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## ROLE OF THROMBOXANE A<sub>2</sub> AND PLATELET ACTIVATING FACTOR IN EARLY HAEMODYNAMIC RESPONSE TO LIPOPOLYSACCHARIDE IN RATS

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The mechanism of early pulmonary and systemic haemodynamic response to intravenous infusion of LPS from *Escherichia coli* was investigated in anaesthetised Wistar rats. 10 mg of LPS given at a rate of 4 mg/kg/min but not at a rate of 1 mg/kg/min induced an increase in pulmonary arterial pressure (PAP) and a fall in systemic arterial pressure (SAP). Pretreatment with a PAF receptor antagonist; WEB 2170 (5 and 25 mg/kg) inhibited both PAP and SAP responses to LPS (4 mg/kg/min) while an inhibitor of thromboxane synthesis; Camonagrel (10 and 20 mg/kg) abolished PAP response without a major effect on SAP response to LPS. In conclusion, both PAF and TXA<sub>2</sub> mediate LPS induced rise in pulmonary arterial pressure while LPS-induced fall in systemic arterial pressure is mediated by PAF.

**Key words:** *lipopolysaccharide, haemodynamic response, thromboxane, platelet-activating factor, rat*

### INTRODUCTION

Bacterial endotoxin from the outer membrane of Gram-negative bacteria e.g. lipopolysaccharide (LPS) is responsible for the development of hypotension and vasoplegia of septic shock. These late vascular effects of LPS were shown to be associated with the induction of nitric oxide synthase (NOS-2) (1—3). However, mechanisms which are involved in immediate pulmonary hypertension and systemic hypotension induced by LPS injection (4) have not been fully characterized. Moreover, the immediate haemodynamic response to LPS was sometimes treated as an unimportant epiphenomenon (5).

It was proposed that nitric oxide (NO) produced by endothelial NOS-3 contributed to the immediate LPS-induced hypotension (6), but in other studies non-selective NOS inhibition was shown to potentiate, paradoxically, LPS-induced immediate hypotension (7, 8). Katori (9) pointed to a possible involvement of bradykinin in mediating LPS-induced hypotension, but again other authors disagree with that opinion (10).

On the other hand, LPS-induced rise in pulmonary arterial pressure was ascribed to thromboxane A<sub>2</sub> (TXA<sub>2</sub>) (11), which is in line with findings of an increased level of thromboxane A<sub>2</sub> metabolites in septic shock (12). Increased generation of platelet activating factor (PAF) was also demonstrated in patients with septic shock, however, PAF antagonists inhibited (13, 14) or had no effect on early haemodynamic response to LPS (15).

Our recent observation on the pneumotoxic properties of PAF and TXA<sub>2</sub> in endotoxaemia in rats (16) prompted us to investigate the role of these mediators in the early hemodynamic response to intravenous infusion of LPS from *Escherichia coli* in anaesthetised rats.

## MATERIALS AND METHODS

### *Animals*

Male Wistar rats weighing 300—450g. (Lod: WIST BR, from Animal Laboratory at Polish Mother Memorial Research Institute Hospital in Łódź, Poland) were used for experiments. Animals were maintained at  $22 \pm 1^\circ\text{C}$ , with a 12/12 light/dark cycle and were allowed water and standard rat chow *ad libitum* before experiment.

### *Surgery and instrumentation.*

Rats were anaesthetised by intraperitoneal injection of thiopental, (120 mg/kg) and fentanyl, (500 µg/kg).

Tracheotomy was performed and polyurethane cannula was inserted into the trachea. Rats were ventilated with room air at the rate of 75 breaths/min with the tidal volume of 10 ml/kg (rodent ventilator Ugo Basile No 7025). Both jugular veins were cannulated with polyurethane catheters for drug administration, the left carotid artery was cannulated for mean systemic blood pressure (SAP) measurements. Sternal thoracotomy was performed to expose heart and lungs, and lobar branch of the left pulmonary artery was cannulated for mean pulmonary artery pressure (PAP) measurement. Catheters in the left carotid artery and in the lobar branch of the pulmonary artery were tied securely with surgical thread and connected to pressure transducers (Isotec, Plugsys Mini Case Type 609-Hugo Sachs Elektronik). Both SAP and PAP were continuously recorded on a chart recorder (Linear 0585). Calibration of the pressure transducer was performed

with the mercury column manometer before each experiment. After the surgery and cannulation had been completed a period of 30 minutes was allowed for stabilization of systemic and pulmonary arterial pressures.

