Br. J. Pharmac. (1982), **77**, 055–058

EFFECT OF TMB-8 ON THE PULMONARY VASOCONSTRICTOR ACTION OF PROSTAGLANDIN $F_{2\alpha}$ AND THE THROMBOXANE MIMIC, U46619

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1 TMB-8 (8-(N,N-diethylamino)octyl-3,4,5 trimethoxybenzoate HCl), an intracellular calcium antagonist, had no direct action on the pulmonary vasculature of the perfused canine lung lobe preparation.

2 The pulmonary pressor response to the thromboxane mimic, U46619, was not affected by TMB-8.

3 The vasopressor response to prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) was significantly attenuated but not completely blocked by TMB-8.

4 We conclude that the pulmonary pressor response to $PGF_{2\alpha}$ is dependent on both intracellular and extracellular calcium pools for contraction and that U46619 facilitates either solely extracellular calcium influx or mobilizes an intracellular calcium pool not inhibited by TMB-8.

Introduction

Contraction of vascular smooth muscle is dependent on the availability and mobilization of both extracellular and/or intracellular calcium pools (Hurwitz, Joiner & Von Hagen, 1967; Deth & van Breeman, 1974). Thus, calcium provides a key regulatory mechanism over the vascular contractile process (Bolton, 1979). However, different pressor agents use different calcium stores in order to achieve maximal contraction (van Breeman, Aaronson, Loutzenhiser & Meisheri, 1980).

The pulmonary vascular responses to prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) and the thromboxane mimic, U46619 (Coleman, Humphrey, Kennedy, Levy & Lumly, 1979), were shown to be dependent, in part, on calcium influx from the extracellular calcium pool (Angerio, Fitzpatrick, Kot, Ramwell & Rose, 1981). The inability of verapamil to block completely the $PGF_{2\alpha}$ pulmonary pressor response raised the possibility that the intracellular calcium pool also contributed to the vasoconstrictor action of these two agents. The availability of intracellular calcium during smooth muscle contraction is reported to be blocked by TMB-8 (8-(N,N-diethylamino) octyl-3, 4, 5 trimethoxybenzoate HCl) (Malagodi & Chiou, 1974; Chiou & Malagodi, 1975).

The purpose of this study was to use TMB-8 to determine indirectly the relative contribution of the intracellular calcium pool to the pressor response induced by $PGF_{2\alpha}$ and U46619 in the canine pulmonary circulation.

Methods

Mongrel dogs of either sex, ranging in weight from 16–21 kg, were anaesthetized with sodium pentobarbitone (Nembutal, 30 mg/kg) and maintained on positive pressure ventilation by use of a Harvard respirator and cuffed endotracheal tube. The ventilation rate was 15/min and the tidal volume varied from 225–275 ml depending on the size of the animal. The right femoral artery was catheterized for recording systemic arterial pressure and as a blood source for priming the extracorporeal pump. The right femoral vein was catheterized as a route for administering additional anaesthetic agent.

A left thoracotomy was performed at the fifth intercostal space. The circulation to the left lower lung lobe was isolated from the remainder of the pulmonary circulation by cannulating the lobar artery and the lobar vein. The isolated lobe was constantly perfused with autologous citrated (10% v/v of 3.8% citrate) blood by use of a peristaltic pump. Pulmonary venous blood drained passively into a

0007-1188/82/090055-04 $01.00

reservoir (Temptrol Q130, Bentley Labs Inc.) maintained at 37°C and was continuously recirculated through the lobe. The volume of the entire system was approximately 200 ml.

Mean lobar arterial pressure was monitored at the inflow cannula. Resting mean lobar pressure was maintained between 10 and 21 mmHg by varying the flow rate through the lobe. Once lobar pressure was stabilized, flow rate remained constant throughout the experiment.

$PGF_{2\alpha}$, U46619 ((15S)-hydroxy-11α,9α (epoxymethano) prosta-5z, 13E-dienoic acid) and 8-(N,N-diethylamino)-octyl-3, 4, 5 trimethoxybenzoate HCl (TMB-8) were supplied by the Upjohn Company. Noradrenaline (NA, (−)-arterenol HCl) was supplied by Sigma. Stock solutions of the $PGF_{2\alpha}$ (1.0 mg/ml in ethanol) were evaporated to dryness under nitrogen and dissolved in 0.9% w/v NaCl solution to produce a 100 μg/ml concentration of the substance. U46619 was dissolved in ethanol to provide a 1 mg/ml solution; this stock solution was diluted in saline to 10 μg/ml. Stock solutions of NA (1.0 mg/ml in saline) were diluted to 10 μg/ml in saline. TMB-8 was stored desiccated at 10°C and solubilized in saline to a concentration of 1.0 mg/ml. All agents were prepared daily. Verapamil was obtained from Knoll Pharmaceuticals and prepared daily by dissolving in 0.85% saline.

