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Atypical β-adrenoceptors mediate isoprenaline induced vasodilatation in the rabbit uterine vascular bed.

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Summary
β-Adrenoceptor subtype(s) mediating isoprenaline-induced vasodilator responses in the perfused rabbit uterine vascular bed was characterized. Isoprenaline induced dose-dependent reduction in perfusion pressure with $-\log ED_{50}$ value of 5.8 ± 0.1. Propranolol (10^{-6} M) had little or no effect on isoprenaline-induced vasodilatation. Similarly, cyanopindolol (10^{-6} M) did not antagonize the vasodilator response. CGP 12177, an agonist on β_3- or β_2- adrenoceptor mediated responses. The results would indicate that isoproterenol-induced vasodilation in the perfused rabbit (oestrus or pregnant) uterine vascular bed was mediated by atypical β-adrenoceptors which may not be identical with those in adipose tissues or gastro-intestinal tract. In addition, the fact that pregnancy did not enhance isoprenaline-induced vasodilatation would suggest that these receptors may not be involved in the hyperaemia of pregnancy.

Résumé
Les sous-types β adrenorecepteurs qui medient la vasodilatation induite par l'isoprenaline dans la couche uterine vascularisée perfuséede lapin a été characterisé. L'isoprenaline a induit une pression de perfusion dependant de la dose, avec une valeur du $\log ED_{50}$ de 5.8 ± 0.1. Le propanolol (10^{-6} M) a u un effet très faible ou nul sur l'isoprenaline qui induit la vasodilatation. Similairement, le cyanopindolol (10^{-6}M) n'a pas u d'effet antagonistesur la response vasodilatrice. Le CGP 12177, un antagoniste, des l'adrenorecepteurs β_3 a imité l'effet vasodilitatrice de l'insoprenaline dans cette preparation. Le $\log ED_{50}$ a été 6.3 ± 0.1. Le propanolol (10^{-6}M) n'a eu aucun effet sur la response vasodilatatrice du GG 121177. La grossesse n'a pas accrut la vasodilatation induite par l'Isoprenaline, de plus, elle n'a pas mas qué les responses medieies par les recepteurs β_1 on β_2. Les resultats indiqueraient, que la vasodilation induite par l'Isoproterenol dans les couches uterines vascularisée perfusée des lapins (en oestrus ou enceinte) a été medie par les adrenorecepteurs a typique β qui ne serait pas Identique avec ceux des tissues adipeux u du tract gastro-intestinal. Deplus, le fait que la grossesse n'a pas accrut la vasodilatation induite par l'isoprenaline suggererait que les recepteurs ne seraient pas impliques dans l'hyperemie pendant la grossesse.

Introduction

Lands *et al* in 1967 [1] classified β-adrenoceptors into β_1- and β_2-subtypes based on the rank order of agonist potencies in some smooth muscle preparations. The discovery of subtype-selective agonists and antagonists have since confirmed this sub classification. β-adrenoceptors in vascular smooth muscles were initially classified as β_2-adrenoceptors and could mediate vasodilatation [2-8].

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Recent studies have shown that in addition to the conventional β- adrenoceptor subtypes, (i.e., β_1- and β_2-adrenoceptors), a third type, β_3- adrenoceptors exists [9]. The existence of this receptor subtype has been confirmed by functional and molecular biology approaches [10,11]. β_3-adrenoceptors differ from β_1- and β_2-adrenoceptors in being relatively resistant to antagonism by propranolol, and selectively activated by compounds such as BRL 37344 (12), ZD 7114 (13), CL 316,243 (14) and SR58611A (15). These receptors are found in adipose tissues, where they mediate lipolysis [12, 16, 17, 18] and in smooth muscles of the gastrointestinal tract, and airways where they mediate smooth muscle relaxation [15, 18-21]. Atypical β-adrenoceptors are also present in blood vessels where they mediate vascular smooth muscle relaxation [21-24]. However, the atypical β-adrenoceptors in vascular smooth muscles may not be identical with those in intestinal smooth muscles and adipose tissues [24].

