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EFFECT OF GENDER ON DEVELOPMENT AND DIURNAL RHYTHM OF PROSTAGLANDIN RECEPTORS IN RAT AORTA

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1 The prostaglandin endoperoxide H_2 analogue, U-46619 (15-hydroxy-11, 9-epoxymethano-prosta-5Z, 13E-enoic acid), was used to determine the effect of gender on the isotonic contractile response of the superfused rat aorta preparation over a 24 h period and at 10 and 16 weeks of age.

2 The maximal responses of the male aortae were $20 \pm 3\%$ and $36 \pm 4\%$ ($P < 0.001$) greater than the female at 10 and 16 weeks, respectively. Similarly, sensitivity of the male aorta to the PGH_2 analogue increased with age.

3 The male but not the female exhibited a diurnal rhythm in which both the maximum contractile response and sensitivity were significantly decreased at night.

4 We conclude that these gender differences may be related to the secretion of androgen, since reported peak serum testosterone over a 24 h period and testosterone changes with maturation are coincident with the maximum response of male aortae to the PGH_2 analogue.

Introduction

The male rabbit aorta is believed to be more sensitive to 'Rabbit Aorta Contracting Substance' than the female aorta (Piper & Vane 1969). This labile contracting material is a mixture of the unstable arachidonate metabolites, namely endoperoxides (PGG_2 and PGH_2) and thromboxane A_2 (Hamberg, Svensson & Samuelsson, 1975). These unstable compounds are difficult to synthesize. Therefore, the stable synthetic endoperoxide analogue (U-46619), which is said to mimic both PGH_2 and TxA_2 (Coleman, Humphrey, Kennedy, Levy & Lumly, 1979), has been used with prostaglandins D_2, E_2 and $F_{2\alpha}$ to demonstrate gender differences in the response of the rat aorta preparation; no gender difference was observed with 5-hydroxytryptamine and noradrenaline (Karanian, Moran, Ramey & Ramwell, 1981). The prostaglandin endoperoxide analogue is significantly more potent than the other prostaglandins and therefore was chosen as a model compound to evaluate the effect of development and diurnal rhythm on the endoperoxide receptors of the rat aorta.

Methods

Spiral strips of thoracic aorta were prepared from intact male and female Wistar rats at 10 and 16 weeks of age (Charles River). The animals were killed by a blow to the head and the thoracic aorta was rapidly excised, trimmed of fat, blood, and connective tissue in Krebs-Ringer bicarbonate buffer at room temperature. Spiral strips 2.5 cm long and 0.25–0.50 cm wide were cut at a pitch of 45°. Each strip was superfused with equilibrated Krebs-solution bubbled with 95% O_2 and 5% CO_2 at 37°C under 1.0 g tension. Changes in tissue length were measured isotonically with a transducer and recorded (Harvard No. 256 and 480).

Four tissues were superfused in parallel (5.0 ml/min) with Krebs-Ringer bicarbonate buffer (mM): 118.2 NaCl, 4.7 KCl, 2.2 $MgCl_2$, 3.5 $CaCl_2$, 25.0 $NaHCO_3$, 1.0 NaH_2PO_4, and 11.1 glucose. The following antagonists were added to the buffer (per ml): propanolol (Sigma) 2.0 mg, methysergide (Sandoz) 0.2 mg, atropine (Matheson, Coleman, and Bell) 0.1 mg, and phenoxybenzamine (Smith, Kline, and French) 0.1 mg; indomethacin (Merck, Sharpe, and Dohme) was superfused at 2.0 μg/ml. The stable prostaglandin endoperoxide analogue (15-hydroxy-11,9-epoxymethano-prosta-5Z,13E-dienoic acid) was obtained from Upjohn. Noradrenaline was from Sigma. All drugs were freshly prepared in 0.9% w/v NaCl solution (saline) and the doses are expressed in nanograms.

