-activating factor (PAF) is a unique bioactive phospholipid which has been identified as 1-*O*-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine (reviewed in [1–3]). It causes aggregation, infiltration and degranulation of blood cells, increased vascular permeability, smooth muscle contraction and hypotension, and is now considered to be an important lipid mediator in various inflammatory and allergic reactions [1–3].

PAF is known to be synthesized in various tissues and cells including inflammatory cells such as polymorphonuclear leukocytes (PMN) upon appropriate stimulation. Dual biosynthetic routes for PAF have already been proposed [2] and the properties of the enzymes involved in these reactions have also been studied by several investigators. The activation of acetyl-CoA/1-alkyl-GPC acetyltransferase in stimulated cells [4–6] would be favorable for the production of PAF through the remodeling of the preexisting alkylacyl-GPC in these cells.

Despite the accumulating knowledge concerning the enzyme activities, however, the mechanism of the regulation of PAF synthesis is not fully elucidated yet. For example, the control mechanism of the substrate availability in living cells still remains quite obscure. In fact, little information is available concerning the origin as well as the metabolic regulation of acetyl-CoA in PAF-producing cells. It is very important to investigate these points in detail in order to better understand the modulatory mechanism of PAF production, especially in vivo.

In this study, we examined the effects of ketone bodies, which are known to be transporters of acetyl units in blood, on the production of PAF in human PMN. We found that the addition of pathologically significant levels of acetoacetate and β-hydroxybutyrate augment the PAF production. The possible relationship between these observations and several pathological situations is discussed.

2. MATERIALS AND METHODS

Acetoacetic acid (lithium salt), DL-β-hydroxybutyric acid (sodium salt), DL-α-hydroxybutyric acid (sodium salt), γ-hydroxybutyric acid (sodium salt), α-ketobutyric acid (sodium salt), L-(+)-lactic acid (lithium salt), pyruvic acid (sodium salt), phorbol myristate acetate (PMA) and zymosan were obtained from Sigma (St. Louis, MO) A23187 was from Calbiochem (La Jolla, CA) Acetic acid (sodium salt), acetone and glucose were from Wako Pure Chem Ind. (Osaka, Japan) 1-*O*-Hexadecyl-2-acetyl-*sn*-glycero-3-phosphocholine (C16:O-PAF) was purchased from Bachem (Bubendorf, Switzerland) A PAF-antagonist, CV3988 was a generous gift from Dr Tsushima, Takeda Chemical Inc (Osaka, Japan) [^{3}H]Arachidonic acid (192 Ci/mmol) and leukotriene B_4 [^{3}H]assay system were obtained from Amersham (Amersham, UK).

PMN were isolated from normal human donors as described in [7] PMN (2 5 or 5×10^6 cells) in 0.5 ml or 1 ml of 20 mM Hepes-Hanks balanced salt solution containing 5 6 mM glucose (pH 7 4) were incubated at 37°C in the presence or absence of 5 mM ketone bodies or related compounds. Ten minutes later, PMN were stimulated by the addition of A23187 (final 1 μM), opsonized zymosan (final 4 mg/ml) or PMA (final 250 ng/ml) After a further 10 min incubation, chloroform/methanol (1.2) was added to the incubation mixture and lipids were extracted by the method of Bligh and Dyer [8]

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Abbreviations: PAF, platelet-actirating factor; PMN, polymorphonuclear leukocytes; PMA, phorbol myristate acetate, LTB_4, leukotriene B_4, DMSO, dimethyl sulfoxide

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In some experiments, PMN were incubated in Hepes-Hanks solution containing 20% autologous serum which had been inactivated by heat (56°C for 30 min) and acid (pH 2.5-3.0 for 15 min) [9] treatments After the stimulation with opsonized zymosan in the presence or absence of acetoacetate, cells were spun down (2000 rpm for 2 min) at 4°C Total lipids both from sedimented cells and from supernatants were extracted as described above

Individual phospholipids were separated from each other by TLC developed with chloroform/methanol/water (40:24.4). The area corresponding to the authentic PAF was scraped off and then extracted with chloroform/methanol/water (1 2.0 8) After the extraction by the method of Bligh and Dyer [8], the chloroform layer was removed and evaporated to dryness The residue was resuspended in Hepes-buffered Tyrode's solution (pH 7 4) containing 0 25% BSA The amount of PAF was measured by estimating the aggregating activity towards washed rabbit platelets [10], using C16:O-PAF as a standard. The aggregation of rabbit platelets by PAF fraction was completely inhibited by the pretreatment of cells with 8 μM CV3988. Furthermore, he phospholipase A_2 treatment of samples abolished the activity These results indicate that the aggregation was really due to PAF

