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OXYGEN CONSUMPTION OF HUMAN BLOOD PLATELETS

II. EFFECT OF INHIBITORS ON THROMBIN-INDUCED OXYGEN BURST*

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SUMMARY

The effect of selected inhibitors on the thrombin-stimulated burst and the basal oxygen consumption of washed human platelets were investigated and compared with inhibition of the release reaction. Cyanide (0.2 mM) caused complete inhibition of the basal respiration, but only 15 % inhibition of the thrombin-stimulated burst of oxygen consumption. Similar differential inhibitory effects were observed with oligomycin, antimycin, rotenone and *N*-ethylmaleimide. Prostaglandin E_1 (0.03 mM) and acetylsalicylic acid (0.8 mM) had little effect on basal respiration, but inhibited the thrombin-stimulated burst of oxygen consumption. *N*-Ethylmaleimide (0.4 mM) inhibited the release of calcium from platelets by 90 %, while prostaglandin E_1, acetylsalicylic acid and the above mitochondrial inhibitors caused no more than 30 % inhibition of the release reaction. Our results provide evidence that basal respiration and a portion of the thrombin-stimulated burst of oxygen consumption are involved in respiratory chain phosphorylation, and that this component of the thrombin-stimulated burst may be coupled to the maintenance of the release reaction.

INTRODUCTION

It has been demonstrated that an energy requirement exists for the release of platelet constituents as well as the subsequent aggregation of the platelets and the contraction of the aggregated platelet mass [1–5]. In the present study, inhibitors of mitochondrial metabolism were used to investigate the cellular locus of oxygen consumption induced by thrombin [6] and the relationship between the oxygen burst and

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the energy metabolism of the platelets. Inhibitors also were used to probe the relationship between the release reaction induced by thrombin and the accompanying burst of oxygen consumption.

METHODS

Preparation of washed platelet suspensions, the polarographic determination of oxygen uptake, and the measurement of the release reaction were conducted as described in the preceding paper [6]. All studies were performed with a final concentration of 1.9 units/ml of thrombin. Because of the decreased thrombin effect during storage of platelets at 4° C [6], all experiments with inhibitors were preceded and followed with controls of thrombin alone.

MATERIALS

Prostaglandin E_1 was kindly provided by Dr John Pike of the Upjohn Co., Kalamazoo, Michigan, and a stock solution was prepared by dissolving 1 mg of prostaglandin E_1 per ml of a 10% ethanol/0.2 mg/ml Na_2CO_3 solution and stored at 4 °C. Thrombin was obtained as previously described [6].

RESULTS

Inhibition of oxygen consumption by inhibitors of mitochondrial metabolism

The basal respiration of human platelets was highly sensitive to mitochondrial inhibitors, in contrast to the thrombin-stimulated burst of oxygen consumption

TABLE I

EFFECTS OF INHIBITORS ON OXYGEN CONSUMPTION AND THE RELEASE REACTION INDUCED BY THROMBIN

Inhibitor	Concn (mM)	Percent inhibition*		
		Oxygen consumption		Release reaction**
		Basal rate	Burst rate	
Cyanide	0.03	25	0	—
	0.2	100	15	15
	4.0	100	50	—
Oligomycin	0.1 μg/ml	100	0	—
	10 μg/ml	100	65	20
Antimycin	0.01 μg/ml	100	0	—
	0.5 μg/ml	100	40	15
Rotenone	0.01	100	35	30
2-Deoxy-D-glucose	20	0	0	15
Prostaglandin E_1	0.001	25	70	15
Acetylsalicylic acid	2.8	0	90	35
N-Ethylmaleimide	1.0	100	40	90

* The results are mean values of at least two determinations for each inhibitor.

** Determined by the amount of calcium released.

(Table I). The differential effects of the mitochondrial inhibitors on oxygen consumption is illustrated in Fig. 1 with cyanide. The basal respiration was completely inhibited by 0.2 mM cyanide, but the thrombin-stimulated burst was inhibited only 15 %.

Inhibition of the two types of oxygen consumption by oligomycin (Fig. 2), cyanide, rotenone and antimycin were proportional to their concentration. Inhibition of the basal respiration by oligomycin was released by the addition of uncoupling agents, 2,4-dinitrophenol or pentachlorophenol (Fig. 3).

