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GENDER DIFFERENCES IN PROSTAGLANDIN RECEPTORS OF RAT AORTA

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The maximal contractile response to the prostaglandin endoperoxide H_2 analogue U46619, prostaglandins E_2, D_2 and $F_{2\alpha}$ and the sensitivity of the superfused aorta preparation to these drugs (except $PGF_{2\alpha}$) is greater in the male than the female rat. In contrast, gender differences were not observed in the response to noradrenaline or 5-hydroxytryptamine. In previous studies, testosterone unlike oestrogen or progesterone, increased the response of both rabbit and rat aorta to U46619. We conclude that prostaglandin receptors in rat thoracic aorta may be hormonally regulated.

Introduction The isolated aorta of the male rabbit is thought to be more sensitive to 'Rabbit Aorta Contracting Substance' than that of the female (Piper & Vane, 1969). This labile material is a mixture of the unstable arachidonate metabolites, prostaglandin endoperoxides (PGG_2, PGH_2) and thromboxane A_2 (TxA_2) (Hamberg, Svensson & Samuelsson, 1975) which have not been synthesized. However, a stable synthetic PGH_2 analogue (Upjohn U46619) is available and with it we have confirmed the observations of Piper & Vane in the rabbit isolated aorta (Sintetos, Ramwell & Ramey, 1978). Here, we show that a gender difference is also seen in a more convenient preparation, the rat aorta, that it is a general phenomenon for all the prostaglandins active in this preparation, and that gender differences are not seen with either noradrenaline or 5-hydroxytryptamine (5-HT).

Method Spiral strips of the thoracic aorta were prepared from intact male and female Wistar rats at 16 weeks of age. The animals were killed by a blow to the head and the vessel was excised, trimmed of fat, blood and connective tissue at room temperature in Krebs bicarbonate (mM): NaCl 118.2, KCl 4.7, $MgCl_2$ 1.2, $CaCl_2$ 3.5, $NaHCO_3$ 25, NaH_2PO_4 1 and dextrose 11.1. Spiral strips 2.5 cm long and 0.25 to 0.5 cm wide were cut at a pitch of 45° and superfused with equilibrated Krebs solution bubbled with 95% O_2 and 5% CO_2 at 37°C under 1 g tension for 2 h. Changes in tissue length were then measured isotonically with a transducer and recorded (Harvard No. 356 and 480).

Tissues were superfused in parallel (5 ml/min). The drug antagonists used were (per/ml): propranolol 2.0 mg (Sigma), methysergide 0.2 mg (Sandoz), atropine 0.1 mg (Matheson, Coleman & Bell) and phenoxybenzamine 0.1 mg (Smith, Kline & French). Indomethacin was superfused at 2 μg/ml (Merck, Sharp & Dohme). The prostaglandin endoperoxide PGH_2 analogue (15-hydroxy-11$_\alpha$,9$_\alpha$-epoxy-methano, prosta-5Z, 13E-dienoic acid), prostaglandins $F_{2\alpha}$, E_1, E_2, D_2 and B_2 were from Upjohn. Noradrenaline and 5-hydroxytryptamine (serotonin, 5-HT) were from Sigma. All drugs were freshly prepared in 0.9% w/v NaCl solution (saline) and the doses were expressed in nanograms.

The contractile response of the aorta to the prostaglandins, noradrenaline and 5-HT was determined. Phenoxybenzamine and methysergide were removed from the inhibitor mixture to permit use of noradrenaline and 5-HT, respectively. Only one agonist was used in each isolated aorta preparation. Maximum responses of the aorta to the prostaglandins were reproducible over a period of 6 h. All experiments were conducted during the day and within a 6 week period to avoid diurnal and seasonal variations.

The maximum response to the prostaglandins, noradrenaline and 5-HT is expressed as a percentage of the maximum response of the male to $PGF_{2\alpha}$ (this was assigned the value of 1), and all other responses were compared accordingly. The mean log ED_{50}s were calculated from concentration-response curves (not shown). All data are presented as mean ± s.e. and were analyzed by Student's *t* test (Table 1).

Results Significant sex differences were observed in the response of the rat aorta to the PGH_2 analogue U46619, prostaglandins $F_{2\alpha}$, E_2 and D_2. The maximum contractile responses of the preparation from the male were at least 25% greater ($P < 0.01$) than those from the female (Table 1). In contrast to the female, the male aorta showed no differences in the maximum responses to the prostaglandins. The reason for the variation in the female responses is not known. Another gender difference was that the sensitivity of the male aorta to U46619, PGE_2 and PGD_2 (but not $PGF_{2\alpha}$) was significantly greater ($P < 0.01$) than in the female. The aortae from females exhibited a significant decrease in the maxima of the sigmoidal

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dose-response curves to U46619, $PGF_{2\alpha}$, PGD_2 and PGE_2 in comparison to the males. No responses were obtained to PGE_1 or PGB_2 in the 0.5 to 64 μg dose range.