The contractile responses of aortae of 10 and 16 week old rats to the endoperoxide analogue and to noradrenaline were determined. Phenoxybenzamine was removed from the mixture of inhibitors to permit use of noradrenaline. Tissues were then tested over a

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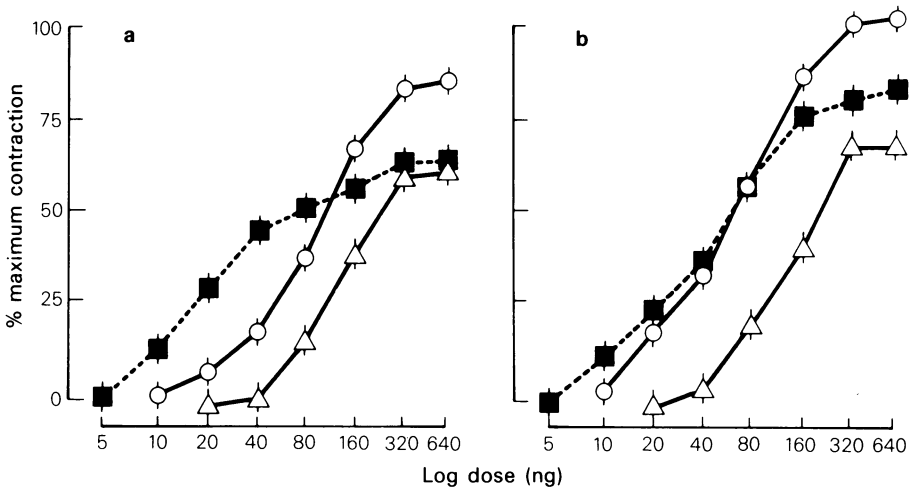

Figure 1 (a) Log dose-response curves for contraction of rat aorta to PGH_2 analogue in male (○, $n = 12$), female (△, $n = 12$), and to noradrenaline (■, $n = 16$) at 10 weeks of age. (b) Log dose-response curves for contraction of rat aorta to PGH_2 analogue in male (○, $n = 12$), female (△, $n = 12$), and to noradrenaline (■, $n = 16$) at 16 weeks of age. Each point represents the mean, and the vertical line, 1 s.e.mean.

24 h period with aortae from 16 week old animals. Only one agonist was used on any isolated preparation. All experiments were conducted within an 8 week period in order to avoid seasonal variations.

The responses to the endoperoxide analogue (PGH_2) and noradrenaline are expressed as a percentage of the maximum response of the male aorta to the endoperoxide analogue, which was assigned a value of one (1). All other responses were compared accordingly. The mean ED_{50} was calculated from dose-response curves. All data are presented as mean ± s.e. and analysed for significance ($P < 0.05$) by Students' *t* test (two-tailed, for unpaired data).

Results

Contractile responses to endoperoxide at 10 weeks

The isotonic contractile response of aortae to the stable prostaglandin endoperoxide analogue in 10 week old rats of either sex is shown in Figure 1a. The log dose-response curves for both male and female aortae possess the same slope. However, the male dose-response curve was displaced to the left of the female. The maximum contractile response of the female ($n = 12$) was 80 ± 3% of the male ($n = 12$, $P < 0.001$). The ED_{50} values for the male and female to PGH_2 are shown in Table 1.

Contractile response to endoperoxide at 16 weeks

At 16 weeks, the male response to PGH_2 was increased by 16 ± 3% ($P < 0.01$) as compared to the 10 week old male. In contrast, there was no significant change in the response of the corresponding female preparation. Thus, the gender difference in the response to PGH_2 was increased at 16 weeks; the female was 64 ± 4% ($n = 12$, $P < 0.001$) of the male maximum (Figure 1b). The ED_{50} value was significantly lower in the male but not the female aorta preparation. The same relationship existed in sen-

Table 1 Effect of gender and age on ED_{50} values for the endoperoxide analogue and noradrenaline

Age	*Agonist*	ED_{50}* *Male*	*Female*
10 week	PGH_2 analogue	94 ± 10†	149 ± 14
	Noradrenaline	26 ± 4‡	29 ± 4‡
16 week	PGH_2 analogue	62 ± 10†	132 ± 16
	Noradrenaline	38 ± 8‡	44 ± 8‡

*Mean concentration of agonist (± s.e.mean) required to produce 50% of the maximum contractile response (expressed in ng).