### *Experimental protocol and data analysis.*

In pilot experiments two groups of rats received the same total dose of LPS (10 mg/kg) but given at different infusion rates, i.e. 1 mg/kg/min ( $n = 4$ ) or 4 mg/kg/min ( $n = 4$ ). The second rate of LPS administration (total dose of 10 mg/kg at a rate of 4 mg/kg/min) was chosen for further experiments because only at that rate of infusion LPS produced a haemodynamic response we wanted to study, i.e. an immediate rise in PAP and a fall in SAP.

The involvement of PAF and TXA<sub>2</sub> in LPS response was investigated using a PAF-receptor antagonist, WEB 2170 (5 mg/kg or 20 mg/kg i.v.) and a TXA<sub>2</sub> synthase inhibitor, Camonagrel (CAM) (10 mg/kg or 25 mg/kg i.v.), respectively. Both WEB 2170 and CAM were injected intravenously 30 min prior to the infusion of LPS.

A period of initial 40 minutes that followed LPS infusion was considered to represent the early haemodynamic response to LPS. SAP and PAP measurements taken at intervals of 0; 2.5; 5; 10; 20; 40 minutes after starting of LPS infusion were analysed.

Results were calculated as arithmetical means  $\pm$  SEM of changes in SAP ( $\Delta$ SAP) or PAP ( $\Delta$ PAP) from time „0” which were induced by the experimental procedures. Differences between means were evaluated by the unpaired Student T-test, which was preceded by F-test to confirm the homogeneity of variances in groups. P value of less than 0.05 was considered to be statistically significant.

### *Reagents*

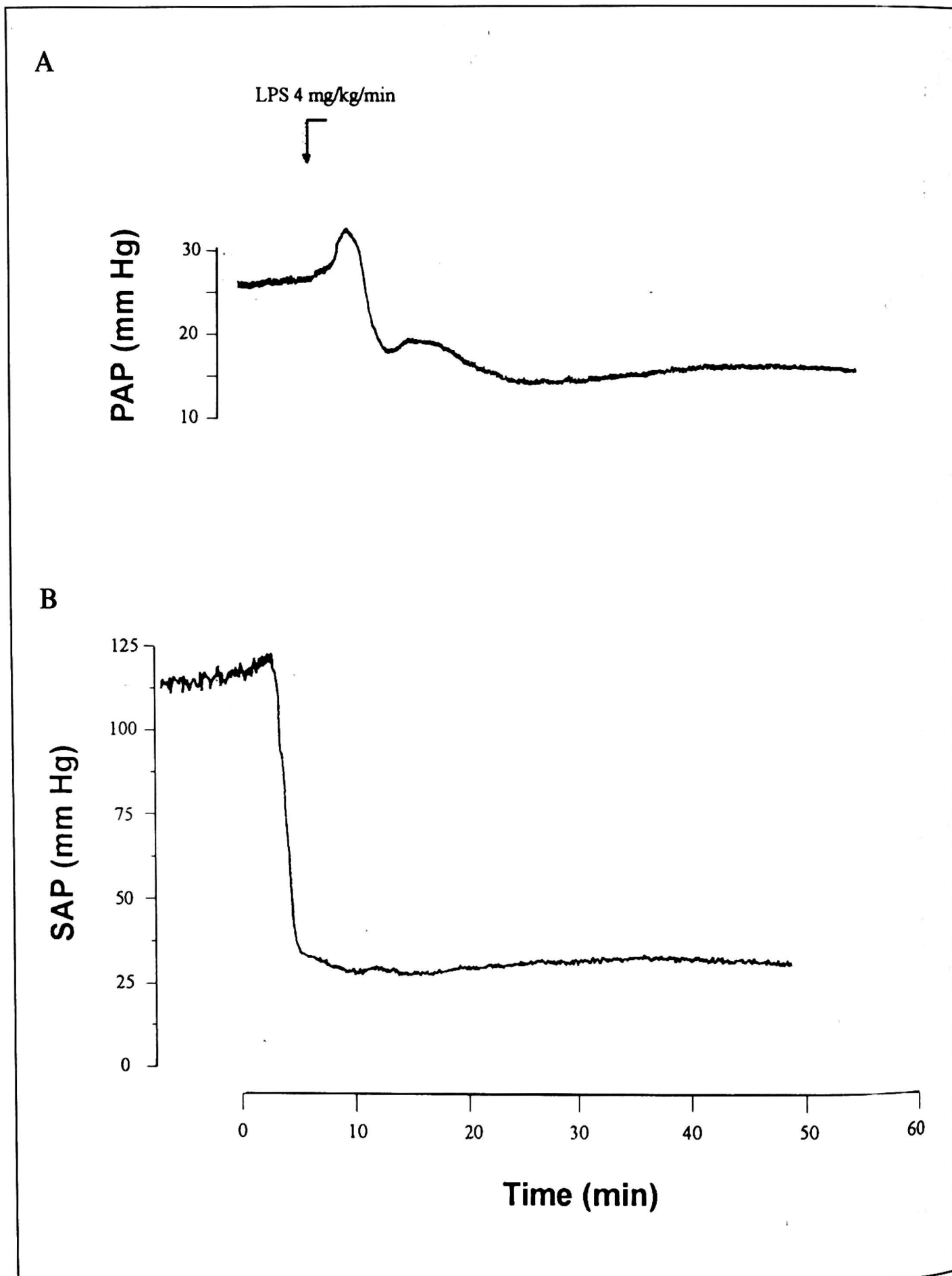
Lippopolysaccharide (LPS, *Escherichia coli* serotype 0127:B8) was purchased from Sigma Chemical Co. International, Thiopental from Biochemie GmbH Germany, Fentanyl from Polfa, Poland. Camonagrel was a gift from Ferrer International S.A. Spain. WEB 2170 was a gift from Boehringer Ingelheim, Germany.

