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EFFECT OF EPINEPHRINE ON GLUCOSE METABOLISM AND HYDROGEN PEROXIDE CONTENT IN INCUBATED RAT MACROPHAGES

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Abstract—The effects of epinephrine on glucose metabolism and hydrogen peroxide content were examined in incubated rat macrophages. An increase in the activities of hexokinase and citrate synthase and a reduction in that of glucose-6-phosphate dehydrogenase was found in resident, inflammatory and activated macrophages incubated for 1 hr in the presence of epinephrine. Glucose utilization by incubated resident, inflammatory and activated macrophages was augmented markedly by the addition of epinephrine, whereas lactate formation was depressed. Under the same conditions, there was a 2.6-fold increment of hydrogen peroxide content and of [U-^{14}C]glucose decarboxylation in activated macrophages incubated for 40 min. Similar results were obtained when pyruvate and oxoglutarate was substituted for glucose. These findings suggest that epinephrine may increase hydrogen peroxide production in activated macrophages possibly through a mitochondrial mechanism other than the pentose–phosphate pathway. Between 40 and 90 min of incubation, the content of hydrogen peroxide decreased markedly, and there was no detectable glucose utilization in the presence of epinephrine. These observations are consistent with the idea that this catecholamine stimulates both hydrogen peroxide production and metabolism, the first process being dependent on mitochondrial fuels.

The role played by glucose in macrophage function is well recognized. Studies carried out in incubated [1] and cultured [2] murine macrophages have shown that these cells utilize glucose at very high rates. The oxidation of this metabolite through the pentose–phosphate pathway provides NADPH for hydrogen peroxide (H_2O_2) production by NADPH-oxidase activity [3, 4]. Hydrogen peroxide is associated with the process of phagocytosis [5] and is responsible for the antimicrobial and antitumoral activities of macrophages [6, 7].

Evidence has been presented that stress may influence the function of the immune system of humans and experimental animals [8]. Changes in macrophage function *in vitro* induced by stress have been demonstrated recently [9]. Most of the investigations on this subject have focused on the role of glucocorticoids [10–12]. However, during stress conditions, the sympathetic nervous system is stimulated and epinephrine is secreted from the adrenal glands [13]. There are many indications that this hormone can modulate several parameters of human immunity by acting on β-adrenoceptors [14, 15]. Despite previous investigations [16], systematic studies on the effect of epinephrine on macrophage metabolism and function have yet to be carried out.

Histochemical and ultrastructural analyses have demonstrated that sympathomimetic agents cause cardiac necrosis [17]. It is known that both isoprenaline and epinephrine can undergo oxidation, producing oxygen free radicals [18]. Reports by Noronha–Dutra and Steen [19] support the idea that catecholamines may provoke cell damage by lipid peroxidation. Mononuclear phagocytes have been reported to trigger lymphocyte activation and thus the complex of the immune response [6]. Consequently, any factor which alters macrophage function may lead to impairment of the immune function [20]. Whether epinephrine can cause the same effects in macrophages that are found in cardiac muscle remains to be elucidated.

From the studies mentioned above three questions were raised: (1) Does epinephrine affect glucose metabolism in macrophages? (2) What is the effect of epinephrine on H_2O_2 production by these cells? (3) Is there any correlation between these two metabolic processes in macrophages stimulated by epinephrine? These points were addressed in the present study. For this purpose, the maximal activities of hexokinase, glucose-6-phosphate dehydrogenase and citrate synthase, which are considered key enzymes of glycolysis, the pentose–phosphate pathway, and the Krebs cycle, respectively [21], were determined in rat macrophages incubated for 1 hr in the presence of epinephrine. The rates of glucose utilization, lactate formation and [U-^{14}C]glucose decarboxylation were also measured under similar conditions. The stimulation of hydrogen peroxide production by epinephrine was evaluated in incubated cells, to which either glucose, pyruvate or oxoglutarate was added to serve as the unique fuel.

MATERIALS AND METHODS

Animals. Male Wistar rats weighing 130–150 g

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(about 2 months old) were obtained from the Instituto Butantan, São Paulo. The rats were maintained at 23° and under a light/dark cycle of 12/12 hr.

