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Release of labile cyclo-oxygenase products of arachidonic acid from kidney by endotoxin[1]

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Summary. The possible release of prostaglandin (PG)-like substances was studied in isolated perfused kidneys from intact and from intrarenal endotoxin (Lipopolysaccharide-LPS)-injected rabbits, using the venous outflow superflow superfuse assay organ technique. Injection of LPS into the renal artery of an LPS-pretreated kidney caused a release of thromboxane A_2 (TXA_2) and prostacyclin (PGI_2)-like materials into the venous effluent as verified by the responses of the specific assay organs. No detectable release of these substances was found in the venous outflow of LPS-injected intact kidney. The possible role of labile cyclo-oxygenase products of arachidonic acid in the Shwartzman reaction is discussed.

It has been shown that many hemodynamic events induced by LPS are accompanied by an increased release of PGs from the lung[2]. This observation led to the assumption that PGs might be involved in pathological derangements of the tissues by LPS. The results of a recent study indicate that LPS may cause the release of PGs from the lung in in vivo but not in vitro conditions[3]. It has been shown that kidney contains stable and unstable PGs and this organ can readily metabolize these lipids[4]. The present study was undertaken to investigate the possible action of LPS on the release of PGs from isolated perfused intact and LPS-pretreated rabbit kidney.

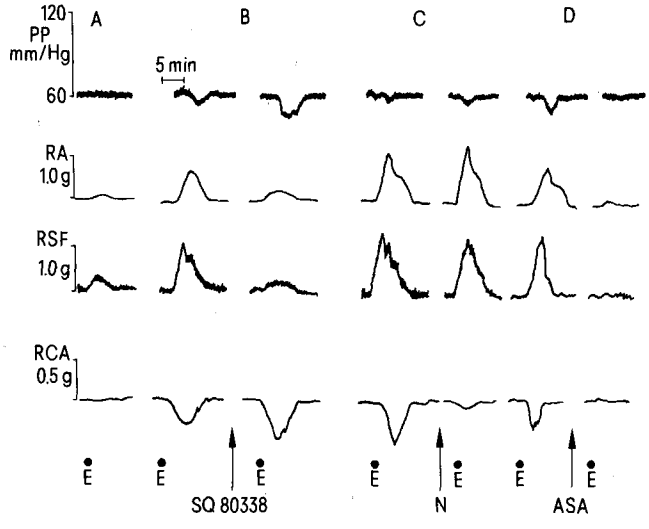
Material and methods. The experiments were carried out on adult rabbits of either sex weighing 2.0–3.0 kg. The animals were anesthetized with sodium pentobarbital (35 mg/kg i.v.) and the left kidneys were isolated, perfused with warmed (37 °C) and oxygenated (5% CO_2 in O_2) Krebs' solution as described previously[5]. A group of rabbits were prepared for LPS treatment. For this procedure the animals were anesthetized with sodium pentobarbital and after the anesthesia was established an incision was made on the left flank and the kidney was exposed. LPS (*E. coli*, lipopolysaccharide W.0111:B_4, Difco Laboratories) was injected intracortically in the upper pool of the kidney at the dose of 100 µg in 0.1 ml saline. In a group of animals (4 rabbits) only saline (0.1 ml) was injected intracortically. After that the flank was surgically sutured and the animals were kept in room temperature in separate rabbit cages. They were allowed food freely, and ordinary water. After 24 h the LPS-injected kidneys were isolated, perfused with Krebs' solution. The venous return was continously superfused over a series of assay organs prepared in cascade[6]. The contamination of the venous outflow by urine was eliminated by a polyethylene cannula inserted into the ureter.

Spirally cut rabbit aorta (RA)[7], rat stomach fundus (RSF)[8] and rabbit coeliac artery (CA)[9] were used as assay organ. These assay organs were selected for the separation of TXA_2 and PGI_2-like material in the kidney outflow. TXA_2 consistently produced a contractile response in 3 assay organs[4,9] while PGI_2 a definite relaxation in CA with extremely low concentrations[9]. The initial tension applied was 0.5–1.0 g, and the contractions were recorded on a Grass polygraph (Model 79

D) through force-displacement transducers (Grass FT-03). Perfusion pressure (PP) of the kidney was also recorded by a Statham pressure transducer (P 23 Dc). Kidneys were allowed to perfuse with normal Krebs' solution and the assay organs were superfused with the venous outflow of the kidney for a 1-h equilibration period, then LPS was applied through the renal artery by bolus injections. Aspirin (lysine acetylsalicylic acid: ASA, Bayer, Germany) as an inhibitor of PG-biosynthesis[10], nicotine (nicotine tartrate:N, Geigy, Switzerland) as an inhibitor of PGI_2-biosynthesis[11] and SW 80338 (Squibb, USA) as an inhibitor of TXA_2 synthesis[12] were added to the perfusion medium, depending on the experimental procedure. Sodium salt of PGI_2 (Upjohn, USA) was dissolved in 0.1 M Na_2CO_3 at the concentration of 10^{-4}M (pH:11) and further dilutions were made in Krebs' solution immediately before use.