LPS, Camonagrel and WEB were dissolved in 0.9% NaCl immediately before experiments.

## RESULTS

### *Characteristics of early haemodynamic response to LPS infusion*

Baseline values of systemic (SAP) and pulmonary (PAP) arterial pressures were  $108.8 \pm 16.7$  mmHg and  $23 \pm 3.3$  mmHg, respectively ( $n = 25$ ). As shown on original tracings from experiment (*Fig. 1*) intravenous infusion of LPS at a rate of 4mg/kg/min induced an immediate increase in PAP (*Fig. 1A*) and a fall in SAP (*Fig. 1B*). PAP began to rise within the first minute of LPS infusion, reached the maximum at about 2.5 minutes after the beginning of LPS



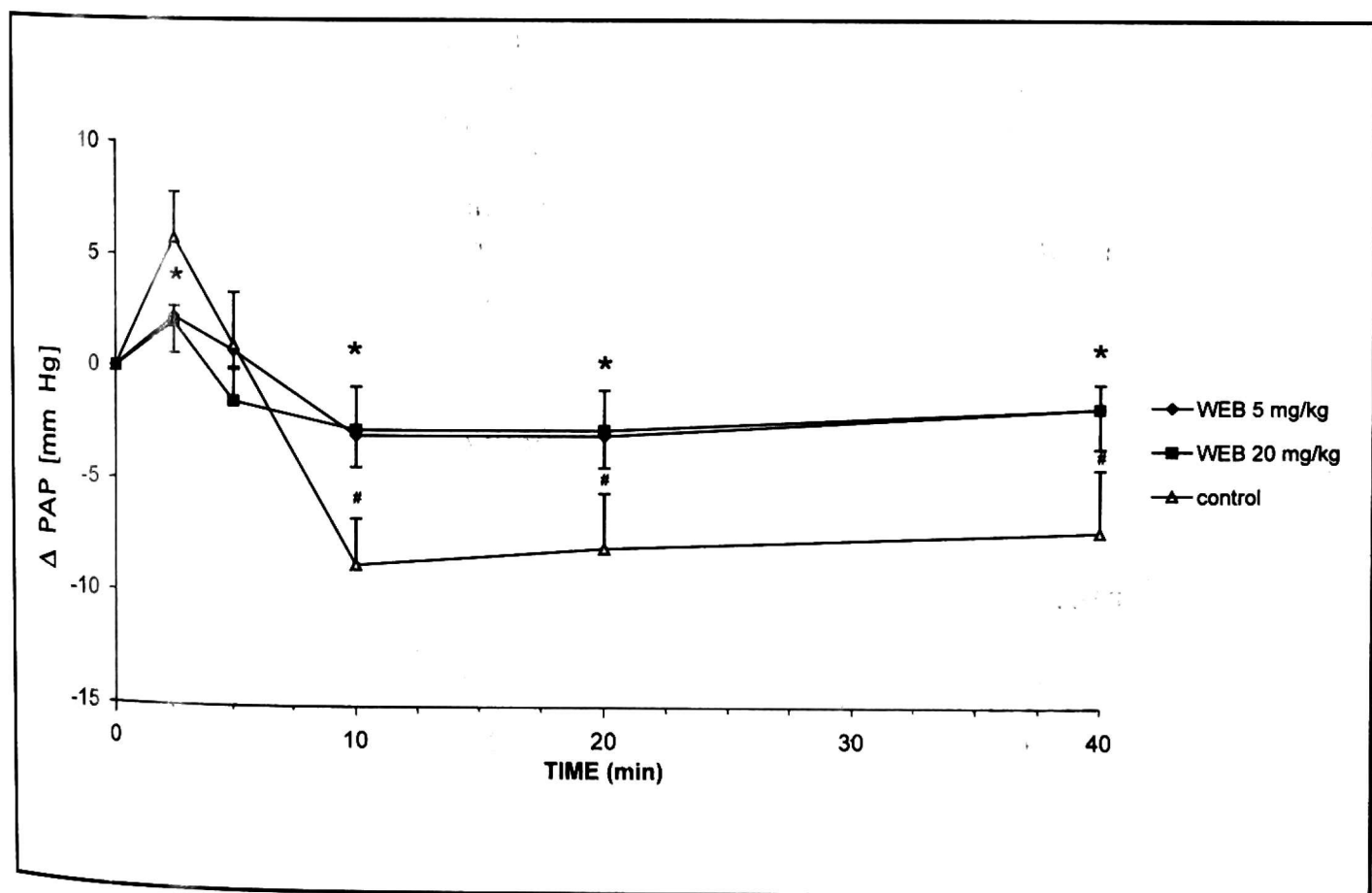
*Fig. 1A:* Original tracing from an *in vivo* experiment in anaesthetised Wistar rat showing a transient rise and subsequent fall in  $\Delta$ PAP induced by 10 mg/kg of LPS infused i.v at a rate of 4 mg/kg/min.