Chemicals and enzymes. All chemicals and enzymes were obtained from the Sigma Chemical Co. (U.S.A.). [U-^{14}C]Glucose was purchased from Amersham (U.K.). Eagle's culture medium was obtained from GIBCO (U.K.).

Experimental procedure. The rats were killed between 8:00 and 11:00 a.m. by decapitation without anesthesia. After laparatomy, macrophages from the intraperitoneal cavity were collected.

Peritoneal cell preparation. Three types of macrophages were studied: resident, inflammatory and activated. Inflammatory macrophages were obtained by intraperitoneal lavage with 6 mL of sterile phosphate-buffered saline (PBS), pH 7.2, 4 days after the i.p. injection of 3 mL of sterile thioglycollate broth (4%). The same procedure was used to obtain activated macrophages, but the rats were pretreated with 25 mg of ONCO-BCG (Bacille Calmette-Guérin), 7 days prior to the harvest. Cell viability was confirmed by Trypan Blue exclusion (>95%). This procedure was described previously by Costa Rosa *et al.* [22].

Cell culture. Harvested macrophages of the three types were pooled in sterile 20-mL plastic tubes, and centrifuged at 750 *g* for 7 min. The cells were then resuspended in Eagle's minimum essential medium (MEM) supplemented with 10% (v/v) fetal bovine serum (FBS), 2 mM glutamine and 20 μg streptomycin/mL. The cells were transferred into 12-well plates having 1.0×10^6 cells/well in 3.0 mL of culture medium (MEM). After 4 hr at 37° in 95% air/5% CO_2, adherent macrophages were washed vigorously three times with PBS and cultured for 48 hr. After this period, the cells were removed from the flasks by using 10 mM lidocaine [23], centrifuged at 750 rpm at 4°, and incubated. The culture was utilized to eliminate possible contamination with polymorphonuclear cells.

Incubation procedure. Macrophages were incubated (1.0×10^6 cells/flask) at 37° in Krebs–Ringer medium with 2% (w/v) defatted bovine serum albumin and in the presence of either pyruvate (2 mM), glucose (10 mM) or oxoglutarate (2 mM). Epinephrine was prepared in aqueous solution with 0.1 mM ascorbic acid to avoid its oxidation [24]. The hormone was added into the incubation medium at a 43 μM concentration. A previous study [24] showed that concentrations of this hormone varying from 1 to 100 μM present a profound pressure-raising effect; therefore, an intermediary pharmacological dose was utilized. After incubation (which varied from 0 to 90 min), the cells were disrupted by 0.2 mL of 25% perchloric acid (PCA). Protein was removed by centrifugation and the supernatant was neutralized with 40% KOH and a tris-(hydroxymethyl) aminomethane/KOH (0.5 to 2 M) solution for measurement of the metabolites. In another set of experiments, the cells were incubated for 1 hr in the presence of epinephrine, and the enzyme activities were then measured as described below. The absence of Trypan Blue in more than 93% of the cells indicated a low rate of cell death during incubation under control conditions.

Test for cytotoxicity. To evaluate the cytotoxicity effect of epinephrine, activated macrophages were incubated in the presence of the hormone (43 μM) for 90 min. After this period, the cells were collected and centrifuged at a low speed. A microscopic (Nikkon) analysis of the percentage of cells containing Trypan Blue was carried out.