†Significantly different from all groups of both ages ($P < 0.01$), Students' *t* test.

‡Significantly different from the endoperoxide group of either age ($P < 0.01$), Students' *t* test.

sitivity to the PGH_2 analogue at 16 weeks (i.e., males > females, $P<0.001$) as compared to the 10 week old animals (Table 1).

Noradrenaline contractile responses

No significant gender differences were observed between the maximum response or in the slopes of the dose-response curve to noradrenaline at either 10 or 16 weeks of age. Therefore, these data were pooled and expressed as a combined dose-response curve. At 10 weeks, (Figure 1a), the noradrenaline dose-response curve is flatter than that of the PGH_2 analogue. The noradrenaline ED_{50} was lower ($P<0.001$) than the ED_{50} value for either gender response to the PGH_2 analogue (Table 1). The maximum response to noradrenaline was increased $11\pm5\%$ ($P<0.05$) in the 16 week old rats vs the 10 week old group. However, the ED_{50} values at 16 weeks of age are significantly lower for noradrenaline ($P<0.01$) than the ED_{50} for PGH_2 in the male or female (Table 1).

Diurnal variation

At 16 weeks of age, a significant diurnal rhythm in response to the PGH_2 analogue was observed; the change in response over 24 h was due to a decrease in the male response at night to $70\pm4\%$ ($n=8$, $P<0.001$) of the maximum male response in daylight hours. In contrast, there were no changes in the contractile responses of the female aorta over a 24 h period ($P>0.05$) (Figure 2). In addition, the ED_{50} was higher ($P<0.01$) for the male aorta in the nocturnal period than during daylight.

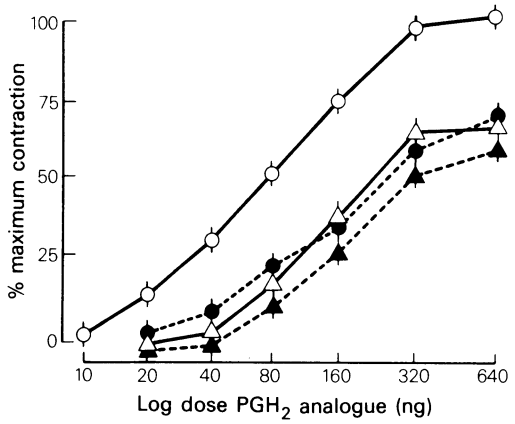

Figure 2 Log dose-response curves for contractions of rat aorta to PGH_2 analogue; male (○, $n=8$), and female (△, $n=8$) aortae from the day are illustrated with male (●, $n=8$), and female (▲, $n=8$) curves from the night (20 h 00 min – 08 h 00 min). Each point represents the mean and vertical line, 1 s.e.mean. The ED_{50} values for these curves were for the day, 62 ± 10 (♂) and 132 ± 16 (♀); and for the night, 150 ± 15 (♂) and 159 ± 18 (♀).

Discussion

The contractile response of the male rat aorta to the prostaglandin endoperoxide analogue of PGH_2 was found to be greater than that of the female at 10 and 16 weeks of age (Figure 1). Development of the contractile response was primarily by the male aorta which also exhibited a diurnal response to the PGH_2 analogue. There appears to be a significant decrease in the intrinsic activity and sensitivity of the male receptor during the nocturnal period (Figure 2). This may indicate a diurnal rhythm in both receptor number and affinity in the male aorta but not in the female for the PGH_2 analogue.