The perfused rabbit uterine vascular bed is constricted by isoprenaline [25]. However, when perfusion pressure was raised with noradrenaline infusion, isoprenaline induced weak vasodilator responses which were not affected by pronethalol suggesting that the relaxation was not mediated via β-adrenoceptors. The possibility, therefore, exists that isoprenaline-induced weak vasodilator responses which were not affected by pronethalol suggesting that the relaxation was not mediated via β-adrenoceptors. The possibility, therefore, exists that isoprenaline-induced vasodilatation in the perfused rabbit uterine vascular bed is mediated via atypical (β_3-) adrenoceptors. The main objective of this study was to characterize β-adrenoceptors mediating isoprenaline-induced vasodilatation in the perfused rabbit uterine vascular bed. In addition, since vasodilator responses are enhanced during pregnancy [26], the effect of pregnancy on β-adrenoceptor-mediated vasodilator response was also examined. The hypothesis was that if β-adrenoceptor mediated responses were enhanced during pregnancy, it may contribute to the hyperemia of pregnancy.

Material and methods

Adult female rabbits, weighing 2.5-3.5 kg, were used in this study. Each rabbit was anaesthetized with sodium pentobarbitone (35 mg/kg I.V) followed by exsanguination. The abdominal cavity was opened and the uterine vascular bed carefully isolated. The uterine artery branches were severed close to their point of entry into the uterine horn. The uterine artery was cannulated and the whole vascular bed was then placed on a warm glass surface (conical flask through which water at 37 ^{0}C was circulated). The preparation was pefused with Kreb's solution at a rate of 6 ml/min using a masterflex flow inducer. The Kreb's solution was the following composition (mM): NaCl, 119; KCL, 4.7; $NaHCO_3$, 25; KH_2PO_4, 1.2; $MgSO_4$, 1.2, $CaCl_2$, 2.5 and dextrose, 11. The solution was gassed with a 95% O_2 and 5% CO_2 mixture. Perfusion pressure was recorded through a dynamometer UF1 transducers on a Grass polygraph (model 7H).

In order to record vasodilator responses, perfusion pressure was raised by an infusion of nor-epinephrine, 10^{-5}M(ascorbic acid, was included in the Kreb's solution to prevent oxidation) and when a stable level of vasoconstriction had been reached, bolus injections (in a volume not exceeding 100µl) of isoproterenol were given via a port close to the pump. Vasodilator responses were obtained in the absence and in the presence of propranolol (10^{-6}M) or cyanopindolol (10^{-6}M). Each antagonist was allowed to equilibrate with the tissue for 30 min before re-establishing agonist dose-response curves. Only one antagonist was tested on any one preparation. Where necessary, antagonist potency was expressed as $-\log K_B$ value where K_B was calculated using the relationship

$$K_B = \frac{[\text{Antagonist}]M}{\text{Dose ratio} - 1}$$

In other series of experiments, a group of rabbits were mated and used between 22 and 24 days of pregnancy. The isolation and setting up of the tissues from these animals were as described in the previous section.

Drugs

Compounds used in this investigation include propranolol hydrochloride (Sigma), (±)-arterenol (noradrenaline) hyrochloride, (±)-CGP 12177A hydrochloride (all from Research Biochemicals International (Natick, MA), u46619 (Cayman) and cyanopindolol (courtesy of Sandoz Pharma, Basel). U46619 was dissolved in absolute ethanol while all the compounds were dissolved in distilled water.

Statistical analysis

Data are presented as mean ± standard error of 'n' observations. Where necessary, differences between mean values were tested for significance using student's 't' test (paired or unpaired). The difference is assumed to be significant when $p < 0.05$.