In comparison to inhibitors of oxidative metabolism, 20 mM 2-deoxy-D-glucose had no effect on either the basal or the thrombin-stimulated burst of oxygen consumption.

Inhibition of oxygen consumption by inhibitors of platelet function

Prostaglandin E_1 and acetylsalicylic acid inhibited the thrombin-stimulated burst of oxygen consumption, but only prostaglandin E_1 inhibited basal respiration (Table I). We also observed that prostaglandin E_1 could inhibit the thrombin-stimulated oxygen consumption after the initiation of the oxygen burst. A pharmaco-

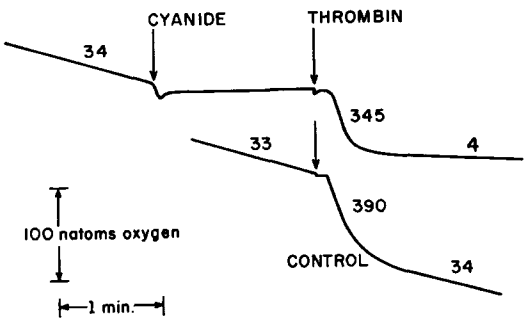

Fig. 1. The effect of cyanide on platelet oxygen consumption. The platelet concentration was $2.9 \cdot 10^9$ cells/ml. Addition of cyanide (0.2 mM) and of thrombin (1.9 units/ml) is indicated by the arrows. The numbers on the tracings express the oxygen consumption as natoms per min.

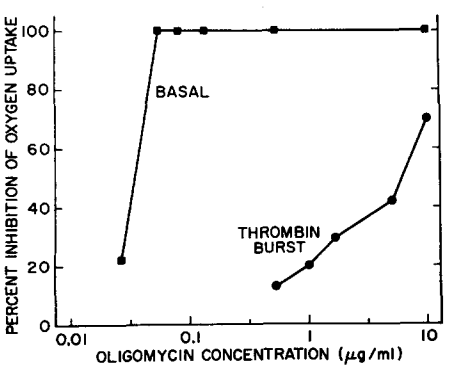

Fig. 2. The inhibition of basal respiration and the thrombin-stimulated burst of oxygen consumption as a function of the concentration of oligomycin. Each point is the mean of two determinations. The concentration of thrombin was 1.9 units/ml.

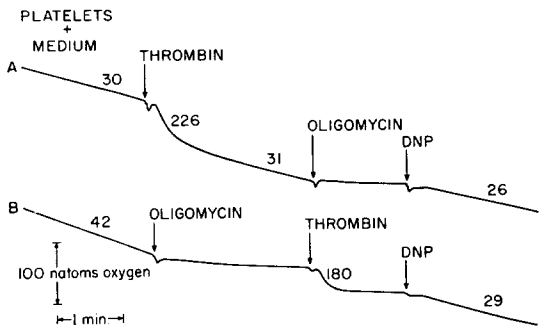

Fig. 3. Inhibition of platelet oxygen consumption by oligomycin and the release of the inhibition by 2,4-dinitrophenol. Platelet concentration was $2.9 \cdot 10^9$ cells/ml. The arrows indicate additions of thrombin (1.9 units/ml), oligomycin (0.5 μg/ml) and 2,4-dinitrophenol (DNP; 0.5 mM). The numbers on the tracings express the oxygen consumption as nanoatoms per min.

logical concentration of acetylsalicylic acid, 0.28 mM (4 mg/100 ml), inhibited the thrombin-stimulated burst by 60 % and also increased the delay in response to thrombin (Figs 4A and 4B). When acetylsalicylic acid (0.28 mM) and antimycin (0.5 μg/ml) were used in combination (Fig. 4D), the thrombin-stimulated oxygen burst was completely inhibited; whereas alone, acetylsalicylic acid or antimycin caused 60 and 40 % inhibition, respectively (Figs 4B and 4C).