Enzyme activity assays. Enzyme activities were measured as described previously [1, 21, 25, 26]. The cells were homogenized in a glass homogenizer containing 0.4 mL of extraction medium for each enzyme. The extraction medium for hexokinase (EC 2.7.1.1) contained 50 mM Tris–HCl, 1 mM EDTA, 30 mM mercaptoethanol and 20 mM $MgCl_2$ at pH 7.4. The extraction medium for citrate synthase (EC 4.1.3.7) and glucose-6-phosphate dehydrogenase (EC 1.1.1.49) contained 50 mM Tris–HCl and 1 mM EDTA; the final pH was 7.4 for citrate synthase and 8.0 for glucose-6-phosphate dehydrogenase. To all enzyme assays, 0.05% (v/v) Triton X-100 was added to complete the extraction of the enzymes. For the assay of hexokinase, the following medium was used: 75 mM Tris–HCl, 7.5 mM $MgCl_2$, 0.8 mM EDTA, 1.5 mM KCl, 4.0 mm β-mercaptoethanol, 0.4 mM creatine phosphate, 1.8 U creatine kinase, 1.4 U glucose-6-phosphate dehydrogenase, 0.4 mM NADP, pH 7.5. The assay medium for citrate synthase consisted of 100 mM Tris–HCl, 0.2 mM 5,5′-dithiobis-2-nitrobenzoic acid (DTNB), 15 mM acetyl CoA and 0.5 mM oxaloacetate, pH 8.1. The assay medium for glucose-6-phosphate dehydrogenase was composed of 86 mM Tris–HCl, 6.9 mM $MgCl_2$, 0.4 mM NADP, 1.2 mM glucose-6-phosphate, 1.2 U 6-phosphogluconate dehydrogenase, pH 7.6. The final volume of the assay mixtures in all cases was 1.0 mL. Citrate synthase was assayed by following the rate of change in absorbance at 412 nm and the remainder of the enzymes at 340 nm. All spectrophotometer measurements were carried out at 25°. Preliminary experiments established that extraction and assay procedures produced maximum enzyme activities as described in Crabtree *et al.* [27].

Hydrogen peroxide. The production of H_2O_2 was measured by a modification of the method described by Pick and Mizel [28]. The cells were incubated in siliconized flasks (25 mL), in 1 mL PBS, in the presence of either glucose, pyruvate or oxoglutarate, under an atmosphere of 5% CO_2/95% air at 37°. when indicated in the tables, phorbol-myristate-acetate (PMA) was added to the medium at a concentration of 10 ng/mL. After a 1-hr incubation, a solution of phenol red and horseradish-peroxidase (HRPO) was added to the medium to quantify the hydrogen peroxide content. After 10 min, the reaction was stopped with 100 μL of 1 N NaOH and the amount of H_2O_2 formed was measured spectrophotometrically at 620 nm.

Metabolite measurements. Neutralized extracts of the incubation medium were analyzed for measurements of lactate [29] and glucose [30]. The $^{14}CO_2$ produced from [U-^{14}C]glucose was collected as described previously [31].

Statistical analysis. Results are expressed as means

Table 1. Effect of epinephrine on the activities of hexokinase (HK), citrate synthase (CS) and glucose-6-phosphate dehydrogenase (G6PDH) in resident, inflammatory and activated macrophages

Enzymes	Additions	Enzyme activities (nmol/min/10^{10} cells) Macrophages: Resident	Inflammatory	Activated
HK	None	270.4 ± 10.7	522.0 ± 28.2	558.3 ± 37.6
	Epinephrine (43 μM)	592.1 ± 40.1*	677.4 ± 30.0*	739.7 ± 31.5*
CS	None	31.3 ± 1.3	18.4 ± 2.0	50.2 ± 4.2
	Epinephrine (43 μM)	60.1 ± 3.7*	34.7 ± 3.1*	95.4 ± 3.9*
G6PDH	None	10.1 ± 0.9	9.3 ± 0.8	20.6 ± 1.3
	Epinephrine (43 μM)	4.5 ± 0.5*	3.3 ± 0.2*	3.5 ± 0.4*

Values are means ± SEM of twelve rats. The cells were incubated for 1 hr in Krebs–Ringer medium with no saturation (none) or in the presence of epinephrine. For details, see Materials and Methods.

* Significantly different from control (no additions) ($P < 0.05$).

± SEM. Comparisons between groups were assessed by ANOVA and Student's *t*-test at a significance level of $P < 0.05$.

