Br. J. Pharmac. (1980), **70**, 295–299

THROMBOXANE B_2 INHIBITS THE PULMONARY INACTIVATION OF PROSTAGLANDIN E_2 IN THE DOG

THOMAS M. FITZPATRICK, LAWRENCE S. FRIEDMAN, PETER A. KOT & PETER W. RAMWELL

Georgetown University Medical Center, Department of Physiology and Biophysics, 3900 Reservoir Road, N.W., Washington, D.C. 20007, U.S.A.

1 The systemic vasodepressor response to intravenously administered prostaglandin E_2 (PGE_2, 0.3, 1.0 and 3.0 μg/kg) is potentiated during intravenous infusion of thromboxane B_2 (TXB_2, 1.0 μg kg^{-1} min^{-1}) in the anaesthetized dog.

2 The augmented haemodynamic response returns toward control values following cessation of the TXB_2 infusion.

3 The systemic haemodynamic responses to intra-arterially administered PGE_2, $PGF_{2\alpha}$ and PGI_2 as well as intravenously administered $PGF_{2\alpha}$ and PGI_2 are not altered by TXB_2 infusion.

4 This study suggests that TXB_2 inhibits the pulmonary inactivation of PGE_2.

5 Arachidonic acid metabolites may interact, producing haemodynamic responses differing from their individual effects.

Introduction

Prostaglandin E_2 (PGE_2), a product of prostaglandin endoperoxide (PGG_2 and PGH_2) isomerization, depresses systemic arterial pressure (SAP) by directly dilating the resistance vessels of the circulation (Nakano & McCurdy, 1967; Malik & McGiff, 1976). Since PGE_2 is approx. 90% inactivated by a single passage through the pulmonary circulation (Ferreira & Vane, 1967) the magnitude of hypotension is dependent upon its route of administration or site of its synthesis. The lungs, therefore, play a strategic role in regulating the concentration of PGE_2 reaching the heart and systemic vasculature.

In addition, depending upon the stimuli, endoperoxides may be converted to several other compounds. Thromboxane A_2 (TXA_2), a potent platelet aggregator and constrictor of vascular smooth muscle (Hamberg, Svensson & Samuelsson, 1975), is a potential product of endoperoxide transformation. Due to structural instability however ($T_{\frac{1}{2}}$ = 32 s in aqueous solution), TXA_2 quickly converts to the more stable thromboxane B_2 (TXB_2) (Hamberg *et al.*, 1975). It was recently demonstrated that TXB_2 inhibited the pulmonary degradation of PGE_2 when infused through the rat isolated lung (Boura & Murphy, 1978a). Since there is a probable concomitant release of PGE_2 and TXB_2 by the lung during anaphylaxis (Anggard & Samuelsson 1965; Palmer, Piper & Vane, 1973; Dawson, Boot, Cockerill, Mallen & Osborn, 1976; Crutchley, Piper & Seale, 1977) and endotoxin shock (Dusting, Moncada & Vane, 1979) we investigated whether interaction between TXB_2 and PGE_2 occurs in the lungs of intact anaesthetized dogs.

Methods

Mongrel dogs (15 to 20 kg) of either sex were anaesthetized with intravenous sodium pentobarbitone (30 mg/kg). Airway patency was maintained with a cuffed endotracheal tube. A Swan-Ganz catheter was positioned in the pulmonary artery. The distal lumen of this catheter was used for monitoring pulmonary arterial pressure (PAP) and the proximal lumen for injecting test compounds intravenously (i.v.). The right femoral artery was catheterized for measurement of systemic arterial blood pressure. A Cordis 'pig-tail' catheter was inserted through the left femoral artery into the left ventricle for the intra-arterial (i.a.) administration of test compounds. The left femoral vein was catheterized and used for TXB_2 infusion.

The extent of PGE_2 inactivation by the lung was determined by measuring the SAP response to intravenous and intra-arterial PGE_2 administered before and during TXB_2 infusion (1 μg kg^{-1} min^{-1}). Neglecting its own metabolism, we estimated that the TXB_2 blood concentration after a 30 min infusion to be less than 400 ng/ml. This TXB_2 concentration alone produced no change in SAP or PAP.