On the other hand, no gender difference was observed in either intrinsic activity or sensitivity to noradrenaline at 10 and 16 weeks. Both sexes appear to have a greater affinity for noradrenaline than for the PGH_2 analogue but the intrinsic activity of noradrenaline receptors was less than that seen with the male PGH_2 receptors (Table 1). One similarity between the noradrenaline and the male PGH_2 response is that both the intrinsic activity and affinity increased with maturation. However, the changes in the male PGH_2 receptors resulted in a greater gender difference than the relatively slight but significant increase observed in the noradrenaline response from 10 to 16 weeks of age.

Defelice & Joiner (1975) found a marked sex difference in the response of the rat aorta to adrenaline; they observed a significantly greater maximum tensile response to adrenaline in the male rat aorta, with a greater sensitivity to adrenaline occurring in the female vessel. However, our studies were conducted with noradrenaline and not adrenaline and moreover, the superfusate contained the cyclooxygenase inhibitor indomethacin, which blocks the production of intramural prostaglandins.

The question arises as to whether the changes in the PGH_2 response may be due to genotypic or phenotypic differences *per se* or more directly to hormonal effects. The latter possibility seems likely because the peak level of contractility found during daylight in the male rat, increases with sexual maturation and occurs at a time of highest plasma testosterone over a 24 h period (Kinson & Chung-Ching, 1973). In addition, preliminary investigations in both the rabbit and rat aorta (Sintetos, Ramwell & Ramey, 1978, Karanian, Ramey & Ramwell, 1980) show that pretreatment with testosterone enhances the response to the PGH_2 analogue.

Tuttle (1976) initially made the observation that the contractile response of the isolated aorta of the

rat to noradrenaline increased up to one year of age followed by an 8 fold decrease in contractility by 2 years. This is in accord with our observation that from 10 to 16 weeks of age, there occurs a marked increase in the response of the male rat aorta to PGH_2.

Testosterone also increases the collagen and elastin in aorta of intact male rats (Wolinsky, 1972b) but does not affect the non-fibrous components (i.e. smooth muscle) of the male rat aorta (Wolinsky, 1972a). Changes in the fibrous components of the carotid artery with testosterone treatment are associated with a resistance to stretch (i.e., increased resistance as demonstrated by passive-stress strain curves) (Cox & Fischer, 1978). Whether androgens affect contractility by changing these vascular components is not clear. In view of the greater response to PGH_2 found in the male, androgens are unlikely to decrease contractility in this preparation by acting on these passive components. We were unable to detect any significant differences in dry weight of aortae of comparable age.

The greater responsiveness of male aortae to PGH_2 may be due to differences in excitation-contraction coupling. The action of prostaglandins on vascular smooth muscle may be particularly dependent upon superficial calcium in contrast to catecholamines which are not significantly altered by changes in this calcium pool (Paton & Daniel, 1967; Altura & Altura, 1976). A preliminary study has indicated that the PGH_2 analogue mobilizes primarily extracellular calcium and only a fraction of the intracellular calcium in rabbit aorta (Loutzenhizer & Van Breeman, 1980). This analogue (Gerrard, Butler, Graff, Stoddard & White, 1978), $PGF_{2\alpha}$ (Carsten & Miller, 1977), and PGE_2 (Carsten & Miller, 1978) have also been described as possessing Ca^{2+} ionophore activity. Defelice & Joiner (1975) have characterized a superficial store of calcium in the male aorta capable of binding three times more Ca^{2+} than in the female, as indicated by $^{45}Ca^{2+}$ desaturation studies. This gender difference in Ca^{2+} binding (Defelice & Joiner, 1975), which is enhanced by testosterone, may indicate that agonists which primarily mobilize this store may exhibit sex differences in their effect. In contrast, no gender differences have been shown in the tightly-bound intracellular Ca^{2+} which is mobilized by most autocoids (i.e., the catecholamines). This is consistent with our observation that a gender difference was not seen with noradrenaline.

We conclude that since the PGH_2 analogue-induced response generally mirrors the response of the rat aorta to other prostaglandins, these prostaglandin receptors may also exhibit a male diurnal rhythm and changes with development which may be androgen-mediated.

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