RESULTS AND DISCUSSION

Enzyme activities in macrophages incubated in the presence of epinephrine. The effects of epinephrine on enzyme activities in resident, inflammatory and activated macrophages are shown in Table 1. The addition of epinephrine to the incubation medium caused an increase in the activity of hexokinase in the resident (2.2-fold), inflammatory (29%) and activated (32%) macrophages, and of citrate synthase (2-fold) in all cell types studied. In contrast, epinephrine reduced glucose-6-phosphate dehydrogenase activity in the resident (55%), inflammatory (64%) and activated (83%) macrophages. These findings indicate that this hormone increases the capacity of glucose utilization, and oxidation through the Krebs cycle in macrophages. However, epinephrine may decrease the capacity of the flux of substrates through the pentose–phosphate pathway; this was particularly noticeable in the activated macrophages where glucose-6-phosphate dehydrogenase activity was inhibited by 80%.

Effects of epinephrine on glucose utilization and lactate formation by resident, inflammatory and activated macrophages. Glucose utilization by incubated macrophages was increased markedly when epinephrine was added to the medium (Table 2). In the resident cells, this hormone was shown to double glucose utilization in all of the intervals studied. The inflammatory macrophages incubated in the presence of epinephrine showed increased glucose utilization, as compared to control, after 10 (2.5-fold), 20 (4.4-fold), 30 (5.3-fold) and 40 (6.0-fold) min. After 40 min, there was no additional increment of glucose utilization. Activated macrophages responded even more dramatically to epinephrine addition with 3.4-, 5.4-, and 6.6-fold increases after 10-, 20- and 30-min incubations. Thereafter, these cells did not show additional significant glucose consumption. The response of glucose utilization to epinephrine stimulus was greatest in the resident cells, intermediary in the inflammatory cells and weakest in the activated macrophages, demonstrating that epinephrine increases glucose utilization in the three cell types, as reported by other authors who investigated skeletal muscle [32]. The inability of

Table 2. Effect of epinephrine on glucose utilization and lactate formation in incubated resident, inflammatory and activated macrophages

Period (min)	Glucose utilization (nmol/10^6 cells) Control	Epinephrine (43 μM)	Lactate formation (nmol/10^6 cells) Control	Epinephrine (43 μM)
		Resident macrophages		
0–10	31 ± 2	73 ± 8*	58 ± 2	47 ± 1
0–20	67 ± 3	111 ± 7*	110 ± 7	103 ± 9
0–30	102 ± 6	182 ± 20*	212 ± 10	192 ± 9
0–40	130 ± 10	253 ± 21*	271 ± 18	264 ± 20
0–50	163 ± 14	307 ± 24*	309 ± 18	300 ± 21
0–60	182 ± 17	348 ± 22*	382 ± 9	352 ± 20
		Inflammatory macrophages		
0–10	42 ± 4	107 ± 10*	70 ± 2	61 ± 5
0–20	77 ± 6	342 ± 21*	143 ± 12	151 ± 10
0–30	113 ± 8	598 ± 33*	240 ± 10	218 ± 22
0–40	152 ± 16	911 ± 45*	298 ± 21	306 ± 23
0–50	198 ± 20	972 ± 87*	413 ± 17	298 ± 10
0–60	214 ± 20	967 ± 75*	420 ± 13	290 ± 21
		Activated macrophages		
0–10	59 ± 4	201 ± 13*	51 ± 2	70 ± 2
0–20	115 ± 9	622 ± 40*	109 ± 2	122 ± 10
0–30	148 ± 13	983 ± 46*	132 ± 18	254 ± 21
0–40	197 ± 21	997 ± 87*	183 ± 10	291 ± 19
0–50	239 ± 20	988 ± 73*	240 ± 15	285 ± 22
0–60	287 ± 24	985 ± 99*	298 ± 14	296 ± 21

Values are means ± SEM of nine incubations.

* Significantly different from control ($P < 0.05$).

macrophages to utilize glucose after 30–40 min when incubated in the presence of epinephrine will be discussed later.