Initially, control systemic arterial pressure responses to intravenous (0.3, 1.0 and 3.0 μg/kg) and

0007-1188/80/100295-05 $01.00

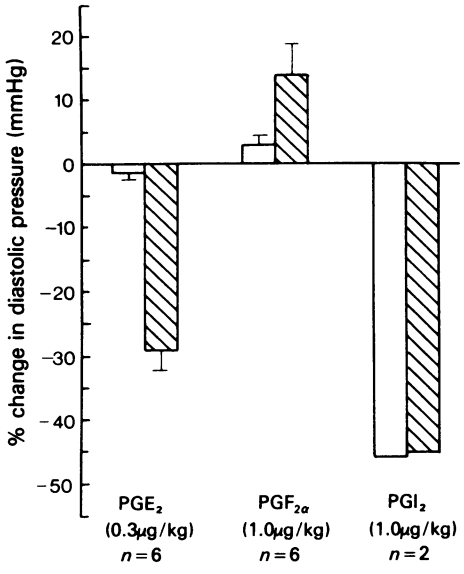

Figure 1 A comparison of the control responses to prostaglandin E_2 (PGE_2^*), $PGF_{2\alpha}^*$ and PGI_2, when administered by intravenous (open columns) and intra-arterial (hatched columns) routes. Vertical bars represent s.e. mean *Significant at $P < 0.05$.

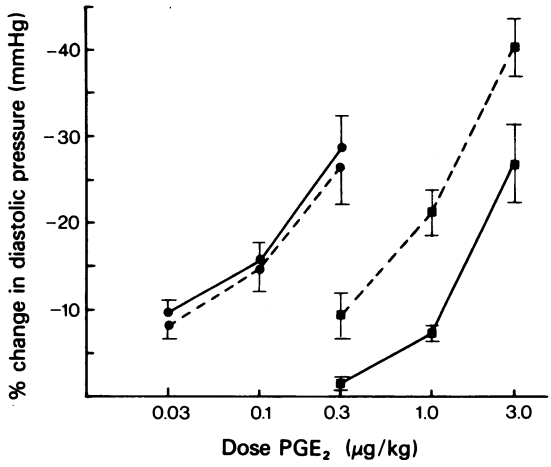

Figure 2 Log dose-responses to prostaglandin E_2 (PGE_2) administered intravenously (■) and intra-arterially (●) before (——) and during (-----) infusion of thromboxane B_2. Vertical bars represent s.e. mean.

intra-arterial (0.03, 0.1 and 0.3 μg/kg) PGE_2 ($n = 16$) were obtained. For purposes of comparison, intravenous (1.0 μg/kg) and intra-arterial (1.0 μg/kg) responses to PGI_2 ($n = 2$) and $PGF_{2\alpha}$ ($n = 6$) were also measured.

In order to establish whether the effect of TXB_2 was reversible, PGE_2 ($n = 6$) (i.v. 1 μg/kg) was administered at 5 min intervals following termination of the TXB_2 infusion.

Stock solutions of TXB_2, PGI_2, PGE_2 and $PGF_{2\alpha}$ were prepared in ethanol (1 mg/ml) and stored at −20°C. Solutions of PGE_2, $PGF_{2\alpha}$ and PGI_2 were dried under nitrogen and diluted to concentrations of 10 μg/ml (i.a. PGE_2) and 100 μg/ml (PGI_2, $PGF_{2\alpha}$ and intravenous PGE_2) with isotonic saline. TXB_2 was prepared in a similar manner at concentrations that varied in proportion to the weight of the dog.

Data were analyzed by Student's t test for paired data. A $P < 0.05$ was considered significant.

Results

Control percentage changes in systemic arterial diastolic blood pressure to intravenously and intra-arterially administered PGE_2, $PGF_{2\alpha}$ and PGI_2 are compared in Figure 1. PGE_2 responses, compared at 0.3 μg/kg, represent the only common intravenous and intra-arterial dose level employed. The systemic hypotensive action of PGI_2 was the same by either route of administration. In contrast, both PGE_2 and $PGF_{2\alpha}$ elicited a significantly ($P < 0.05$) greater response when administered intra-arterially.