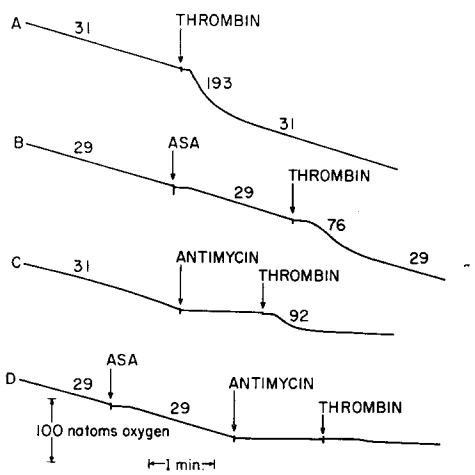

Fig. 4. The effects of acetylsalicylic acid and antimycin on oxygen consumption of platelets. Platelet concentration was $1.75 \cdot 10^9$ cells/ml. Arrows indicate additions of thrombin (1.9 units/ml), acetylsalicylic acid (ASA; 0.28 mM) and antimycin (0.5 μg/ml) in all tracings. The numbers on the tracings express the oxygen consumption as nanoatoms per min.

Inhibition of the release reaction

The effects of mitochondrial inhibitors, platelet function inhibitors and a sulfhydryl reactant on the release reaction are presented in Table I. When acetylsalicylic acid (0.28 mM) and antimycin (0.5 μg/ml) were used in combination, the release reaction was inhibited 50 %; whereas alone, maximum inhibition was 20 %.

DISCUSSION

Inhibitors of mitochondrial respiration completely blocked the basal respiration of washed platelets, whereas inhibitors of platelet function, such as prostaglandin E_1 and acetylsalicylic acid had little effect on basal respiration. In contrast, inhibitors of platelet function caused a greater degree of suppression of the thrombin-induced burst rate than the mitochondrial inhibitors.

It is highly likely that the inhibition by oligomycin of basal and the thrombin-stimulated burst of oxygen consumption in the platelet reflects the action of this inhibitor on mitochondrial enzymes. Evidence for the site of action of oligomycin in mitochondria is the release of blocked electron transport by uncouplers of oxidative phosphorylation [7]. Hackenbrock et al. [8] have shown that such effects also are demonstrable in the intact Erhlich ascites tumor cell. Antimycin and rotenone have been observed to inhibit respiration in intact tumor cells [9, 10], similar to their action on isolated mitochondria. The present experiments thus indicate that respiration in human platelets is coupled to the synthesis of ATP via respiratory chain phosphorylation.

Mürer [2] has shown that a combination of glycolytic and mitochondrial inhibitors suppressed the release reaction induced by thrombin 70–80 %, whereas either class of inhibitors alone had minimal effects. Our results indicate that the release reaction is partially dependent on mitochondrial respiration, because compounds that inhibit respiration also partially inhibit the release reaction (Table I).

A possible explanation of the effect of prostaglandin E_1 and acetylsalicylic acid on platelet oxygen consumption is that these compounds act indirectly by inhibiting the release reaction and thereby reducing the requirement for ATP synthesis via respiratory chain phosphorylation. In contrast, the mitochondrial inhibitors have a direct effect on oxygen consumption. Their effect on the release reaction presumably is indirect by reducing the supply of ATP needed to maintain the release of platelet constituents.

Smith and Willis [11] have shown that thrombin stimulates the de novo synthesis of prostaglandins in human platelets and that acetylsalicylic acid selectively inhibits the production of prostaglandins [12]. Our results (Table I) show that acetylsalicylic acid also inhibits the thrombin-induced oxygen burst. Therefore, it is conceivable that part of the thrombin-stimulated oxygen burst is associated with the de novo synthesis of prostaglandins, since prostaglandins are synthesized from polyunsaturated fatty acids by the incorporation of molecular oxygen [13, 14]. It is also possible that the non-mitochondrial oxygen uptake is associated with either mixed function oxidases or other enzymes utilizing oxygen directly.

We conclude from our studies that the burst of oxygen consumption induced by thrombin is the result of multiple events. Part of the oxygen consumption appears to be involved in respiratory chain phosphorylation, possibly related to the maintenance of the release reaction. Thus, it is highly probable that the ATP necessary for the maintenance of the release reaction arises from both respiratory chain and glycolytic phosphorylations.

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