*Fig. 1B:* Original tracing from an *in vivo* experiment in anaesthetised Wistar rat showing an immediate fall in  $\Delta$ SAP induced by 10 mg/kg of LPS infused i.v at a rate of 4 mg/kg/min.

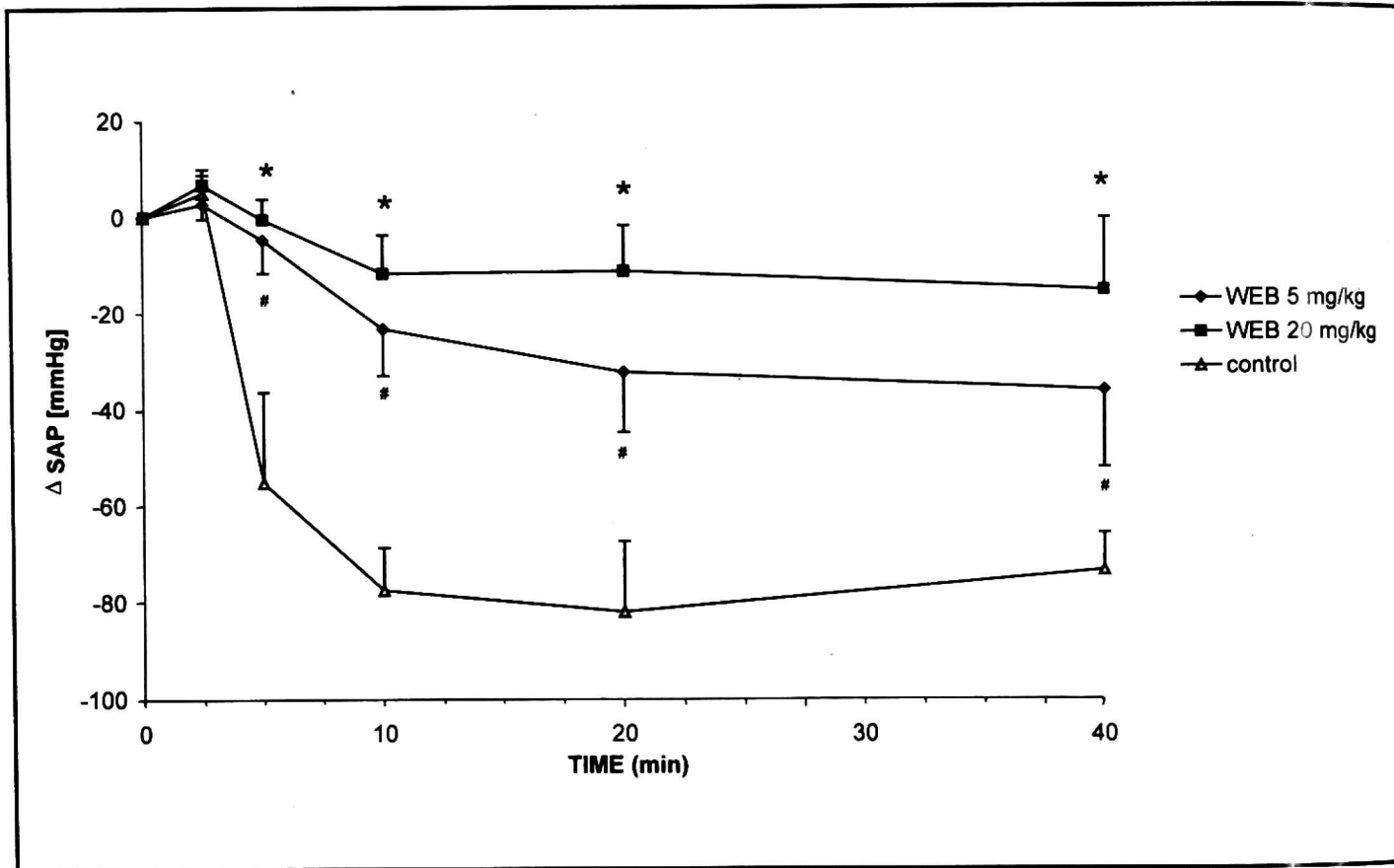
infusion ( $\Delta\text{PAP} = 5.75 \pm 2.04$  mmHg) and then started to fall (*Fig. 1A*). When PAP started to decrease, a parallel fall in SAP was observed (*Fig. 1B*). 10 min after the start of LPS infusion SAP reached the lowest value ( $\Delta\text{SAP} = -77.5 \pm 8.84$  mmHg) and remained at approximately the same low level for the next 30 minutes.