Initial studies were performed to assess the direct effect of TMB-8 alone on the pulmonary vascular smooth muscle tone. TMB-8 was added to the isolated circuit in increasing concentrations ranging from 60–125 μM while constantly monitoring lobar arterial pressure.

Secondly, we determined the optimal concentration of TMB-8 which produced maximal attenuation of the submaximal pressor response induced by $PGF_{2\alpha}$ (1.25 μg/kg) in the isolated lung lobe preparation. The percent attenuation of the $PGF_{2\alpha}$ pressor response was plotted against the TMB-8 concentration and a dose-response curve was obtained (Figure 1). The results of the dose-response trials determined the concentration of TMB-8 which would be employed throughout the course of these experiments (95–110 μM) and are consistent with results obtained in previously reported *in vitro* experiments (Malagodi & Chiou, 1974; Chiou & Malagodi, 1975).

In the final series of experiments, $PGF_{2\alpha}$ (1.25 μg/kg), U46619 (0.1 μg/kg) and NA (1.0 μg/kg) were administered by bolus injection directly into the arterial inflow cannula. These specific doses of $PGF_{2\alpha}$, U46619, and NA were selected because each had previously been shown to produce a consistent and reproducible submaximal pressor response ($PGF_{2\alpha}$, U46619, 33% of maximum; NA, 50% of maximum) in the isolated lung lobe preparation (Rose, Kot, Ramwell, Doykos & O'Neill, 1976).

Following the pulmonary vascular response to each agonist, the pulmonary artery pressure was allowed to return to baseline values (10 min). TMB-8 was then added directly to the reservoir and allowed to circulate for 4 min before the administration of a second dose of the agonists. The circulating concentration of TMB-8 in these studies ranged between 95–110 μM based on the results of the dose-response curve cited above. Pulmonary arterial pressure responses to each of the agonists tested were obtained before and after the addition of TMB-8, and are reported as a percentage change from baseline. Data were analysed by Student's paired *t* test and significance was assigned at $P < 0.01$.

Results

In 5 dogs, TMB-8 alone was added to the isolated lung lobe preparation. Concentrations of TMB-8 ranging from 60–125 μM, resulted in no change in the resting vascular smooth muscle tone. In an additional 8 dogs, dose-response curves were obtained to assess the concentration-dependent inhibitory effect of TMB-8 on the $PGF_{2\alpha}$ pressor response (Figure 1). The maximum inhibitory effect of TMB-8 was observed between 90–115 μM.

The pulmonary vasoconstrictor response to $PGF_{2\alpha}$ was first determined in 9 dogs before administration of TMB-8. The mean increase in lobar arterial pressure was 43.0 ± 4.3%. The systemic arterial pressure remained unchanged. $PGF_{2\alpha}$ increased lobar arterial pressure by only 15.4 ± 1.5% following the addition of TMB-8 (Figure 2). This represents a significant

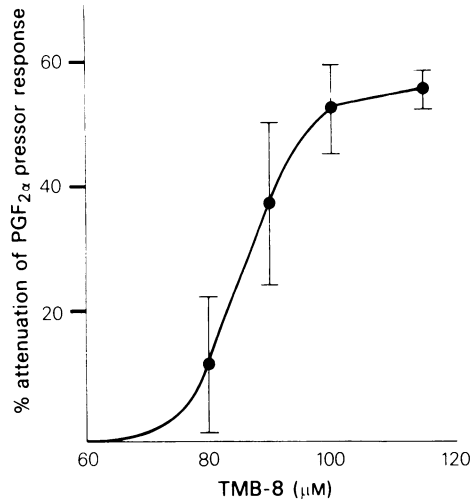

Figure 1 Inhibitory effect of increasing concentrations of TMB-8 on the prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) pressor response in the canine isolated lung lobe preparation. Vertical bars represent the s.e.mean in 8 dogs.

attenuation of the pulmonary pressor response.

The pulmonary pressor action of the thromboxane mimic, U46619 (0.1 μg/kg), was determined in 5 dogs. The increase in lobar arterial pressure before TMB-8 was 57.4 ± 10.3% and after TMB-8 was 58.3 ± 15.4% (Figure 2). No significant difference in the pulmonary pressor response was found in the presence or absence of TMB-8. No tachyphylaxis was found with $PGF_{2\alpha}$ or U46619 alone.

NA is believed to use exclusively intracellular calcium stores during phasic contraction of smooth muscle (Bolton, 1979; van Breeman *et al.*, 1980).