Assuming that for each mol of glucose utilized 2 mol of lactate are produced, it is possible to calculate the percentage of glucose conversion into lactate during incubation, in the three cell types; for 100% conversion, the rates of lactate formation were 2-fold higher than those of glucose utilization. In resident and inflammatory macrophages, almost 100% (97%; mean of twelve measurements) of the glucose was converted into lactate when these cells were incubated under control conditions (Table 2). The addition of epinephrine to the medium decreased the conversion of glucose into lactate to 47% (mean of six measurements) in the resident macrophages and to 19% (mean of six measurements) in the inflammatory cells, as compared with control (no addition). Clearly, the reducing effect of epinephrine on lactate formation was more pronounced in the inflammatory cells. Incubated activated macrophages converted 47% (mean of six measurements) of the glucose into lactate under control conditions (Table 2). The observation that the activation process of macrophages is accompanied by a significant decrease of lactate production from glucose is an important finding. When epinephrine was added to the medium, this proportion diminished to about 13.8% (mean of six measurements), in accordance with what was verified in the resident and inflammatory cells, this hormone also markedly decreased lactate formation from glucose in the activated macrophages. This metabolic fate suggests that, in the presence of epinephrine, pyruvate produced from glucose is mainly oxidized through the Krebs cycle. To investigate this possibility, the rate of [U-^{14}C]glucose decarboxylation was measured in activated macrophages after 30 min of incubation. The results obtained (expressed as μmol/10^6 cells) were: 0.49 ± 0.03 and 1.26 ± 0.06 (mean $\pm$ SEM of five incubations) for control and epinephrine addition, respectively. Therefore, this catecholamine does enhance glucose oxidation through the tricarboxylic acid (TCA) cycle by 2.6-fold. In the studies concerning muscle [33], a higher number of adrenergic receptors was found in the mitochondria-rich muscles as compared to white fibers. Spencer and colleagues [34] also found that epinephrine increases the concentration of TCA cycle intermediates in human skeletal muscle. In addition, propranolol, a well-known β-adrenergic receptor antagonist, was proved to inhibit heart mitochondria respiration [35]. The implications of these metabolic effects of epinephrine on H_2O_2 content of incubated activated macrophages were examined next.

Effect of epinephrine on hydrogen peroxide production by activated macrophages. The results of hydrogen peroxide content in activated macrophages incubated in the presence of 10 mM glucose are shown in Table 3. Incubations carried out in the absence of glucose for 90 min showed the following rates of H_2O_2 production: Control: No addition, 0.80 ± 0.03 nmol/10^6 cells; PMA addition, 0.97 ± 0.10; Epinephrine: No addition, 1.2 ± 0.10; plus PMA, 1.31 ± 0.12. Therefore, in the absence of exogenous substrates, there was no significant production of H_2O_2. These findings permit the evaluation of the metabolic requirements of these cells for hydrogen peroxide formation and metabolism. The content of H_2O_2 in the cell depends on its rate of production and metabolism. Therefore, in the experiments described here, both processes have to be considered to discuss the effect of epinephrine on hydrogen peroxide content of the incubated macrophages. PMA is well known to increase hydrogen peroxide production by stimulating protein kinase C and NADPH oxidase activities [36]. This phorbol ester was used so that a comparison could be made between its effect and that of epinephrine on H_2O_2 formation. It is notable that the catecholamine potentiated the effect of PMA especially when a 1-hr incubation period was used (Table 3). The mechanisms involved, however, remain to be examined.

Epinephrine increased H_2O_2 content markedly in activated macrophages (by 2.6-fold, in either the presence or absence of PMA, in the first 30 min of incubation; it had only a mild effect in the interval between 30 and 40 min of incubation (Table 3), after which, the content of H_2O_2 gradually decreased. Coincidentally, glucose utilization was not measurable after this same interval (30–40 min) when the cells were incubated in the presence of epinephrine. These findings suggest that H_2O_2 production stimulated by this catecholamine may be related to glucose metabolism.

