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# Regulation of bradykinin $B_2$-receptor expression by oestrogen

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**1** Tissue kallikrein is overexpressed in the kidney of female rats, this sexual dimorphism being associated with a greater effect of early blockade of bradykinin $B_2$-receptors on female blood pressure phenotype. We evaluated the effect of ovariectomy and oestradiol benzoate (50 μg $kg^{-1}$ every two days for two weeks) on the vasodepressor response to intra-arterial injection of bradykinin (150–900 ng $kg^{-1}$) and on the expression of bradykinin $B_2$-receptors.

**2** Ovariectomy reduced the magnitude of the vasodepressor response to bradykinin and unmasked a secondary vasopressor effect. Oestrogen replacement restored the vasodepressor response to bradykinin in ovariectomized rats.

**3** The vasodepressor responses to sodium nitroprusside (3–18 $\mu g\ kg^{-1}$), acetylcholine (30–600 ng $kg^{-1}$), desArg$^9$-bradykinin (150–900 ng $kg^{-1}$) or prostaglandin $E_2$ (30–600 ng $kg^{-1}$) were significantly reduced by ovariectomy. Oestrogen restored to normal the responses to desArg$^9$-bradykinin, acetylcholine and prostaglandin $E_2$, but not that to sodium nitroprusside.

**4** $B_2$-receptor mRNA levels were decreased by ovariectomy in the aorta and kidney and they were restored to normal levels by oestrogen. Neither ovariectomy nor oestradiol affected receptor expression in the heart and uterus.

**5** These results indicate that oestrogen regulates $B_2$-receptor gene expression and function. Since kinins exert a cardiovascular protective action, reduction in their vasodilator activity after menopause might contribute to the increased risk of pathological cardiovascular events. Conversely, the cardioprotective effects of oestrogen replacement might be, at least in part, mediated by activation of the kallikrein-kinin system.

**Keywords:** bradykinin; bradykinin $B_2$ receptors; blood pressure; ovariectomy; oestrogen; mRNA; vasodilatation

## Introduction

Oestrogen exerts protective effects against the development of cardiovascular diseases in women (Colditz *et al*., 1987; Stampfer *et al*., 1991; Barrett-Connors & Bush, 1991). In addition, sex hormones influence the progression of high blood pressure in man and in experimental animal models (Dhal *et al*., 1975; Jams & Wexler, 1979; Leher *et al*., 1993; Luscher *et al*., 1987; Crofton *et al*., 1989; Kauser & Rubanyi, 1995). The protective action of oestrogen may be mediated by interference with both lipoprotein metabolism (Wagner *et al*., 1991; Riedel *et al*., 1993) and vascular function (Williams *et al*., 1990; Gilligan *et al*., 1994; Lieberman *et al*., 1994; Sudhir *et al*., 1996). Acute intravenous administration of oestradiol causes vasodilatation in both the tail artery of rats (Shan *et al*., 1994) and coronary (Reis *et al*., 1994) or forearm arterial circulation of humans (Gilligan *et al*., 1994). In addition, oestrogen acutely reverses abnormal acetylcholine-induced vasoconstriction of atherosclerotic coronary arteries (Williams *et al*., 1992; Reis *et al*., 1994), while hormonal replacement therapy improves endothelium-dependent vasodilatation in the brachial arteries of postmenopausal women (Lieberman *et al*., 1994). Oestrogen-induced vasodilatation is mediated by interaction of this hormone with its receptors at the level of vascular endothelial and smooth muscle cells (Venkov *et al*., 1996; Kim-Schulze *et al*., 1996) with subsequent stimulation of endothelial nitric oxide synthase (Yallampalli *et al*., 1994) and activation of ATP-sensitive potassium channels (Standen *et al*., 1989; Brayden & Nelson, 1992).