In order to assess the *in vivo* action of TMB-8 in the isolated lung lobe preparation, we challenged the NA-induced contraction with TMB-8. In 3 dogs, TMB-8 attenuated the pulmonary vasoconstrictor response induced by NA. The control vasopressor response to NA (1.0 μg/kg) was 16.7 ± 0.3%. Following addition of TMB-8 the increase in lobar arterial pressure was reduced to 9.0 ± 0.5% (Figure 2). Addition of verapamil with the TMB-8 did not further attenuate the NA pressor response.

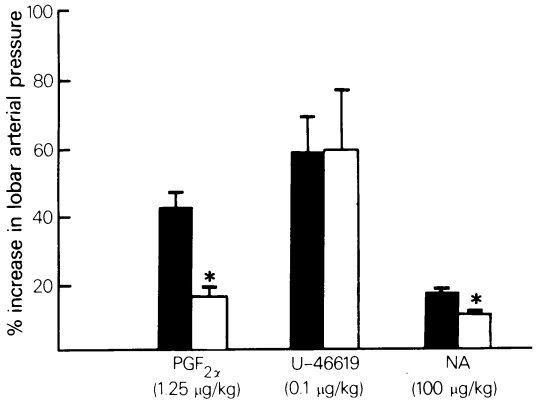

Figure 2 Mean percentage change in lobar arterial pressure produced by bolus injections of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), U46619 and noradrenaline (NA) before (solid columns) and after (open columns) administration of TMB-8 (95–110 μM). Doses of each substance are below each set of columns. Values are mean; vertical lines show s.e.mean. *Significance equals $P < 0.01$.

Discussion

Calcium antagonists provide a useful means of determining which calcium sources are primarily used during vascular smooth muscle contraction (Bolton, 1979; van Breeman *et al.*, 1980). In a previous study performed in our laboratory (Angerio *et al.*, 1981), we have shown that the extracellular calcium antagonist, verapamil (Kohlhazdt, Bauer, Krause & Fleckenstein, 1972), attenuated the pulmonary vasoconstrictor actions of $PGF_{2\alpha}$ and U46619. The inability of verapamil to block completely the pressor response to these agents suggested that intracellular calcium stores were also important to the vasoconstrictor activities of these two agonists.

Lack of previous data concerning the action of TMB-8 *in vivo* necessitated a series of preliminary trials to assess the possible direct action of TMB-8 on the vascular smooth muscle of the isolated lung lobe preparation and to determine the action of TMB-8 on the intracellular calcium pool. We demonstrated that TMB-8 exerted little or no effect on the resting vascular smooth muscle tone in the isolated pulmonary circulation. TMB-8 attenuated the vasoconstrictor response induced by NA. The dependence of the NA-induced vasoconstrictor response on the mobilization of intracellular calcium stores (Deth & van Breeman, 1977; Bolton, 1979; van Breeman *et al.*, 1980) suggests that TMB-8 may indeed be an intracellular calcium antagonist as previously demonstrated by *in vitro* experiments (Malagodi & Chiou, 1974; Chiou & Malagodi, 1975). The absence of further attenuation in the presence of the slow channel calcium blocker, verapamil, demonstrates that the extracellular calcium influx is non-contributory to the NA pressor response. This provides further evidence implicating TMB-8 as an intracellular calcium antagonist.

Therefore, TMB-8 afforded us the opportunity to evaluate the role of intracellular calcium mobilization in the pulmonary vascular response to $PGF_{2\alpha}$ and U46619. Since blood flow through the isolated lobe is maintained at a constant level, alterations in pulmonary arterial pressure are a direct reflection of the degree of vascular smooth muscle activity. TMB-8 attenuated the pulmonary vasopressor action of $PGF_{2\alpha}$ by 64.0 ± 5.9%. We conclude that the mobilization of intracellular calcium contributes significantly to $PGF_{2\alpha}$ vasoconstriction. These findings are consistent with our previous study which indicated that the extracellular calcium influx accounts for 40% of the pulmonary vasoconstrictor action of $PGF_{2\alpha}$ (Angerio *et al.*, 1981).

Whereas the vascular smooth muscle action of $PGF_{2\alpha}$ is dependent on the mobilization of calcium from both the intracellular and extracellular calcium pools, the absence of any inhibitory action by TMB-8 on the pulmonary pressor response to U46619 indicates that this thromboxane mimic used predominantly extracellular calcium stores or is partially dependent on an intracellular calcium store not inhibited by TMB-8.

We are grateful to Edward C. Howard, C.C.P., Chief Perfusionist at Georgetown University Hospital, for his invaluable technical assistance.

This work was supported by NHLBI grant HL-18718 and USPHS Training Grant HL-07213.

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(Received November 10, 1981.
Revised April 1, 1982.)