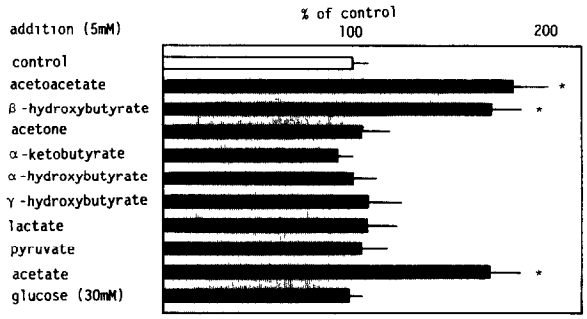

Fig.1. Effects of ketone bodies and related compounds on the production of PAF in A23187-stimulated human PMN PMN were incubated with or without these compounds (final 5 mM) for 10 min In the case of glucose, a high concentration (final 30 mM) was employed Then, cells were stimulated with A23187 (1 μM) for 10 min The amounts of produced PAF were measured as described in section 2 The data were expressed as the mean percentages ±SD from 5 to 6 determinations *Significantly different from control ($P<0$ 001), Student's t-test.

3. RESULTS AND DISCUSSION

Fig.1 shows the effects of ketone bodies and related compounds on the production of PAF in A23187-stimulated human PMN. The addition of 5mM acetoacetate, β-hydroxybutyrate as well as acetate augmented the PAF production by 1.7-1.8 times. In contrast, the addition of α-ketobutyrate, an isomer of acetoacetate, or α- and γ-hydroxybutyrate, isomers of β-hydroxybutyrate, did not affect the PAF production. A rather inactive ketone body, acetone, also failed to stimulate PAF synthesis, at least at 5 mM. Lactate, pyruvate and a high concentration (30 mM) of glucose were also found to be ineffective. Furthermore, the addition of free arachidonic acid (20:4), in the presence of 1% human serum albumin, did not affect the PAF synthesis up to 200 μM and a higher concentration of free 20:4 showed only an inhibitory effect, at least under these experimental conditions (data not shown).

The dose dependencies of the augmented production of PAF by acetoacetate, β-hydroxybutyrate and acetate were next examined. Only a slight acceleration of PAF synthesis in A23187-stimulated PMN was observed in the presence of 50 μM of these 3 compounds (data not shown). On the other hand, the amounts of produced PAF significantly increased with higher concentrations (500 μM, 5 mM and 20 mM) of these compounds (data not shown). The enhancement was maximal at 5 mM in each case. This concentration is similar to the concentration of ketone bodies in which utilization by several peripheral tissues reached the plateau [11].

The effects of acetoacetate on the production of PAF in PMN stimulated with several stimuli are shown in table 1. The addition of acetoacetate did not affect the PAF level in control cells. The acceleration was observed only for stimulated cells. The amounts of PAF produced by the stimulation with A23187, opsonized zymosan and PMA, all increased in the presence of 5 mM acetoacetate. The most prominent augmentation was observed in the case of a physiological stimulus, opsonized zymosan (the stimulation index was 2.5), though the absolute amount of PAF was lower than that produced in the case of A23187-stimulation.

In order to establish the effects of acetoacetate under conditions closer to those in vivo, we next examined the effects of acetoacetate in the presence of autologous serum. Fig.2 shows the effects of acetoacetate on the

Table 1

Effects of acetoacetate on the production of PAF in human PMN stimulated with several stimuli

Stimuli	Acetoacetate (5 mM)		Stimulation index
	−	+	
	pmol/2 5×10^6 cells/10 min		
none	0.05 ± 0 01	0 05 ± 0.01	1 0
DMSO (0 5%)	0.05 ± 0 03	0.05 ± 0 03	1 0
A23187 (1 μM)	29 9 ± 1 7	49.3 ± 4 0	1.7
opsonized zymosan (4 mg/ml)	1 23 ± 0 13	3 08 ± 0 48	2 5
PMA (250 ng/ml)	0 37 ± 0 03	0 71 ± 0 19	1 9

PMN (2 5×10^6 cells/0 5 ml or 5×10^6 cells/ml) were incubated with or without 5 mM acetoacetate for 10 min Then, cells were stimulated with A23187 (1 μM), opsonized zymosan (4 mg/ml) or PMA (250 ng/ml) for 10 min A23187 and PMA were diluted with DMSO and then used (the final concentration of DMSO in the incubation mixture did not exceed 0 5%) The values are means ±SD from 4 to 5 determinations