Figure 2 shows the log dose-responses to intravenous and intra-arterial administration of PGE_2 before and during the infusion of TXB_2. The systemic depressor response to intravenous PGE_2 was significantly ($P < 0.05$) augmented at each of the three dose levels tested. However, the log dose-responses to intra-arterial administration of PGE_2 were unaffected by the TXB_2 infusion.

To determine whether the effect of TXB_2 was specific for PGE_2, the systemic arterial diastolic pressure responses to PGE_2, $PGF_{2\alpha}$ and PGI_2 were compared before and during TXB_2 infusion. Figure 3 illustrates the percentage change in arterial diastolic pressure elicited by intravenous bolus injections (1.0 μg/kg) of each of the three test compounds. Only the systemic arterial diastolic pressure response of PGE_2 was significantly ($P < 0.05$) augmented during TXB_2 infusion.

The ability of TXB_2 to potentiate the systemic hypotensive action of intravenously administered PGE_2 was rapidly reversible. In six animals PGE_2 (1.0 μg/kg) was administered before, during and at 5 min intervals following termination of the TXB_2 infusion. Within 5 min after stopping the TXB_2 infusion, the systemic depressor response elicited by PGE_2 was significantly ($P < 0.05$) diminished and continued to be reduced during the subsequent 15 min (Figure 4). At 20 min after the end of the TXB_2 infusion, the PGE_2 response was not significantly different from control.

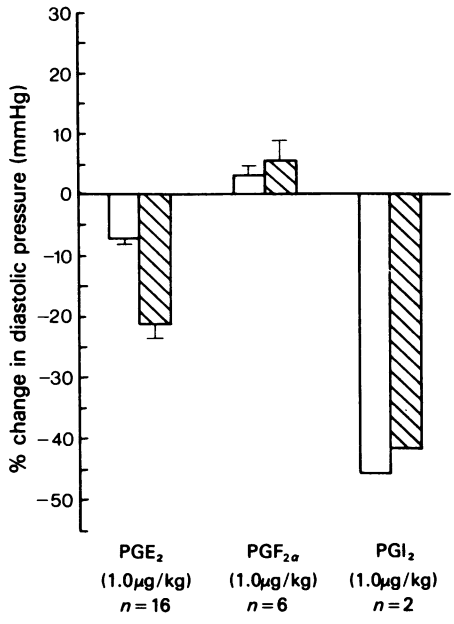

Figure 3 Comparison of response to intravenously administered prostaglandin E_2 (PGE_2^*), $PGF_{2\alpha}$ and PGI_2 before (open columns) and during (hatched columns) infusion of thromboxane B_2 (TXB_2). The difference in $PGF_{2\alpha}$ response is not significant. Vertical bars represent s.e. mean *Significant at $P < 0.05$.

Discussion

The magnitude of the systemic arterial depressor response to PGE_2 when administered intra-arterially is normally several times greater than the response elicited by an equivalent dose administered intravenously (Waldman, Alter, Kot, Rose & Ramwell, 1978). The i.a.-i.v. difference is explained by 15-prostaglandin dehydrogenase (15-PGDH)-mediated degradation in the lung (Ferreira & Vane, 1967; Anggard & Samuelsson, 1967). 15-PGDH is not unique to the lung but its presence there is important in regulating the concentration of several prostaglandins that affect the heart and systemic circulation.

The present study demonstrates that the systemic vasodepressor response produced by intravenous PGE_2 is augmented in the intact canine circulation during TXB_2 infusion. The shift of the log dose-response to intravenous PGE_2 suggests that greater concentrations of this substance are reaching the systemic circulation.

However, TXB_2 does not alter the vascular response to intra-arterially administered PGE_2 and the duration of the potentiated PGE_2 response was similar to that of the control response. Furthermore, peripheral inactivation of PGE_2 appears to be unaffected by TXB_2 and the potentiating action appears specific for PGE_2 since TXB_2 does not affect the SAP responses of $PGF_{2\alpha}$ and PGI_2.

Induction of pulmonary PGE_2 synthesis by TXB_2 could account for the augmented vascular response that we observed. However, Boura & Murphy (1978a) have demonstrated that TXB_2 does not increase prostaglandin release when perfused through the isolated lung of the rat.

