The effects of thromboxane receptor antagonists on oestrogen-induced uterotrophic responses in the spontaneously hypertensive rat

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1 The possible role of thromboxane in the uterotrophic response to oestrogen, in the spontaneously hypertensive rat was investigated by use of the thromboxane receptor antagonists EP092, AH23848 and BM 13.505.

2 The parameters studied were uterine blood flow (measured by the microsphere technique), uterine wet and dry weights and the concentrations of cytosolic and nuclear oestrogen receptors.

3 The antagonists attenuated oestradiol-induced uterine blood flow and significantly reduced both wet and dry uterine weight. These changes were accompanied by decreases in nuclear oestrogen receptor levels.

4 The results suggest a supportive role for thromboxane in oestradiol-induced uterine growth.

Keywords: Uterus; hypertensive; thromboxane; oestrogen; thromboxane receptor antagonists; spontaneously hypertensive rat; oestrogen receptor

Introduction

The spontaneously hypertensive rat (SHR) is an established strain that develops hypertension several months after birth (Okamoto, 1969). The SHR is considered to be the most acceptable experimental model thus far developed of essential hypertension since neither drug treatment nor surgical intervention are required to induce a chronic hypertensive state. The exact aetiology of the hypertension is unknown but it probably involves a balance of cardiac and peripheral factors. Increased vascular resistance has been attributed to a plethora of factors: encroachment into the vessel lumen caused by arterial wall hypertrophy (Folkow *et al.*, 1973); augmented humoral sensitivity (Lais *et al.*, 1974); sympathetic nerve hyperactivity (Bohlen, 1979) and arterial rarefaction (Hutchins, 1979).

In this work the role of thromboxane, the production of which is most commonly associated with potent platelet aggregation and induction of vascular and extra-vascular smooth muscle contraction, in the uterotrophic response to oestrogen in the non-pregnant SHR is investigated with the view to extending this study to the pregnant animal. The contribution made by thromboxane was assessed by the use of known thromboxane receptor blocking drugs namely: EP092 (Armstrong *et al.*, 1984); AH23848 (Brittain *et al.*, 1985) and BM 13.505 (Stegmeier *et al.*, 1986). Comparisons are made to earlier results from this laboratory using the normotensive CD.

Methods

Mature, virgin, female spontaneously hypertensive rats, SHR strain (BP > 180 mmHg) from the animal house, University of Bradford, were used throughout and were housed in light (07h 00min–19h 00min) and temperature (18°C) controlled rooms, food and water were available *ad libitum*. All the animals used in this study were bilaterally ovariectomized at least 21 days before further experimentation and were randomly assigned to groups. All the animals used were of similar body weight (around 240 g); there was no significant difference between the weights within the groups or between groups.

Measurement of blood flow

The microsphere technique (previously described by Phaily & Senior, 1978a) was used to determine blood flow in the rat anaesthetized with sodium pentobarbitone (60 mg kg⁻¹). This dose was selected to ensure anaesthetic control throughout the experimental procedure and minimal cardiovascular changes. The microspheres used were 15 µm in diameter (NEN-Trac, New England Nuclear, Boston, MA, U.S.A.), uniformly labelled with Sc⁴⁶ and suspended in 10% (w/v) dextran containing 0.1% (w/v) Tween 80. Uterine blood flows were calculated from wet weight of tissue. In all cases blood flow between the adrenals and kidneys was balanced, indicating a uniform distribution of microspheres. Cardiac output was also unaffected by the treatments. When dry weights are quoted the tissue was dried until the weight remained constant.