### Effects of PAF receptor blockade on the early haemodynamic response to LPS

As illustrated in *Fig. 2A* and *2B*, pretreatment with WEB 2170 was associated with a pronounced and dose-dependent inhibition of  $\Delta\text{PAP}$  and  $\Delta\text{SAP}$  responses induced by LPS. In rats receiving higher dose of WEB 2170 (20 mg/kg) ( $n = 4$ ) LPS-induced fall in SAP was nearly completely prevented, (*Fig. 2B*) and PAP increase was diminished. Interestingly, the late fall in PAP below baseline value was also inhibited by pretreatment with WEB 2170 (*Fig. 2A*).



*Fig. 2A*: Changes in  $\Delta\text{PAP}$  induced by 10mg/kg of LPS infused at a rate of 4 mg/kg/min in control rats (triangles) and in rats pretreated with 5mg/kg (squares) or 20 mg/kg of WEB 2170 (circles). Data represent mean values of  $\Delta\text{PAP}$  and vertical bars show SEM. # represent statistically significant ( $p < 0.05$ ) differences between  $\Delta\text{PAP}$  in rats pretreated with WEB 2170 5 mg/kg as compared to control rats. \* represent statistically significant ( $p < 0.05$ ) differences between  $\Delta\text{PAP}$  in rats pretreated with WEB 2170 20 mg/kg as compared to control rats.



*Fig. 2B:* Changes in  $\Delta$ SAP induced by 10 mg/kg of LPS infused at a rate of 4 mg/kg/min in control rats (triangles) and in rats pretreated with 5 mg/kg (rombuses) or 20 mg/kg of WEB 2170 (squares). Data represent mean values of  $\Delta$ SAP and vertical bars show SEM. # represent statistically significant ( $p < 0.05$ ) differences between  $\Delta$ SAP in rats pretreated with WEB 2170 5 mg/kg as compared to control rats. \* represent statistically significant ( $p < 0.05$ ) differences between  $\Delta$ SAP in rats pretreated with WEB 2170 20 mg/kg as compared to control rats.

#### *Effects of thromboxane synthesis inhibition on the early haemodynamic response to LPS:*

Effects of pretreatment with Camonagrel (CAM) on  $\Delta$ SAP and  $\Delta$ PAP responses to LPS are shown in *Fig. 3*. In the presence of CAM, LPS did not cause rise in PAP but induced only a sustained fall in PAP. LPS-induced fall in SAP was not affected by 10 mg/kg of CAM but was slightly inhibited by 20 mg/kg of CAM.