Results

Isoprenaline did not produce a vasoconstrictor response in the perfused rabbit ovarian vascular bed. However, when perfusion pressure was raised by an infusion of noradrenaline (10^{-5}M)m isoprenaline (10^{-7}-3x10^{-5}mol), dose-dependently reduced the perfusion pressure (Fig. 1). The $-\log ED_{50}$ value was 5.8 ± 0.1. Isoprenaline (10^{-7}-3 x 10^{-5} mol) also produced a vasodilator response when the perfusion pressure was raised with U46619 (10^{-7}M) (Fig. 1). Under this condition, $-\log ED_{50}$ was 6.0 ± 0.02. Thus isoprenaline was equipotent in dilating preparations constricted with nor-adrenaline or U46619.

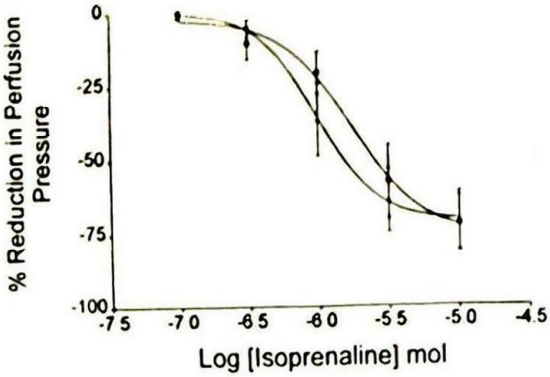

Fig. 1: Vasodilator effects of isoprenaline in the perfused uterine vasuclar bed. Perfusion pressure was raised with either (■) nor-adrenaline (10^{-5}M) or (▲) U46619 (10^{-7}M). Each point on the graph is the mean ± s.e. of 4 experiments.

In preparation where U46619 (10^{-7}M) was used to raise the perfusion pressure, inclusion of phentolamine (10^{-6}M) in the Kreb's solution did not increase the vasodilator response to isoprenaline, indicating that isoprenaline-induced vasodilatation was not limited by a simultaneous activation of α-adrenoceptors (data not shown). CGP 12177 (10^{-7}-3 x 10^{-5} mol) also induced dose-dependent vasodilatation in the perfused uterine vascular bed (Fig. 2). The $-\log ED_{50}$ was 6.3 ± 0.1. The NO synthase inhibitor, L-NOARG (10^{-4}M),had little or no effect on isoprenaline-induced vasodilatation.

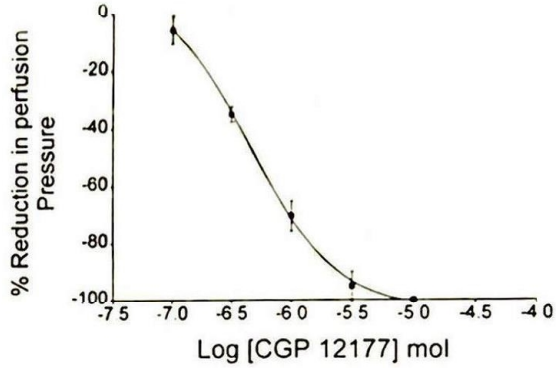

Fig. 2: Vasodilator effects of CGP 12177 in the perfused rabbit uterine vascular bed in the presence of noradrenaline (10^{-5}M) to raise the perfusion pressure. Each point on the graph is the mean ± s.e. of 5 experiments.