No significant gender differences were observed in the response of the superfused rat aorta preparation to either noradrenaline or 5-HT. In both sexes, the maximum response to 5-HT was approximately half ($P < 0.001$) and sensitivity was 330 fold less ($P < 0.001$) than the response to noradrenaline.

Discussion We conclude from Table 1 that prostaglandin receptors and perhaps the associated coupling mechanism appear to be sexually differentiated in the rat aorta preparation. These differences in sensitivity and contractility may be interpreted (Goldstein, Aronow & Kalman, 1974) to mean that both the affinity (U46619, PGD_2, PGE_2) and the number of receptors (U46619, $PGF_{2\alpha}$, PGD_2 and PGE_2) are greater in the male aorta for these prostaglandins.

An attractive explanation for these gender differences is based upon the report of gender differences in tissue calcium of rat aorta (Defelice & Joiner, 1975). These authors found that three fold more calcium binding is present in the loosely bound superficial fraction of the male aorta during ^{45}Ca desaturation studies, in comparison to the female. Testosterone increased the capacity of the aorta to bind this superficial calcium fraction. No gender difference existed in the more tightly bound intracellular calcium. This pool appears to be insensitive to testosterone but is responsive to oestradiol or prolactin. The response of smooth muscle to prostaglandins is well known to be calcium-dependent. The PGH_2 analogue U46619 (Gerrard, Butler, Graff, Stoddard & White, 1978), as well as $PGF_{2\alpha}$ (Carsten & Miller, 1977) and PGE_2 (Carsten & Miller, 1978) appear to possess calcium ionophore activity. A preliminary report indicates that the analogue U46619 mobilizes extracellular calcium and only a fraction of the intracellular in rabbit aorta (Loutzenhiser & Van Breeman, 1980). Earlier work (Paton & Daniel, 1967) indicates a dependence of prostaglandin contraction in smooth muscle upon a loosely held superficial calcium. Recently, exposure of isolated aorta and portal vein of the rat (Altura & Altura, 1976) to a calcium-free medium has been shown to inhibit prostaglandin-mediated contractions (prostaglandins E_2, $F_{2\alpha}$, B_2 and D_2) but not the contractile response to noradrenaline. In our preparation, we observed no sex differences with noradrenaline which as stated above, primarily mobilizes the tightly bound intracellular calcium of rat aorta (Defelice & Joiner, 1975). Thus, gender differences observed in the loosely bound superficial calcium store of rat aorta may contribute to the gender-dependent responses seen here with the endoperoxide analogue and the prostaglandins.

It is also likely that gender differences in response to these prostaglandins are hormonally controlled, since the sex steroids affect the superficial calcium pool and possibly receptor coupling to excitation and contraction. We have shown earlier that the sensitivity of both rabbit and rat aorta to the endoperoxide analogue is increased by testosterone treatment (Sintetos *et al.*, 1978; Karanian, Ramey & Ramwell, 1980). We conclude, therefore, that vascular prostaglandin receptor sensitivity and intrinsic activity may be regulated by gonadal androgens.

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Table 1 The effects of gender on the ED_{50} and maximum response of the superfused rat aorta to various vasoactive agents

Agonists	*ED_{50} (ng)**		*Maximum response (%)†*	
	Male	Female	Male	Female
Noradrenaline	$2.3 \pm 0.4 \times 10$	$2 \pm 0.4 \times 10$	77 ± 3 (14)	74 ± 2 (10)
5-HT	$7.6 \pm 1 \times 10^3$	$8 \pm 1 \times 10^3$	43 ± 4 (7)	47 ± 4 (9)
U46619	$6.0 \pm 0.3 \times 10$§	$8.3 \pm 5 \times 10$	93 ± 5 (12)§	70 ± 3 (12)
PGD_2	$1.4 \pm 0.2 \times 10^4$¶	$2.6 \pm 3 \times 10^4$	88 ± 4 (18)‡	45 ± 2 (18)
PGE_2	$2.8 \pm 0.3 \times 10^4$¶	$4.4 \pm 0.4 \times 10^4$	86 ± 6 (22)‡	49 ± 2 (21)
$PGF_{2\alpha}$	$7.0 \pm 0.9 \times 10^3$	$8.1 \pm 1 \times 10^3$	100 ± 4 (18)‡	25 ± 1 (17)

* Mean concentration ± s.e. of agonist required to produce 50% response;
† Expressed as % of the maximum male contractile response.
¶ Statistical difference from opposite sex for the same agonist: $P < 0.05$;
§ $P < 0.01$;
‡ $P < 0.001$.
The number of experiments is shown in parentheses.

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