Reduced synthesis of the inactivating enzyme 15-PGDH could also account for the increased responsiveness to PGE_2. For instance, rats pretreated with cycloheximide, an inhibitor of 15-PGDH synthesis, demonstrate a reduced inactivation of PGE_2 (Boura & Murphy, 1978b). Since the potentiated response to PGE_2 is significantly reversed within 5 min after termination of the TXB_2 infusion it seems unlikely that 15-PGDH synthesis could be inhibited and re-established within that short time.

TXB_2 itself, is not a substrate for 15-PGDH (Roberts, Sweetman, Morgan, Payne & Oates, 1977), yet it may interfere with the carrier-mediated intracellular prostaglandin transport mechanism postulated by Bito, Wallenstein & Baroody (1976). Blockade, competition or saturation of this process in the pulmonary vasculature by TXB_2 would potentiate intravenously administered PGE_2. Probenecid, an organic acid transport inhibitor reduces the inactivation of PGE_2 in the rat (Bito & Baroody, 1975; Boura & Murphy, 1978b) and dog lungs (Wicks, Ramwell, Kot & Rose, 1978). In addition, clinical indicator dyes such as indocyanine green, sulphobromophthalein, phenol red, Evans blue, methylene blue and bromcresol green once considered inert compounds, inhibit PGE_2 inactivation in isolated lung preparations of the rat (Bito & Baroody, 1975; Bakhle, 1978).

Furthermore, although PGI_2 is a substrate for 15-PGDH in lung homogenate (McGuire & Sun, 1978) and intact liver (Wong, Sun & McGiff, 1979) it is unaffected by transit through the pulmonary circulation (Dusting, Moncada & Vane, 1978a). It has been postulated that a transmembrane transport system for PGI_2 is lacking in the lung (Dusting, Moncada & Vane, 1978b). This would explain why TXB_2 did not affect the PGI_2 vasodepressor response.

Whatever the mechanism by which TXB_2 is acting, it is the first substance of endogenous origin found to be able to inhibit the pulmonary inactivation of PGE_2 in an intact animal.

Prostacyclin is the major arachidonic acid metabolite produced by the lung under physiological conditions (Dusting *et al.*, 1979; Mullane, Dusting, Salmon, Moncada & Vane, 1979). There are circumstances however when the pulmonary production of other AA metabolites increases. Although elevated blood levels of PGE_2 and TXB_2 have yet to be conclusively measured simultaneously in the same ani-

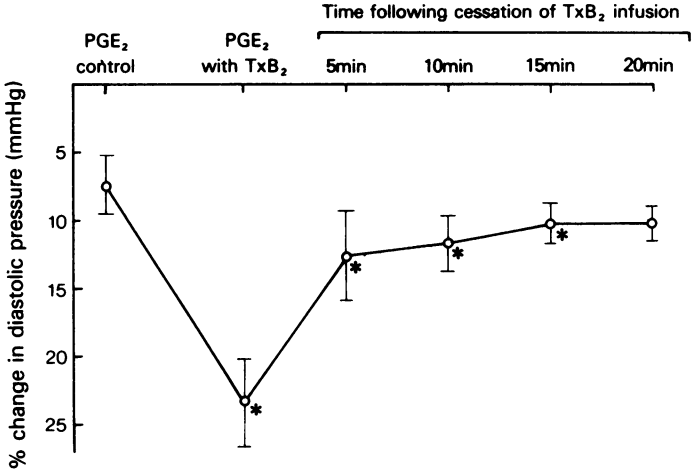

Figure 4 A comparison of the depressor response to prostaglandin E_2 (PGE_2, i.v. 1 μg/kg) before, during and at 5 min intervals following cessation of thromboxane B_2 (TXB_2) infusion. Vertical bars represent s.e. mean; $n = 6$ *Significantly different from control at $P < 0.05$.

mal, PGE_2 is elevated in the dog (Anderson, Jibiz, Fralios, Tsagaris & Kuida, 1972) and baboon (Fletcher, Ramwell & Herman, 1976) following endotoxin shock. Frolich, Ogletree & Brigham (1979) recently found a marked increase of TXB_2 (>200 ng/ml) in the lung lymph of sheep and Harris, Zmudka, Maddox, Ramwell & Fletcher (1979) reported elevated plasma TXB_2 (maximum 2260 ng/ml) in the conscious baboon following endotoxin shock. These studies lead us to speculate that under some pathophysiological conditions, large enough quantities of TXB_2 may be produced to inhibit partially the pulmonary degradation of PGE_2. Their interaction would result in the augmented systemic hypotension we have observed.