A definitive explanation for the inhibition of glucose utilization after 30–40 min still remains to be found. However, the cytotoxic effect of H_2O_2 has been widely reported in several tissues, e.g. brain [37], lung [38], and heart [17, 18]. As indicated by the Trypan Blue exclusion method, the percentages of viable macrophages after incubation for 90 min were 94 ± 2 and 69 ± 2.7% (mean $\pm$ SEM of five incubations), for the control and after epinephrine addition, respectively. As a consequence, the enhanced content of intracellular H_2O_2 due to the effect of epinephrine (probably in the mitochondrial compartment as discussed later) may inhibit the activity of glycolytic enzymes, e.g. glycerol-phosphate dehydrogenase [39] and pyruvate dehydrogenase [40], and hence, the capacity of macrophages to utilize glucose thereby resulting in toxicity. The inability of macrophages to utilize glucose may impair the provision of reducing equivalents for H_2O_2 formation after 30–40 min of incubation, as observed in Table 3. The reduction of the H_2O_2 produced after this interval suggests that this hormone may act as a stimulator of H_2O_2 metabolism. Whether this detoxifying effect of epinephrine is mediated by mitochondrial glutathione peroxidase activity [41, 42] deserves to be examined.

Surprisingly, this catecholamine elevated H_2O_2 formation with a concomitant activation of glucose decarboxylation through the TCA cycle (2.6-fold increase in both processes). To further examine the importance of the Krebs cycle in this phenomenon, the effect of epinephrine on hydrogen peroxide production was evaluated in incubated macrophages

Table 3. Effect of epinephrine on the production of hydrogen peroxide by activated macrophages in the presence and absence of phorbol-myristate-acetate (PMA)

Period (min)	Hydrogen peroxide (nmol/10^6 cells)			
	Control		Epinephrine	
	No addition	Plus PMA	No addition	Plus PMA
0–10	2.1 ± 0.2	4.0 ± 0.2	5.4 ± 0.2*	10.2 ± 0.8*
0–20	5.1 ± 0.3	13.2 ± 0.9	9.1 ± 0.7*	30.7 ± 2.2*
0–30	5.9 ± 0.3	18.4 ± 0.9	15.3 ± 1.1*	66.0 ± 2.1*
0–40	7.5 ± 0.5	27.9 ± 1.1	19.1 ± 0.6*	73.1 ± 2.4*
0–50	12.0 ± 0.7	30.4 ± 1.4	14.8 ± 0.3*	74.7 ± 3.2*
0–60	13.3 ± 0.7	37.4 ± 0.5	3.9 ± 0.2*	53.1 ± 1.5*
0–70	14.3 ± 1.1	41.1 ± 2.3	2.1 ± 0.2*	21.3 ± 0.9*
0–80	16.0 ± 1.0	45.1 ± 2.8	2.2 ± 0.1*	9.3 ± 0.3*
0–90	17.4 ± 0.9	48.3 ± 3.5	1.9 ± 0.2*	4.4 ± 0.5*

Values are means ± SEM of nine incubations. All incubations were carried out in the presence of 10 mM glucose. Incubations performed in the absence of glucose for 90 min showed the following rates of H_2O_2 production: Control: No addition, 0.80 ± 0.03 nmol/10^6 cells; and plus PMA, 0.97 ± 0.10; Epinephrine: No addition, 1.2 ± 0.10; and plus PMA, 1.31 ± 0.12. To verify the amount of H_2O_2 assayed, a catalase control was run for a 50-min period, which represents the period of maximal hydrogen peroxide content. When the cells were incubated with 13,000 U of catalase (Boehringer, Cat. No. 106836), H_2O_2 production could not be detected (N = 4). Results of incubations carried out in the absence of catalase are presented above.

* Significantly different from control ($P < 0.05$) for no addition and in the presence of PMA.

Table 4. Effect of epinephrine on hydrogen peroxide production by activated macrophages incubated in the presence of glucose, pyruvate or oxoglutarate

Period (min)	Hydrogen peroxide (nmol/10^6 cells)		
	Glucose	Pyruvate	Oxoglutarate
0–15	7.30 ± 0.8	7.20 ± 0.4	2.71 ± 0.3
0–30	15.3 ± 1.1	16.0 ± 1.4	7.98 ± 0.45
0–45	17.0 ± 1.0	15.7 ± 1.2	9.01 ± 0.81
0–60	3.9 ± 0.2	5.07 ± 0.3	9.72 ± 0.72

Values are means ± SEM of nine incubations. Epinephrine was added in all incubations at a concentration of 43 μM. The following concentrations of substrates were used: glucose (10 mM), pyruvate (2 mM), and oxoglutarate (2 mM).