Measurement of cytoplasmic and nuclear oestrogen receptors by [³H]-oestradiol exchange

Homogenization, cytosol and nuclear pellet preparation and determination of oestrogen receptors therein was carried out by the method of Marshall & Senior (1987). Briefly, animals were killed by cervical dislocation and the uteri were rapidly removed and homogenized in PE buffer (10 mM phosphate buffer, pH 7.4, containing 1.5 mM EDTA and 2 mM mercaptoethanol). All procedures were performed at 4°C unless otherwise stated. The exchange conditions employed were found to be optimal with respect to maximal exchange with minimal loss of binding sites. Homogenates were centrifuged at 100,000g for 1 h. Aliquots of cytosol were incubated at 30°C for 1 h with 1–20 nM of [³H]-oestradiol ([2,4,6,7-³H]-oestradiol, 86–110 Ci mmol⁻¹) (Radiochemical Centre, Amersham, Bucks) plus or minus 100 fold excess of oestradiol. Separation of bound from free ligand was achieved by the use of dextran-coated charcoal (0.5% dextran (BDH, Poole), 5% Norit A acid washed charcoal w/v in phosphate buffer). The radioactivity in the supernatant was determined by liquid scintillation counting. The assay of nuclear oestrogen receptors involved the preparation of a low speed nuclear pellet (prepared by centrifugation at 800g of homogenate). Aliquots of the nuclear suspension were treated in the same way as in the cytosolic assay. Separation was achieved with a 60% hydroxylapatite suspension in phosphate buffer. After ethanolic extraction of the pellet, radioactivity was determined by liquid scintillation counting (45% efficiency).

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Table 1 The effect of EP092 (10 mg kg⁻¹ i.v., 2 doses) given 10 min before and 1 h 30 min after oestradiol, AH23848 (2.5 mg kg⁻¹ i.v., single dose) and BM 13.505 (20 mg kg⁻¹ i.v., single dose) given 10 min before oestradiol on uterine blood flow and weight in the ovariectomised SHR

Pretreatment	No. of rats	Uterine blood flow (ml min ⁻¹ 100 g ⁻¹)	Uterine weight (mg)	
			Wet	Dry
None (control)	6	15 ± 5	83 ± 6	17 ± 1
Oestradiol alone	6	277 ± 50***	111 ± 9*	25 ± 1**
EP092	6	150 ± 40*	56 ± 3†††**	11 ± 1†††
AH23848	6	160 ± 11†***	92 ± 4	19 ± 0.3†††
BM 13.505	6	148 ± 47*	82 ± 7†	16 ± 3†

Measurements were made 3 h after oestradiol (0.5 µg kg⁻¹ i.v., single dose) administration.

Values are mean ± s.e.mean: * $P < 0.05$, ** $P < 0.005$, *** $P < 0.001$ values are significantly different from those of control group;

† $P < 0.05$, ††† $P < 0.001$ values are significantly different from those of oestradiol alone group.

In this work the term cytoplasmic receptor is used: this refers to the receptor form that separates into the cytoplasmic cell fraction after homogenisation. It represents the unoccupied inactive form of the receptor thought to be located in the nucleus *in vivo* (King & Greene, 1984). The nuclear or activated form of the receptor remains in the nuclear compartment after fractionation. Although receptor localisation is important, in terms of this study receptor measurements made are used in a qualitative manner to assess the effect of drug treatments on the receptor population as a whole, rather than as an attempt to compartmentalise the oestrogen receptor.