#### DISCUSSION

It is well known that in various models of endotoxic shock, intravenous administration of LPS may result in an immediate systemic hypotension and a rise in pulmonary arterial pressure. To induce such a haemodynamic response in our experimental set-up we had to infuse relatively high doses of

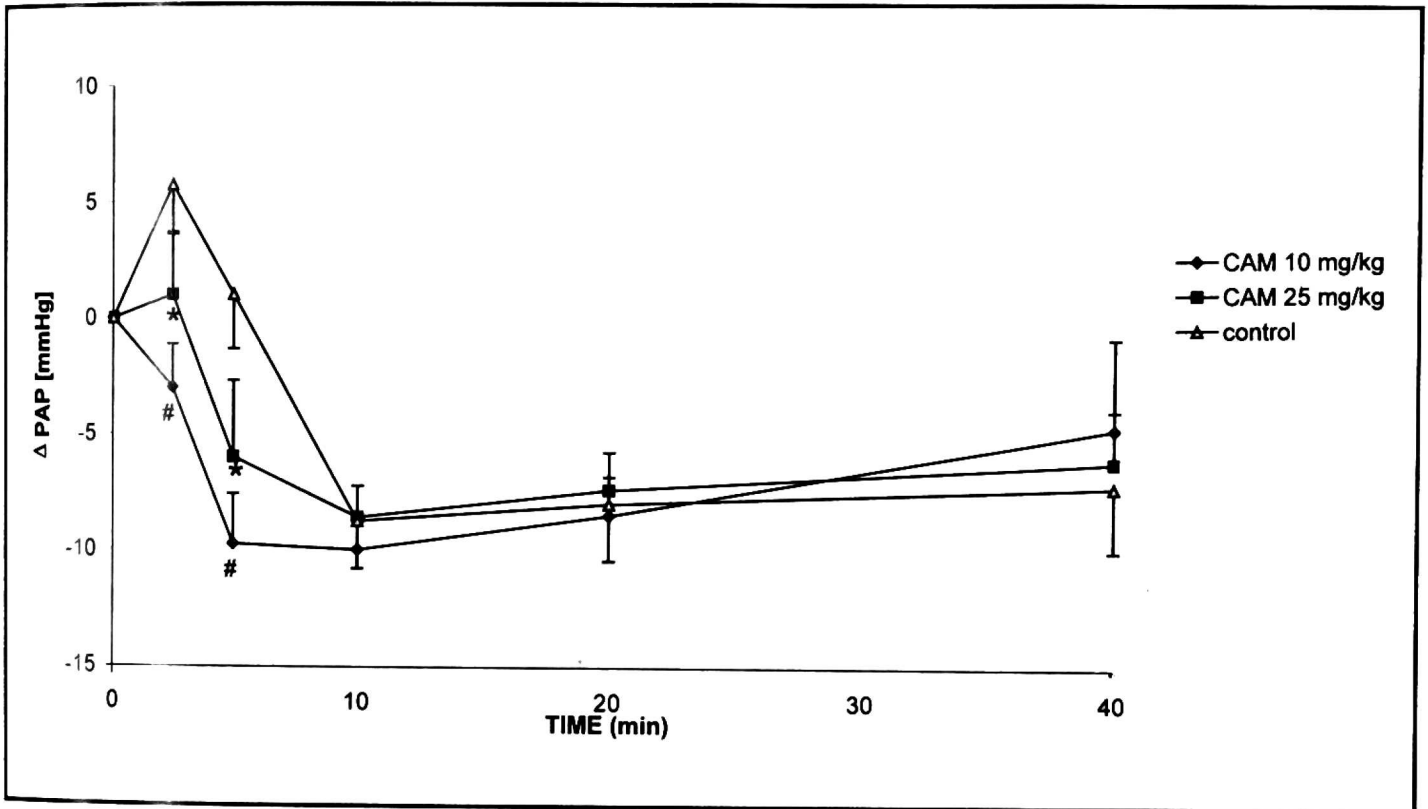


Fig. 3A: Changes in  $\Delta$ PAP induced by 10 mg/kg of LPS infused at a rate of 4 mg/kg/min in control rats (triangles) and in rats pretreated with 10 mg/kg (rombuses) or 25 mg/kg of Camonagrel (squares). Data represent mean values of  $\Delta$ PAP and vertical bars show SEM. # represent statistically significant ( $p < 0.05$ ) difference between  $\Delta$ PAP in rats pretreated with CAM 10 mg/kg as compared to control rats. \* represent statistically significant ( $p < 0.05$ ) differences between  $\Delta$ PAP in rats pretreated with CAM 25 mg/kg as compared to control rats.