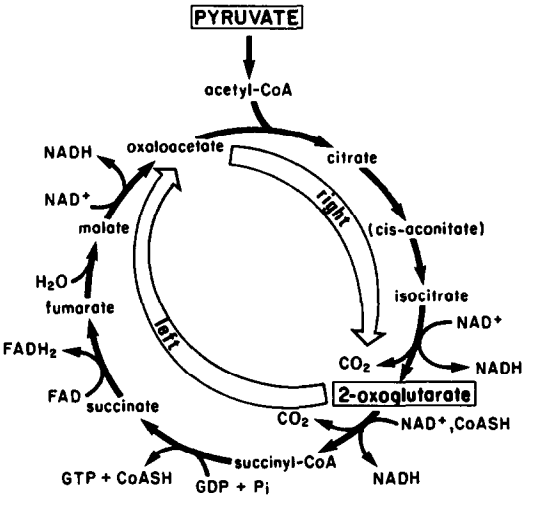

Fig. 1. Pathway of oxidation of pyruvate and oxoglutarate entering by the right- and left-hand sides of the tricarboxylic acid cycle, respectively.

having as unique fuels, pyruvate or oxoglutarate, intermediates of the TCA cycle.

Effect of epinephrine on hydrogen peroxide production in the presence of pyruvate or oxoglutarate. Hydrogen peroxide formation by epinephrine-stimulated macrophages was similar in the presence of both glucose and pyruvate (Table 4). These findings indicate the important role played by the TCA cycle for H_2O_2 production in the presence of epinephrine. Interestingly, this effect seems to be rapidly triggered on the right-hand side of the Krebs cycle (Fig. 1): pyruvate-acetyl-CoA-citrate-aconitate-isocitrate-oxoglutarate [43, 44]. Indeed, oxoglutarate, entering the TCA cycle by the left-hand side (oxoglutarate-succinyl-CoA-succinate-fumarate-malate-oxaloacetate), as shown in Fig. 1 [43, 44], caused a delay in the formation of H_2O_2. The TCA cycle may generate NADH and $FADH_2$ for H_2O_2 production. However, how the formation of hydrogen peroxide actually occurs must be investigated further.

Concluding remarks. The differences observed between inflammatory and activated macrophages

concerning the activities of citrate synthase and glucose-6-phosphate dehydrogenase and the rates of glucose utilization and lactate formation may be related to the functions of these cells. For instance, activated macrophages are the only cells to exhibit a respiratory burst among the three types examined [45].

When we look at the metabolism of glucose in macrophages, it is clear that epinephrine stimulated glucose utilization and its oxidation by the Krebs cycle, as it suppressed the capacity of the flux of substrates through the pentose–phosphate pathway. Hydrogen peroxide production by activated macrophages was increased markedly by epinephrine during 30–40 min of incubation. This effect depended strictly on the provision of exogenous substrates for the cells. Hormonal activation of H_2O_2 formation by macrophages is unlikely to rely on NADPH generated from the pentose–phosphate pathway. The fact that the rates of hydrogen peroxide production were similar when pyruvate or glucose was the sole fuel in the incubation medium suggests the important role played by the TCA cycle in mediating the effect of this catecholamine. The increase in [U-^{14}C]-glucose decarboxylation by epinephrine strongly supports this suggestion. In addition, it seems that an immediate production of H_2O_2 will occur preferentially in the right-hand side of the TCA cycle, whereas the left-hand side may play an important role during a prolonged response (Fig. 1).

Beyond enhancing hydrogen peroxide formation, epinephrine may stimulate H_2O_2 metabolism and this effect does not seem to depend on energy provision. Whether glutathione plays a role (via glutathione peroxidase activity), as shown in the liver [41, 42], for H_2O_2 metabolism in mitochondria of macrophages incubated in the presence of this catecholamine remains to be examined.

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