Metabolic Degradation of Prostaglandin E_1 in the Lung and Kidney of Rats in Endotoxin Shock[1] (37623)

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It is well established that prostaglandins are effectively inactivated by prostaglandin dehydrogenase (PGDH) in the lung and kidney, and thus very low concentrations of prostaglandins are found in the plasma of healthy individuals (1–3). Several investigators (4–7) have postulated that the biosynthesis and release of prostaglandins are increased in different pathological disorders and that prostaglandins act as chemical mediators in inflammatory and immunological reactions. Recently, the plasma prostaglandin levels were also found to be increased in animals in endotoxin shock (8–10). It is possible that the increased plasma concentrations may be due not only to enhanced biosynthesis and release, but also to decreased degradation of prostaglandins in lungs and kidneys in this condition. Since the cellular morphology, and biochemical and physiological functions of the lung and other organs were markedly impaired in animals with advanced shock states (11, 12), the present study was undertaken to determine whether the metabolic degradation of prostaglandin E_1 (PGE_1) is altered in the lung and kidney of rats in lethal endotoxin shock.

Materials. PGE_1 used in this study was supplied by Dr. J. E. Pike, Upjohn Company, Kalamazoo, Mich. ^3H-prostaglandin E_1 (^3H-PGE_1) and *E. coli* endotoxin (lipopolysaccharide batch no. 209684) were purchased from the New England Nuclear Corp., Boston, Mass. and the Difco Laboratories, Detroit, Mich., respectively.

Methods. Twenty-four male Holtzman rats weighing between 200 and 250 g, which previously had been fed *ad libitum,* were fasted over night. A LD_{80} (10 mg/kg) of endotixin was given ip to 12 rats, whereas an equivalent volume of 0.9% NaCl solution was given ip to the remaining 12 rats as controls. Eight hours after the injection of endotoxin or 0.9% NaCl solution, all rats were anesthetized with the ip injection of sodium pentobarbital (35 mg/kg) and systemic arterial pressure was measured with a Statham pressure transducer (P23AA) connected to a polyethylene catheter (PE 90) which was cannulated into a femoral artery. Thereafter, the lungs and kidneys were excised and cooled immediately, and then were homogenized at 4° in 9 vol of Bücher medium (20 m*M* KH_2PO_4, 70 m*M* K_2HPO_4, 27.6 m*M* nicotinamide, 3.6 m*M* $MgCl_2$, pH 7.4) with a Potter-Elvehjem tissue grinder. The homogenates were centrifuged at 10,000*g* for 20 min with a Sorvall centrifuge (Model RC 2). The protein concentration of the supernatant was determined by the method described by Lowry *et al.* (13) and averaged 12.1 ± 0.2 mg/ml. After 25 μCi/ml of ^3H-PGE_1 (28 Ci/mmole), 50 ng/ml of PGE_1 and 2 m*M* of NAD^+ were added, the supernatant was incubated at 37° in a Dubnoff temperature-controlled water bath shaker. Before, 5 and 15 min after the incubation was started, an aliquot (5 ml) of the incubation media was pipetted into tubes containing 0.5 ml of 2 *N* HCl solu-

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tion to terminate the reaction and to acidify to pH 3.0. ^{3}H-PGE_1 and its metabolites were extracted twice with ethyl acetate. The extract was filtered through a Whatman No. 3 paper and washed with distilled water. After evaporation at 40° under reduced pressure, the extract was dissolved in 1 ml of 95% ethanol. An aliquot (100 μl) of the extract solution and 10 μl of PGE_1 standard solution (5 mg/ml) were applied on activated (110° for 1 hr) Silica gel G thin layer chromatography plate, and separated at room temperature using a solvent system (upper layer) of ethyl acetate-isooctane-acetic acid-water (100:20:10:110, v/v). After drying at room temperature, a PGE_1 spot was identified by brief exposure to iodine vapor, and scraped into a counting vial. After dissolving with 2 ml of methanol and then adding 15 ml of counting solution (2), sp act of the samples were counted with a Packard Tricarb liquid scintillation spectrometer (3000 Series). The experimental data in this study were evaluated statistically using the t test (14). Values of p less than 0.05 were accepted as indicating a significant difference between compared values.

Results. Eight hours after the injection of 0.9% NaCl or endotoxin, systemic arterial pressure of control rats and endotoxemic rats was 121 ± 4 and 52 ± 6 mm Hg, respectively. As shown in Fig. 1, the incubation of the lung homogenates from control rats resulted in a rapid disappearance of ^{3}H-PGE_1, and average 31.0% and 11.2% of ^{3}H-PGE_1 remained after 5- and 15-min incubations, respectively. This is essentially in agreement with previous observations from this laboratory (2, 3) and others (15, 16). On the other hand, the incubation of the lung homogenates from endotoxemic rats caused a very slow disappearance of ^{3}H-PGE_1, and an average of 84.2% and 76.4% remained after 5- and 15-min incubations, respectively. This indicates that, as compared with control lung homogenates, the metabolic degradation of ^{3}H-PGE_1 is considerable slower ($p < 0.001$) in endotoxemic lungs.