Drug administration

Oestradiol (oestr-1,3,5-(10)-triene-3,17β-diol; BDH, Poole, U.K.) was dissolved in a 10% (v/v) solution of propylene glycol and was administered intravenously at a dose level of 0.5 µg kg⁻¹ (single dose) into the tail vein of the conscious rat 3.0 h before measurements were made. The following compounds were gifts and are gratefully acknowledged: EP092 ((+)-5-endo(6'-carboxy-hex-2'-enyl)-6-exo-(1'-[N-(phenylthiocarbamoyl)-hydrazono]-ethyl-bi-cyclo[2,2,1]heptane) (Pharmacology Dept., University of Edinburgh) was dissolved in absolute ethanol (5 mg ml⁻¹) plus 2.5 µM sodium hydroxide per mg EP092; the ethanol was then evaporated under nitrogen and the resultant sodium salt was dissolved in aqueous sodium hydroxide (M/400)/sodium chloride (0.9%). EP092 was given 1 h 30 min and 3 h 10 min before measurements were made at a dose level of 10 mg kg⁻¹. AH23848 ([1α(z),2β,5α]-(+)-7-[5-[[1,1'-biphenyl]-4-yl]methoxy]-2-(4-morphinyl)-3-oxocyclopentyl]-4-heptenoic acid) (Glaxo Group Research) was dissolved in 1% (w/v) sodium bicarbonate in sodium chloride 0.9% (w/v). BM 13.505 (4-(2-(4-chlorobenzene)sulphonamide)-ethyl)-benzene acetic acid) (Boehringer Mannheim, GmbH) was obtained in a stock solution of BM 13.505 100 mg; meglumin 65 mg; mannitol 164 mg; Tritriplex III 1 mg in water for injection to 5 ml, all subsequent dilutions were made in water for injection. Both AH23848 (2.5 mg kg⁻¹) and BM 13.505 (20 mg kg⁻¹) were given intravenously 3 h 10 min before measurements were made. The doses of the thromboxane antagonists were chosen after preliminary studies which indicated that at these dosage levels the uterotrophic response was significantly altered. The drug solvents used did not have any effects on the parameters measured and these results have been grouped as controls.

Statistical analysis

The results (expressed as mean ± s.e.mean) were compared by analysis of variance and Student's *t* test (two tailed) (Snedecor & Cochran, 1979).

Results

In the absence of any exogenous oestradiol the thromboxane antagonists had no significant effect on the parameters studied.

Effect of EP092, AH23848 and BM 13.505 on oestradiol-stimulated blood flow (Table 1)

The vehicles used for the thromboxane antagonists had no effect upon the oestradiol response. All three antagonists were observed to cause a reduction in oestradiol-stimulated uterine blood flow; the hyperaemic response was significantly attenuated by AH23848 alone.

Cardiovascular function was not affected by treatment with any of the antagonists; cardiac output and heart rate remained unchanged. Likewise, thromboxane antagonist pretreatment did not affect blood flow, to the organs studied.

As previously reported by Kerr *et al.* (1990a) it was observed that in approximately 40% of hypertensive rats a significant increase in radioactivity in the lung was noted. This enhanced accumulation of microspheres in the lung did not appear to affect flow to any other organ examined. Animals exhibiting pulmonary pooling were not included in the study.

Effect of EP092, AH23848 and BM 13.505 on oestradiol-stimulated uterine weight (Table 1)

All three thromboxane antagonists induced a significant reduction in oestradiol stimulated wet and dry uterine weight. In the case of EP092 the uterine weight was reduced to a level below that seen in the untreated control group.

Effect of EP092, AH23848 and BM 13.505 on oestrogen receptor distribution in the oestrogen stimulated rat uterus (Figure 1)

Cytosolic receptor levels were not significantly affected by the thromboxane antagonists compared with oestradiol alone. All three antagonists showed a tendency to reduce nuclear receptor levels; this attenuation was significant in the case of EP092 (compared with oestradiol alone).

Discussion

It is known that a single injection of oestradiol stimulates a wide range of metabolic events (Clark & Peck, 1979) in the uterus of the ovariectomized rat. Since a time delay is apparent between the administration of oestradiol and the onset of uterine hyperaemia a number of vasoactive mediators have been implicated in the induction of the response to the steroid. Phaily & Senior (1978a,b) suggested that oestradiol-induced