Antagonists

Propranolol (10^{-6}M) did not significantly antagonize isoprenaline-induced vasodilatation (Fig. 3) CGP 12177-induced responses were also not affected by propranolol (10^{-6}M), suggesting that β_1- and β_2-adrenoceptors may also be involved in this response

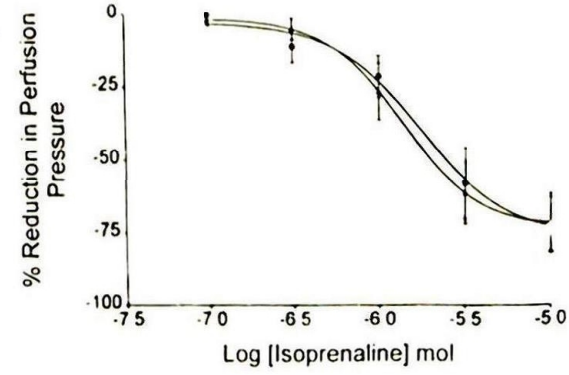

Fig 3 Effect of propranolol (10^{-6}M) on isoprenaline-induced vasodilatation in the perfused rabbit (non-pregnant) uterine vascular bed. The preparation was perfused with nor-adrenaline (10^{-5}M) to raise the perfusion pressure. (■) and (▲) represent vasodilator effects of isoprenaline before and in the presence of propranolol (10^{-6}), respectively (n = 4).

In order to determine whether the propranolol-resistant vasodilatation was medicated via atypical (β_3?) β-andrenoceptors, the effect of cyanopindolol (10^{-6}M) on isoprenaline-induced vasodilatation was studied. The results are summarized in Fig. 4, which shows the cyanopindolol (10^{-6}M) had no significant effect on isoprenaline-induced responses.

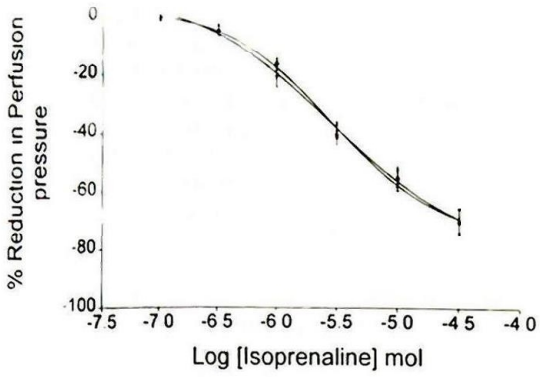

Fig 4 Effect of cyanopindolol (10^{-6}M) on isoprenaline-induced vasodilatation in the perfused rabbit (non-pregnant) uterine vascular bed The preparation was perfused with noradrenaline (10^{-5}M) to raise the perfusion pressure (■) and (▲) represent vasodilator effects of isoprenaline before and in the presence of cyanopindolol (10^{-6}M) respectively (n = 4).

Effect of pregnancy on isoprenaline-induced vasodilatation

In tissues isolated from pregnant rabbits, isoprenaline (10^{-7}-3 x 10^{-5} mol), produced dose-dependent vasodilator responses (Fig. 5). The $-\log ED_{50}$ value was 5.54 ± 0.10. In order to determine whether there was any change in receptor characteristics, the effect of propranolol on isoprenaline-induced vasodilator responses were investigated. Propranolol (10^{-6} M) significantly ($p < 0.05$) reduced isoprenaline-induced vasodilator responses (Fig. 5).

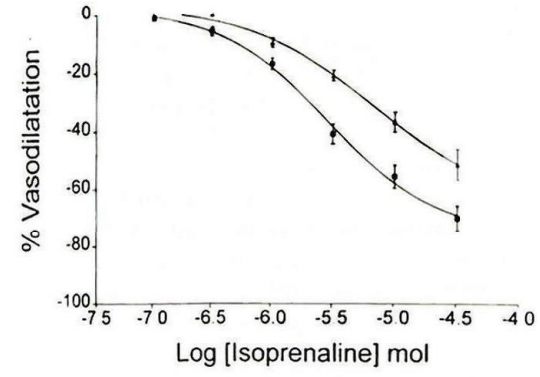

Fig. 5 Effect of propranolol (10^{-6}) on isoprenaline-induced vasodilatation in the perfused pregnant rabbit uterine vascular bed The preparation was perfused with nor-adrenaline (10^{-5}M) to raise the perfusion pressure (■) and (▲) represent vasodilator effects of isoprenaline before and in the presence of propranolol (10^{-6}M), respectively (n = 4)

There was approximate two fold (from 5.54 ± 0.10 to 5.16 ± 0.19) reduction in the potency of isoprenaline. The $-\log K_B$ value was calculated (using mean values) to be 6.13. Cyanopindolol was not effective against isoprenaline-induced vasodilatation. L-NOARG (10^{-5}) also had no effect on isoprenaline-induced vasodilatation in preparation from pregnant rabbits (Fig. 6).