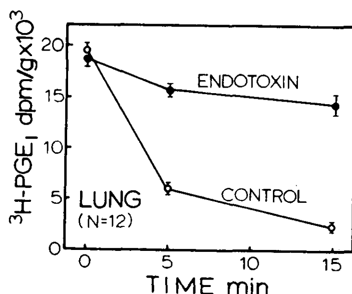

FIG. 1. Metabolic degradation of ^{3}H-PGE_1 by the lung homogenates from control rats and from rats in endotoxin shock. Each dot represents the average values of 12 experiments with SEM.

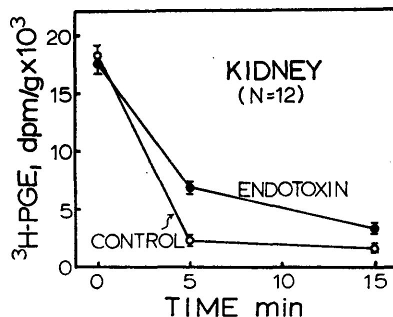

FIG. 2. Metabolic degradation of ^{3}H-PGE_1 by the kidney homogenates from control rats and from rats in endotoxin shock.

As shown in Fig. 2, the incubation of the control kidney homogenates caused a disappearance of ^{3}H-PGE_1 at even faster rate than that of the lung homogenates, an average of 11.8% and 8.9% remained after 5- and 15-min incubations, respectively. However, as seen with the lung homogenates, the rate of the metabolic degradation of ^{3}H-PGE_1 in the shock kidney homogenates was significantly ($p < 0.01$) slower than that in the control kidney homogenates, an average of 36.1% and 14.7% of ^{3}H-PGE_1 remained after 5- and 15-min incubations, respectively. Obviously, the rate of metabolism of ^{3}H-PGE_1 in the shock kidney homogenates was significantly faster than that in the shock lung homogenates.

Discussion. As shown previously (1–3, 15, 16), healthy rat lung and kidney metabolize PGE_1 very rapidly and convert it to 15-keto-PGE_1 by NAD^+-dependent PGDH (1, 2, 16, 17). Thereafter, 15-keto-PGE_1 is further metabolized by Δ^{13}-prostaglandin reductase in the lung and by nonspecific beta- and omega-oxidation in the liver (16). However, the major and initial inactivation of prostaglandins released or administered takes place by the oxidation of the secondary alcohol

group of prostaglandins by PGDH (15–17). This study shows that the rate of the metabolic degradation of PGE_1 in the shock lung and kidney is considerably slower than that in the controls. Although the metabolites of PGE_1 were not elucidated in this study from the previous observations in this laboratory (2, 3) and by others (1, 15–17), the disappearance of the substrate (PGE_1) accounts for its conversion to 15-keto-PGE_1 by PGDH. It has been shown that the LD_{80} of *E. coli* endotoxin caused marked cellular damages and physiological and biochemical changes in the lung and kidney 4–6 hr later (11, 12). The present results showing the impairment of the metabolic degradation of PGE_1 in the lung and kidney appear to concur with the biochemical changes which took place in advanced shock. Very recently, several workers (8–10) reported that the plasma prostaglandin levels are increased in animals in endotoxin shock. It is reasonable to assume that the biosynthesis and release of prostaglandins in a variety of organs and tissues are enhanced in endotoxin shock (4, 6). However, it has been shown that prostaglandins are very effectively metabolized in the lung by a single circulation and approximately 95% of the injected prostaglandins would disappear following a single circulation through the lung (18, 19). Hence, it is reasonable to assume that one of the major mechanisms responsible for the increased plasma prostaglandins levels is a disturbance in the enzymatic activities of PGDH in the lung and kidney of animals in endotoxin shock. Obviously, it is possible that other metabolic processes such as Δ^{13}-prostaglandin reductase and beta- and omega-oxidation enzymes may also be impaired by endotoxin in these animals.

Summary. The metabolic degradation of PGE_1 was studied by the incubation of ^{3}H-PGE_1 with the lung and kidney homogenates from control and endotoxemic rats. The control lung and kidney homogenates metabolized PGE_1 very rapidly, whereas the shock lung and kidney homogenates inactivated PGE_1 at considerably slower rates. It is suggested that the increased levels of prostaglandins found in animals with endotoxin shock may be partly due to the impairment of prostaglandin metabolism in the lungs and kidneys.

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