Figure 1 The effect of EP092 (10 mg kg⁻¹) given 10 min before and 1 h 30 min after oestradiol, AH23848 (2.5 mg kg⁻¹ i.v., single dose) and BM 13.505 (20 mg kg⁻¹ i.v., single dose) given 10 min before oestradiol on cytosolic (a) and nuclear (b) oestrogen receptor distribution in the ovarioectomized SHR. Measurements were made 3 h after oestradiol (0.5 µg kg⁻¹ i.v., single dose) administration. Each column represents the mean of 3–6 determinations, with 6 uteri per determination; vertical bars show s.e.méan. * $P < 0.05$, ** $P < 0.005$, *** $P < 0.001$ values are significantly different from those of control group. † $P < 0.05$ value is significantly different from oestradiol alone group.

hyperaemia is mediated through the complex interaction of several vasoactive substances: histamine; kinins and prostaglandins. Stimulation by oestrogen of prostaglandin synthesis has been widely accepted (Ramwell *et al.*, 1977). An oestradiol-induced increase in thromboxane content of the ovarioectomized rat uterus has also been reported (Jouanen *et al.*, 1985). Ramwell *et al.* (1977) speculated that the latter may be attributed to increased uterine blood flow with a concomitant rise in platelet number. However, direct stimulation of thromboxane production by oestrogen, in the uterine tissue of ovarioectomized rats has been reported (Brown & Poyser,

1985). Therefore, it would be expected that in this study the addition of a thromboxane antagonist (at a dose known to block receptors; Armstrong *et al.*, 1984; Brittain *et al.*, 1985; Stegmeier *et al.*, 1986) to the oestradiol regimen would potentiate the hyperaemic response. However, the converse was found to be true, in that the inclusion of the antagonists attenuated the blood flow response to oestradiol. In the cases of AH23848 and BM 13.505, this phenomenon could be ascribed to the fact that both these compounds have been shown to be partial agonists (Brittain *et al.*, 1985; Patscheke *et al.*, 1987). However, there have been no reports of EP092 displaying partial agonist activity and in the normotensive CD strain this compound was found to enhance the oestradiol-stimulated hyperaemia (Kerr *et al.*, 1991). It is possible that due to the time lag between drug administration and blood flow analysis, adaptive regulatory vascular changes have occurred in the SHR which counteract the influence of the thromboxane receptor blockade. Thus while it would appear that basal vascular thromboxane levels show no significant strain differences (when the antagonists were used without oestradiol they had no effect on blood flow in either species), the thromboxane synthetic capacity of the SHR may exhibit altered reactivity under oestrogen stimulated conditions.

Oestradiol-induced water imbibition also appears to be more sensitive to the inhibitory actions of the thromboxane antagonists than the equivalent response in the CD rat (Kerr *et al.*, 1991). Thromboxane has been implicated in the induction of lysosomal instability (Dorn & Halushka, 1985) and such activity may contribute to the eosinophil-mediated (Tchernitchin *et al.*, 1989) uterine oedematous response. Alternatively thromboxane may exert an anti-chemotactic influence on the eosinophils so preventing the initial migration of their cells to the uterus.

The addition of the thromboxane antagonists to the oestradiol regimen caused a marked reduction in uterine dry weight, which corresponded with decreased levels of nuclear receptor. This phenomenon was also observed, but to a lesser extent, in the CD (Kerr *et al.*, 1991). Interestingly, the total receptor level was lower in the SHR uterus than the CD, even though the weight of the uterus in the SHR is higher than that of the CD when normalised for body weight (Kerr *et al.*, 1991). This paucity of oestrogen receptors in the SHR may explain the increased sensitivity seen to thromboxane antagonists in inhibiting the dry weight response in the uterus. These results suggest that oestradiol may mobilize cellular thromboxane; there is contradictory evidence as to whether this event is genomically mediated as a study by Castracane & Jordan (1976), using systemically administered protein synthesis inhibitors found that these agents did not prevent the stimulation by oestrogen of uterine prostaglandin synthesis. However, Poyser (1979) and Poyser *et al.* (1987) have presented some contradictory evidence in this respect in the guinea-pig, but in turn this prostanoid contributes to the induction of oestrogen-induced uterine growth, presumed to be genomically mediated. This train of events may be of significance in other hormonally regulated tissues.

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