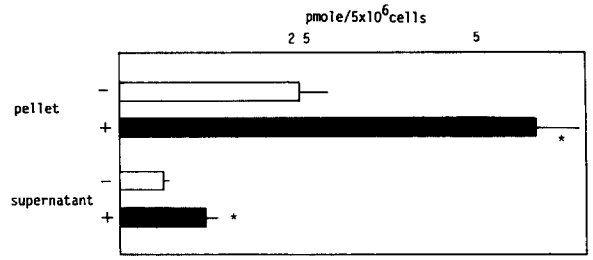

Fig.2. Augmented production of PAF in opsonized zymosan-stimulated human PMN by acetoacetate in the presence of autologous serum. PMN (5×10^6 cells/ml) were incubated in 20% autologous serum-containing Hank's solution for 10 min with or without 5 mM of acetoacetate Then, cells were stimulated with opsonized zymosan (4 mg/ml) for 10 min. After the centrifugation, PAF retained in cells and that released into the supernatant were extracted as described in section 2 The data were expressed as the means ± SD from four determinations *Significantly different from control ($P<0.001$), Student's t-test

production and release of PAF in opsonized zymosan-stimulated PMN in the presence of 20% autologous serum. In the absence of serum or serum albumin, almost all of the produced PAF was retained in cells (data not shown) as previously reported by others [9,12]. On the other hand, ca. 20% of PAF was released into the medium in the presence of serum, which is in general agreement with the previous observations by several investigators [7,9,12,13]. Here we found that the addition of a pathological level of acetoacetate (5 mM) augmented the PAF production induced by opsonized zymosan by 2.3 times even in the presence of autologous serum. Both PAF retained in cells and that released into the medium were increased relatively to the same degree by the treatment of cells with acetoacetate. These observations suggest that the augmented production of PAF by ketone bodies may have some pathophysiological implications in vivo.

The possible involvement of PAF in the pathogenesis of several diseases, such as endotoxin shock, anaphylactic shock, nephritis, ischemia and asthma, has already been suggested by many investigators [14]. Thus, the modulation of the biosynthesis of PAF, especially in vivo, seems to be a very important problem from the pathological viewpoint. In the present investigation, we examined the effects of the clinically important materials, ketone bodies, which are known to be rapidly incorporated and metabolized by peripheral tissues to form acetyl-CoA, a precursor for the acetyl moiety of PAF. Previously, several investigators have shown that the treatments of kidney [15] and heart [16] with acetoacetate (5 mM) and β-hydroxybutyrate (5.5 mM), respectively, markedly increased the intracellular level of acetyl-CoA. These observations are interesting in view of the availability of acetyl-CoA for PAF synthesis, since the Km value of the acetyl-CoA:lyso-PAF acetyltransferase for acetyl-CoA is considerably higher than the acetyl-CoA level in cells (unpublished data). This suggests that the increased level of intracellular acetyl-CoA would lead to the enhanced production of PAF. Concerning this possibility, Benveniste and his colleagues [17–19] have demonstrated the accelerated synthesis of PAF in stimulated macrophages and PMN in the presence of acetate and acetyl-CoA. Similar augmentation was observed in this study for ketone bodies such as acetoacetate and β-hydroxybutyrate, which are regarded as important transporters of acetyl units in the human body. Although the exact amount of acetyl-CoA in PMN has not yet been determined, it is conceivable that such an augmentation is due to an increased level of acetyl-CoA within the cells. We confirmed that neither the release of [^{3}H]20:4 nor the production of LTB_4 was affected by the treatment of cells with 5 mM acetoacetate (data not shown).

The blood levels of ketone bodies from normal subjects are reported to be 44 ± 17 μM (acetoacetate) and 59 ± 33 μM (β-hydroxybutyrate) [20]. These concentrations of ketone bodies, added independently or together (data not shown) to PMN, did not change the amount of PAF produced by A23187-stimulation markedly. On the other hand, the levels of ketone bodies are raised dramatically (>1 mM) in several pathological situations such as diabetes and starvation, in which the lipid catabolism is accelerated [21]. In the case of diabetic ketoacidosis, the concentration of ketone bodies can be more than 5 mM. The levels of ketone bodies even in patients with hyperosmolar non S-ketotic coma are often 10-fold higher than the control level [21]. These concentrations of ketone bodies could be enough to augment the PAF production, as shown in this study, suggesting that amplification of the PAF-mediated biological responses could take place. In this regard, it is interesting to note that the platelet aggregation is potentially elevated in diabetic patients [22,23]. In fact, disseminated intravascular coagulation (DIC) is often observed in patients with diabetic coma [24,25,26]. The increased platelet aggregation is also considered to be very important in the pathogenesis of microangiopathy. Moreover, endotoxemia, which could be mediated by endogenously generated PAF [27,28], is sometimes an important complication of diabetes. Further studies are required to show whether the increased levels of ketone bodies really modify various PAF-induced pathological conditions in vivo.

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