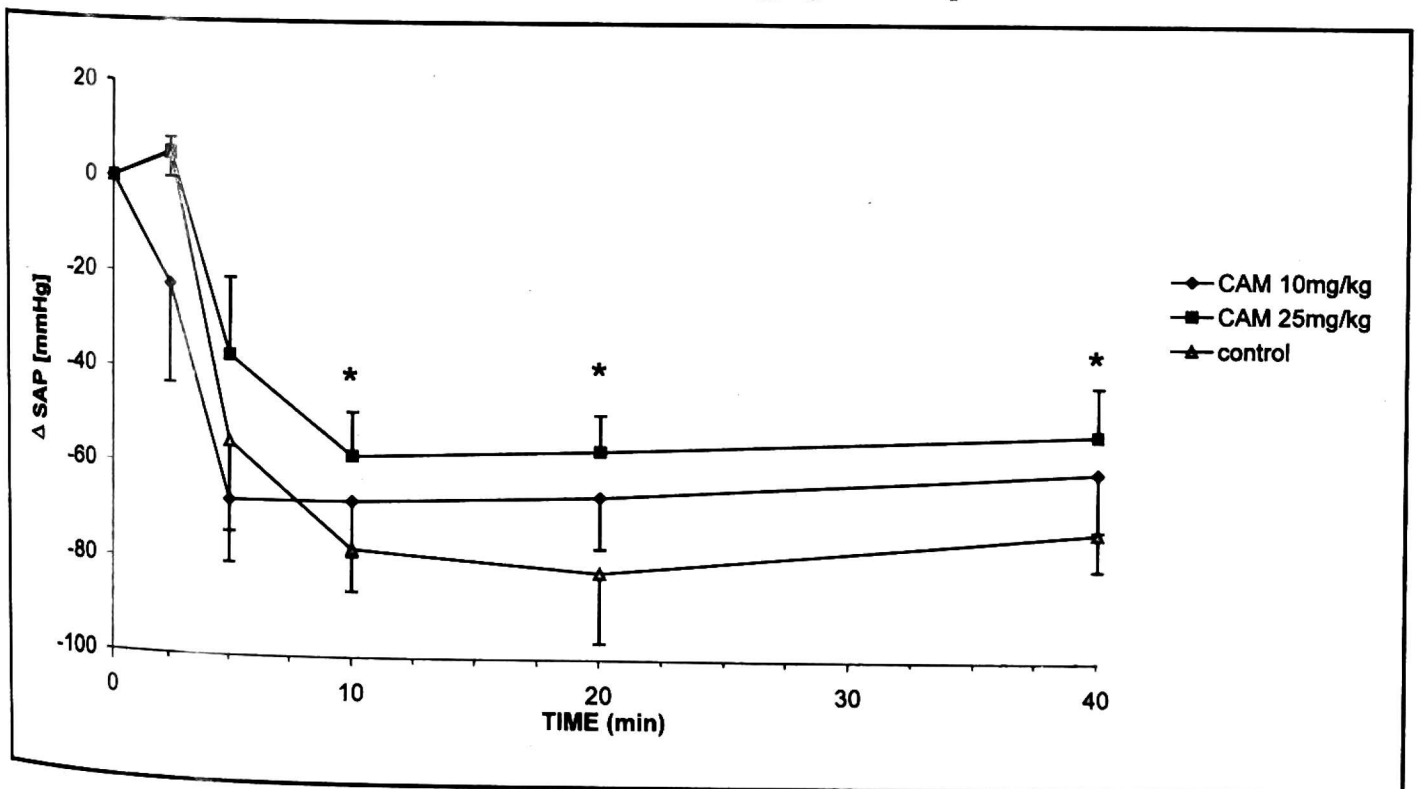


Fig. 3B: Changes in  $\Delta$ SAP induced by 10 mg/kg of LPS infused at a rate of 4 mg/kg/min in control rats (triangles) and in rats pretreated with 10 mg/kg (rombuses) or 25 mg/kg of Camonagrel (squares). Data represent mean values of  $\Delta$ SAP and vertical bars show SEM. # represent statistically significant ( $p < 0.05$ ) difference between  $\Delta$ SAP in rats pretreated with CAM 10 mg/kg as compared to control rats. \* represent statistically significant ( $p < 0.05$ ) differences between  $\Delta$ SAP in rats pretreated with CAM 25 mg/kg as compared to control rats.



LPS (10 mg/kg) given at a high rate of 4 mg/kg/min. Indeed, much more of endotoxin is required to induce cardiovascular responses in rats than in other species (14, 17—19). We showed that a TXA<sub>2</sub> synthase inhibitor, Camonagrel, abolished LPS-induced rise in pulmonary pressure without a major effect on LPS-induced decrease in systemic arterial pressure, whereas a PAF receptors antagonist, WEB 2170, although effectively attenuated systemic hypotension, was a weaker protector against LPS-induced pulmonary hypertension. Accordingly, both TXA<sub>2</sub> and PAF seem to mediate LPS-induced pulmonary hypertension, while only PAF seems to play an important role in mediating the LPS-induced systemic hypotension.