Results and discussion. LPS, when injected through the renal artery of intact kidney at the concentrations of 50–100 µg/ml, did not induce a response in PP but caused a slight contraction in RA and RSF. Direct superfusion of LPS over the assay organs at the same concentrations produced almost the same responses. These findings indicate that LPS did not cause a measurable release of active substance(s) from the kidney, supporting the results obtained in isolated lung tissue[3]. LPS (50–100 µg/ml), however, when tested in LPS-treated kidney produced a fall in PP, a contraction in venous outflow superfused RA and RSF and a relaxation in CA. The contraction of RA and RSF could partly be prevented by SQ 80338 (10^{-6}M) but decrease in PP and relaxation of CA slightly potentiated. Inhibition by SQ 80338, a specific inhibitor of TXA_2-synthetase[12], of the responses in RA and RSF suggests that LPS activates the TXA_2 pathway in LPS treated kidney. The increase in the fall of PP and in the relaxation of CA following SQ 80338 support this speculation, since more substrate would be available for the production of PGI_2. N, however, as specific inhibitor of PGI_2-synthetase[11], significantly prevented the relaxation induced by LPS without altering the responses obtained in PP, RA and RSF. ASA (10^{-5}M), as an inhibitor of cyclo-oxygenase, when added to the perfusion medium completely abolished the responses obtained in all parameters (fig.). However, bolus injection of PGI_2 into the renal artery produced a long-lasting fall in PP and a concentration-dependent relaxation in CA.

Depending on the response of CA, the possible release of PGI_2-like substance by LPS was calculated and found to be 80.0 ± 12.0 pg/ml/5 min (n:28) while no detectable release of PGI_2-like substance was found in intact kidney. These results indicate that LPS can cause the release of labile PGs from isolated perfused kidney pretreated with LPS. The production of generalized Shwartzman reaction is observed in the rabbits after 2 i.v. injections of LPS given 20–22 h apart [13]. A similar generalized Shwartzman reaction is also observed following a 2nd i.v. injection of LPS in the rabbits which were given LPS into the renal cortex beforehand. Light microscope studies have shown local necrosis, leucocytic infiltration and vascular dilatation in LPS-injected kidney (in preparation for publication). Thus, it is assumed that prior injection of LPS into the renal cortex may prepare a basis for the release of PGs (mainly TXA_2 and PGI_2) for the 2nd injection of LPS into the renal artery. The Shwartzman reaction was first described as a local skin lesion basically due to the LPS of an injected bacterial culture[14]. This reaction has also been observed in rabbit colon[15] and heart[16]. Several authors have presented evidence that kinin-peptides, serotonin may have some role in the production of this reaction[16,17,18]. The results presented here suggest that labile cyclo-oxygenase metabolites of arachidonic acid released form LPS pretreated kidney by LPS may participate in the production of generalized Shwartzman-like phenomena.



Recorder tracings from isolated perfused rabbit kidney and venous efferent superfused rabbit aorta (RA), rat stomach fundus (RSF) and rabbit coeliac artery (CA) prepared in cascade. Perfusion pressure (PP) was also recorded simultaneously. Column A shows the effect of endotoxin (E) injected into the renal artery of an intact kidney. Slight contraction was observed in RA and RSF. Direct superfusion of endotoxin over the assay organs produced similar responses indicating that no appreciable release of active material occurred in intact kidney on treatment with endotoxin. Columns B, C, D represents the effect of endotoxin before and after SQ 80338 (10^{-6} M), nicotine: N (10^{-6} M) and aspirin: ASA (10^{-5} M) in different endotoxin-injected rabbit kidneys. Inhibition by SQ 80338, a TXA_2-synthetase inhibitor, of the contractile responses of RA and RSF, and potentiation of the relaxation observed in CA and renal PP indicate the presence of TXA_2 and PGI_2 in the venous return. Prevention by N of the relaxation observed in CA indicates the release of PGI_2. Prevention by ASA of all responses again indicates the release of labile cyclo-oxgenase products (TXA_2 and PGI_2) in the venous outflow of endotoxin-injected perfused kidney by endotoxin. Endotoxin was injected into the renal artery at a dose of 50 µg.

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