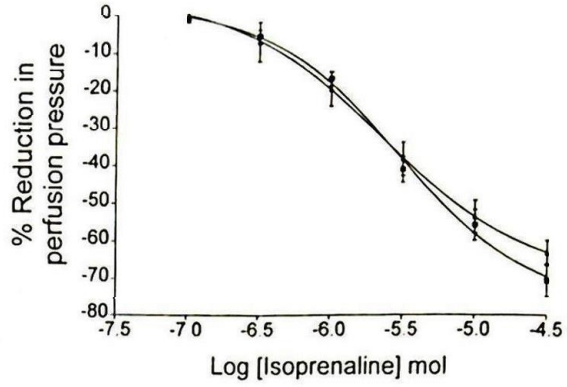

Fig. 6 Effect of L-NOARG (10^{-5}M) on isoprenaline-induced vasodilatation in the perfused pregnant rabbit uterine vascular bed. The preparation was perfused with nor-adrenaline (10^{-5}M) to raise the perfusion pressure (■) and (▲) represent vasodilator effects of isoprenaline before and in the presence of L-NOARG (10^{-5}M), respectively (n = 4)

Discussion

These results show that isoprenaline produced a dose-dependent vasodilatation in the perfused rabbit ovarian vascular bed. This is in agreement with the earlier observations of Graham and Sani [25] even though weak dilator responses were observed in their studies. The reason for the low efficacy is unknown, however, it may be related to the level of tone in the preparations. The concentration of nor-adrenaline (2 x 10^{-6}M) used by Graham and Sani [25] to raise perfusion pressure in their study was about 5-fold less than that (10^{-5}M) used in the present study. An attempt was made in this study to determine if α-adrenoceptor blockade (since perfusion pressure was raised with nor-adrenaline) was involved in isoprenaline-induced vasodilatation in the perfused rabbit uterine vascular bed. This was done by raising perfusion pressure with the thromboxane analogue, U46619 instead of nor-adrenaline. The results showed that isoproterenol produced a vasodilator response when perfusion pressure was raised with U 46619, indicating that α-adrenoceptor blockade was not involved in this response. Even though β-adrenoceptor mediated vascular smooth muscle relaxation were initially believed to be endothelium-independent, later results have shown that β-adrenoceptor mediated vasorelaxation can also be partly dependent on the endothelium. This has been observed in the rat aorta [27] and carotid artery [22], where isoprenaline-induced relaxation was significantly inhibted by L-NOARG. Endothelium-dependency or vascular bed was therefore examined. The results showed that L-NOARG, an inhibitor of NO synthase did not affect isoprenaline-induced vasodilatation suggesting that the response is independent of NO generation. Are the vasodilator responses to the agonists mediated by β_1-, β_2- or β_3- adrenoceptors? Is there a homogenous or heterogeneous population of β-