The mechanism of LPS-induced rise in pulmonary pressure was studied previously, though there is no unanimity in the literature as for the mediators involved. There is strong evidence confirming that LPS-induced release of TXA<sub>2</sub> mediates this response (11, 12). However, some authors dispute the role of thromboxane, and postulate that PAF (14) or leukotrienes (20) are major mediators of LPS-induced pulmonary hypertension. Hereby, we demonstrated involvement of both TXA<sub>2</sub> and PAF in pulmonary response to LPS. A weaker inhibition of LPS-induced rise in PAP by WEB 2170 than by Camonagrel in our experiments *in vivo* corroborates well with our data *in vitro* from the isolated, perfused rat lung. In this preparation even as high concentration of WEB 2170 as 300 μM did not abolish vasoconstricting action of 10 μg of PAF (unpublished data), whereas Camonagrel completely inhibited LPS-induced rise in PAP.

It seems therefore possible that the action of large amount of PAF released into the pulmonary circulation by intravenous infusion of LPS was not fully blocked by WEB 2170 at high dose of 25 mg/kg. On the other hand, explanations for a better attenuation of LPS-induced rise in PAP by Camonagrel than by WEB 2170 observed in our experiments *in vivo* may be as follows. Firstly, in Camonagrel but not in WEB 2170 pretreated rats LPS induced rapid and deep fall in SAP, that possibly diminished right ventricular filling. Secondly, inhibition of thromboxane synthesis cannot be assumed to be a simple ablation of TXA<sub>2</sub> action because there is a possible redirection of PG endoperoxide to other metabolic pathways (21) resulting in an increased production of vasodilatory PGI<sub>2</sub> or PGE<sub>2</sub>. Thirdly, Camonagrel as an inhibitor of TXA<sub>2</sub> synthesis does not eliminate production of other vasoactive lipids such as isoprostanes, which unexpectedly possess NO-dependent vasodilating properties in rat pulmonary artery, (22). Further investigations using both selective thromboxane synthase inhibitors and thromboxane receptor antagonists are needed to fully elucidate the role of TXA<sub>2</sub> in LPS-induced pulmonary vasoconstriction.

The present data shows that PAF plays a pivotal role in mediating haemodynamic changes elicited by LPS, being involved in both pulmonary and



systemic vascular responses to LPS. Indeed the role of PAF may be very complex since PAF has been reported to release not only  $\text{TXA}_2$  (23), but also other mediators such as leukotrienes (24), free radicals (25) and serotonin (26). We demonstrated that  $\text{TXA}_2$  is involved in LPS-induced pulmonary vasoconstriction and it may well be that  $\text{TXA}_2$  is a final mediator of pulmonary response to LPS (23, 27). On the other hand PAF that is considered to be a vasopressor agent may also act as an endothelium and NO-dependent vasodilator in pulmonary and systemic vascular beds (28—31). Our data are consistent with dual action of LPS-released PAF. WEB 2170 inhibited both pulmonary and systemic response to LPS suggesting that PAF released by LPS act as vasoconstrictor in pulmonary and vasodilator in systemic circulation. Indeed, intravenous injection of PAF mimics pulmonary and systemic LPS response (32). Interestingly, it was demonstrated that NO derived from endothelial NOS-3 contributes to PAF-induced (32) or LPS-induced systemic hypotension (33) but in other studies (7, 8) non-specific inhibition of NOS exacerbated LPS-induced hypotension.

We believe that the above differences might arise from the various regiments of LPS used. In the present study, only 4 mg/kg/min of LPS caused an increase in pulmonary pressure and as shown earlier L-NAME pretreatment potentiated systemic hypotension induced by high dose of LPS (17). In the absence of endogenous NO injection of high dose of LPS result in outburst of PAF and other lipid mediators causing excessive vasoconstriction, pulmonary thrombosis and leukocytes plugging which lead to a lethal fall in the systemic blood pressure, because left ventricle is not capable to push blood through pulmonary circulation. The activation of NOS-3 by LPS in pulmonary endothelial cells and pulmonary release of NO constitute the only rescue against LPS-induced, lipid mediated, damage to the lung (16).

On the other hand L-NAME pretreatment of rats injected with lower dose of LPS was not associated with damage of the lung and LPS-induced fall in arterial blood pressure was inhibited (33). In the present work LPS given at a rate of 1 mg/kg/min did not cause an increase in pulmonary pressure in the rat. On the light of the above we are tempting to speculate that only when PAF is released in large amount by LPS it causes pulmonary vasoconstriction in addition to its systemic hypotensive effect.

In conclusion, our study demonstrates that in rat both  $\text{TXA}_2$  and PAF contribute to immediate LPS-induced rise in pulmonary artery pressure, whereas early systemic hypotension after LPS infusion is mediated by PAF.

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