In conclusion, our study demonstrates: (1) TXB_2 augments the systemic vasodepressor response of intravenously administered PGE_2 in the intact dog, (2) TXB_2 appears to inhibit specifically the pulmonary inactivation of PGE_2, (3) inhibition of PGE_2 pulmonary degradation by TXB_2 is reversible. Possibly of greatest significance, it is important to observe potential interactions between arachidonic acid metabolites. The biological activity of the combined compounds may differ from that of the individual components.

This work was supported by U.S.P.H.S. grant HL-18718 and U.S.P.H.S. Training grant HL-07213. We want to thank Yvonne Maddox, Debbie Holden and Stephen Moore for their technical assistance as well as Ellen Costello for her excellent editorial assistance and Amy Hogan for her secretarial skills.

TXB_2, $PGF_{2\alpha}$, PGI_2 and PGE_2 were generously supplied by the Upjohn Company.

References

ANDERSON, F.L., JIBIZ, W., FRALIOS, A.C., TSAGARIS, T.J., & KUIDA, H. (1972). Plasma prostaglandin levels during endotoxin shock in dogs. *Circulation*, **45**, 11–124.

ANGGARD, E. & SAMUELSSON, B. (1965). Biosynthesis of prostaglandins from arachidonic acid in guinea pig lung. Prostaglandins and related factors. *J. biol. Chem.*, **240**, 3518–3521.

ANGGARD, E. & SAMUELSSON, B. (1967). The metabolism of prostaglandins in lung tissue. In *Nobel Symposium*, Vol 2, *Prostaglandins*. ed Bergstrom, S. & Samuelsson, B., New York: Interscience.

BAKHLE, Y.S. (1978). Clinically-used dyes are inhibitors of prostaglandin E_2 inactivation in the isolated lung. *Br. J. Pharmac.*, **64**, 386P.

BITO, L.Z. & BAROODY, R.A. (1975). Inhibition of pulmonary prostaglandin metabolism by inhibitors of prostaglandin biotransport (probenecid and bromcresol green). *Prostaglandins*, **10**, 633–639.

BITO, L.Z., WALLENSTEIN, M. & BAROODY, R. (1976). The role of transport processes in the distribution and disposition of prostaglandins. In *Advances in Prostaglandin and Thromboxane Research*, Vol 1, ed. Samuelsson, B. & Paoletti, R. pp. 297–303. New York: Raven Press.

BOURA, A.L.A. & MURPHY, R.D. (1978a). Thromboxane B_2 inhibits prostaglandin E_2 inactivation by the rat isolated perfused lung. *Clin. exp. Pharmac. Physiol.*, **5**, 387–392.

BOURA, A.L.A. & MURPHY, R.D. (1978b). Some factors affecting inactivation of prostaglandin E_2 (PGE_2) by the rat isolated perfused lung. *Br. J. Pharmac.*, **62**, 411P.

CRUTCHLEY, D.J., PIPER, P.J. & SEALE, J.P. (1977). The nature of prostaglandin-like substances released from guinea-pig lungs in anaphylaxis. *Eur. J. Pharmac.*, **44**, 319–323.

DAWSON, W., BOOT, J.R., COCKERILL, A.F., MALLEN, D.N.B. & OSBORN, D.J. (1976). Release of novel prostaglandins and thromboxanes after immunological challenge of guinea-pig lung. *Nature*, **262**, 699–702.

DUSTING, G.J., MONCADA, S. & VANE, J.R. (1978a). Disappearance of prostacyclin (PGI_2) in the circulation of the dog. *Br. J. Pharmac.*, **62**, 414P.