adrenoceptor in the rabbit uterine vascular bed? Answers to these questions were provided by the antagonist studies. The antagonists used were propranolol and cyanopindolol. Propranolol is a non-selective β_1- and β_2-adrenoceptor antagonist with very little affinity for β_3-adrenoceptors while cyanopindolol is, in addition to being a potent and non-selective β_1- and β_2-adrenoceptor antagonist, also a potent β_3-adrenoceptors antagonist. The results show that propranolol had little or no effect on isoprenaline-induced vasodilator responses indicating that β_1- and β_2-adrenoceptor are not involved in this response. It would therefore appear that atypical β-adrenoceptor mediated the vasodilator effects of isoprenaline in the uterine vascular bed. CGP 12177 is a non-selective β_1- and β_2-adrenoceptor antagonist [28], but an agonist on β_3-adrenoceptors [29,30]. A dose-dependent vasodilator response to CGP 12177 would therefore confirm the presence of an atypical β-adrenoceptors mediating vasodilatation in the vascular bed. However, cyanpindolol did not significantly modify agonist-induced vasodilatation. The concentration of cyanopindolol used in this study (10^{-6}M) significantly antagonized β_3-adrenoceptor mediated responses in the rat distal colon and fundic strips [18,24]. This would therefore suggest that the atypical β-adrenoceptors mediating vasodilatation in the perfused rabbit uterine vascular bed are not identical with those in intestinal smooth muscles or adipose tissues. In this regard, the atypical β-adrenoceptors mediating vasodilatation in the rabbit uterine vascular bed are similar to those mediating vaso relaxation in rat aortic and carotid arterial segments [21,24].

Circulatory adjustments take place during pregnancy to accommodate the needs of the growing foetus. This usually is in the form of an increased cardiac output and reduced peripheral vascular resistance especially in the uterine vascular bed. While the mediator(s) of the reduced vascular resistance is(are) still not well understood, an induction of endothelium-dependent cholinergic vasodilatation could be a contributing factor. This was based on the observation that acetylcholine only dilated uterine vascular bed during pregnancy [27,31]. Similarly, in the rabbit uterine vascular bed, acetylcholine -induced vasoconstrictor responses which were converted into relaxation by hormone (oestrogens) treatment [25]. Even though there is no clear evidence of cholinergic innervation of the uterine bed, electrical stimulation of arterial segments in the presence of an adrenergic neurone blocker and noradrenaline (to raise tone) produced a vasodilator response which was not effected by atropine in non-pregnant animals. However, similar responses by atropine, indicating a contribution from cholinergic activation. β-adrenoceptors mediating vasodilator responses in the uterine vascular beds have not received much attention apparently because of the weak dilator responses obtained in perfused uterine vascular beds or arterial ring segments (25). However, these studies were conducted on tissues from oestrus rabbits or guinea pigs. It is therefore not known whether β-adrenoceptors mediating vasodilator responses would be enhanced during pregnancy. An attempt was therefore made in this investigation to study the effect of pregnancy on β-adrenoceptors mediating vasodilator responses. Uterine vascular beds were obtained for pregnant rabbits (22-24 days) and produced a dose-dependent vasodilatation and, compared with non-pregnant rabbits, there was no significant difference either in the maximum response or potency, indicating that β-adrenoceptors mediating vasodilator response was not enhanced during pregnancy.

The possibility that pregnancy could induce β_1- and β_2-adrenoceptor subtypes in the uterine vascular bed was also examined. The results showed that the propranolol (10^{-6}M) produced a small but significant reduction (dose ratio was approximately 2.5) in isoprenaline-induced vasodilatation. The $-\log K_B$ value calculated using this concentration of propranolol, 6.14, is at least 100 times less than values expected for propranolol in tissues containing β_1- and β_2-adrenoceptors. This would indicate that isoprenaline-induced vasodilatation in the perfused pregnant rabbit uterine vascular bed via an atypical β-adrenoceptors.

It was therefore concluded that atypical β-adrenoceptors mediating isoprenaline-induced vasodilatation in the perfused rabbit uterine vascular bed. These atypical β-adrenoceptors are, however, not identical with those mediating intestinal smooth muscle relaxation or lipolysis in adipose tissues since cyanopindolol did not antagonize the vasodilator effects. Vasodilator responses mediated via these receptors were not increased during pregnancy, indicating that β-adrenoceptor activation is not involved in the hyperaemia of pregnancy.

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