DUSTING, G.J., MONCADA, S. & VANE, J.R. (1978b). Recirculation of prostacyclin (PGI_2) in the dog. *Br. J. Pharmac.*, **64**, 315–320.

DUSTING, G.J., MONCADA, S. & VANE, J.R. (1979). Prostaglandins, their intermediates and precursors: cardiovascular actions and regulatory roles in normal and abnormal circulatory systems. *Prog. Cardiovasc. Dis.*, **21**, 405–430.

FERREIRA, S.H. & VANE, J.R. (1967). Some factors affecting inactivation of prostaglandin E_2 (PGE_2) by the rat isolated perfused lung. *Br. J. Pharmac.*, **62**, 411P.

FLETCHER, J.R., RAMWELL, P.W. & HERMAN, C.M. (1976). Prostaglandins and the hemodynamic course of endotoxin shock. *J. Surg. Res.*, **20**, 589–594.

FROLICH, J.C., OGLETREE, M., & BRINGHAM, K.L. (1979). Pulmonary hypertension correlated to pulmonary thromboxane synthesis. *Fourth International Prostaglandin Conference*, Washington, D.C. Abstract 38.

HAMBERG, M., SVENSSON, J. & SAMUELSSON, B. (1975). Thromboxanes: A new group of biologically active compounds derived from prostaglandin endoperoxides. *Proc. natn. Acad. Sci. U.S.A.*, **72**, 2994–2998.

HARRIS, R.H., ZMUDKA, M., MADDOX, Y., RAMWELL, P.W., & FLETCHER, J.R. (1979). Relationships of TXB_2 and 6-keto-$PGF_{1\alpha}$ to the hemodynamic changes during baboon endotoxic shock. In *Advances in Prostaglandin and Thromboxane Research*, Vol. 7, ed. Sammuelsson, B., Ramwell, P.W. & Paoletti, R. pp. 843–849. New York: Raven Press.

MCGUIRE, J.C. & SUN, F.F. (1978). Oxidation by Rhesus Monkey Lung 15-Hhydroxyl Prostaglandin Dehydrogenase. *Arch. Biochem. Biophys.*, **189**, 92–96.

MALIK, K.U. & MCGIFF, J.C. (1976). Cardiovascular actions of prostaglandins. In *Advances in Prostaglandins. Physiological, Pharmacological and Pathological Aspects*, Vol 3. ed. Karim, S.M.M. pp. 103–104. Lancaster: M.T.P. Press, Ltd.

MULLANE, K.M., DUSTING, G.J., SALMON, J.A., MONCADA, S. & VANE, J.R. (1979). Biotransformation and cardiovascular effects of arachidonic acid in the dog. *Eur. J. Pharmac.*, **54**, 217–228.

NAKANO, J. & MCGURDY, J.R. (1967). Cardiovascular effects of prostaglandin E_1. *J. Pharmac. exp. Ther.*, **156**, 538–547.

PALMER, M.A., PIPER, P.J. & VANE, J.R. (1973). Release of rabbit aorta contracting substance (RCS) and prostaglandins induced by chemical and mechanical stimulation of guinea-pig lungs. *Br. J. Pharmac.*, **49**, 226–242.

ROBERTS, L.J. 2d, SWEETMAN, B.J., MORGAN, J.L., PAYNE, N.A. & OATES, J.A. (1977). Identification of the major urinary metabolite of thromboxane B_2 in the monkey. *Prostaglandins*, **13**, 631–647.

WALDMAN, H.M., ALTER, I., KOT, P.A., ROSE, J.C. & RAMWELL, P.W. (1978). Effect of lung transit on systemic depressor responses to arachidonic acid and prostacyclin in dogs. *J. Pharmac. exp. Ther.*, **204**, 289–293.

WICKS, T.C., RAMWELL, P.W., KOT, P.A. & ROSE, J.C. (1978). Inhibition of prostaglandin-induced pulmonary vasoconstriction by organic acid transport inhibitors. *Proc. Soc. exp. Biol. Med.*, **157**, 677–680.

WONG, P.Y.-K., SUN, F.F. & MCGIFF, J.C. (1978). Metabolism of prostacyclin in blood vessels. *J. biol. Chem.*, **253**, 5555.

(Received September 7, 1979.)