Whether the kallikrein-kinin system, a paracrine mechanism able to stimulate the release of endothelium-derived relaxing factors and prostaglandins (Moncada & Vane, 1978; Nakashima *et al*., 1993), contributes to oestrogen-induced endothelium-dependent vasorelaxation remains to be elucidated. Tissue kallikrein, a member of serine protease family, releases bradykinin or lys-bradykinin from kininogen. Kinins interact with specific receptors named $B_1$ and $B_2$ (Regoli & Barabe, 1980), the majority of cardiovascular effects being mediated by the $B_2$ receptor. The possibility that sex hormones contribute to the transcriptional regulation of genes encoding for components of the kallikrein-kinin system is suggested by the observations that (1) human pancreatic/renal kallikrein gene has potential receptor binding sites for oestrogen and progesterone (Murray *et al*., 1990), (2) renal immunoreactive kallikrein and kallikrein mRNA levels, which are elevated in female compared to male rats, are reduced by ovariectomy, with these alterations corrected by treatment with oestrogen (Madeddu *et al*., 1991), (3) ovariectomy reduces kininogen gene transcript in the liver, while oestrogen replacement increases mRNA levels (Chen *et al*., 1992), and (4) serum angiotensin-converting-enzyme (ACE), also called kinase II because of its ability to degrade bradykinin into inactive fragments, is reduced by 20% in postmenopausal women on oestrogen treatment compared with controls (Proudler *et al*., 1995). The influence of sex hormones on the expression and functional activity of bradykinin $B_2$ receptors has not yet been documented. However, the possibility that this occurs is favoured by the finding of a sexual dimorphism in the cardiovascular response to early blockade of bradykinin $B_2$ receptors by icatibant (Madeddu *et al*., 1996).

To challenge this hypothesis we studied the blood pressure effects induced by bradykinin in ovariectomized female rats with or without oestrogen replacement, with other endothelium-dependent or endothelium-independent vasodilators as reference compounds. In addition, we tested the effects of

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ovariectomy and oestrogen or progesterone replacement on bradykinin $B_2$ receptor expression.

## Methods

Wistar rats (200–250 g) were obtained from Charles River Company (Como, Italy). They were housed at a constant room temperature ($24 \pm 1°C$) and humidity ($60 \pm 3\%$) with a 12 h light/dark cycle. During experiments, rats had free access to chow (sodium, 0.12 mmol $g^{-1}$ chow, Mucedola, Milan, Italy) and tap water. All procedures complied with the standards for the care and use of animal subjects as stated in Guide for the Care and Use of Laboratory Animals (Institute of Laboratory Animal Resources, National Academy of Sciences, Bethesda, MD, U.S.A.) and were approved by the local Animal Care and Use Committee.

### *Blood pressure measurements*

A polyethylene catheter (Clay Adams, Parsippany, NJ, U.S.A.) was inserted into the left carotid artery and advanced to reach the thoracic aorta of rats anaesthetized with ether. Another catheter was inserted into the left femoral artery and advanced into the abdominal aorta. Both catheters were tunnelled under the skin and exteriorized at the back of the neck. The following day, the direct mean blood pressure (MBP) of unanaesthetized, unrestrained rats was measured with a Statham transducer (Gould Instruments, Oxford, CA, U.S.A.) connected to the femoral catheter and it was recorded on a recorder (Quartet, Ugo Basile, Comerio, Italy).

### *Effect of ovariectomy and oestrogen on the vasodepressor response to bradykinin*

Bilateral ovariectomized or sham-operation was performed with rats anaesthetized with ether. Animals were allowed to recover for 4 days. Then, sham-operated rats received a subcutaneous injection of vehicle (sesame oil) every 48 h for two weeks. Ovariectomized rats were randomly assigned to oestradiol banzoate (50 μg $kg^{-1}$, s.c.) or vehicle administration for the same duration. Then, arterial catheters were inserted, as described above, to determine the blood pressure responses to endothelium-dependent and endothelium-independent vasodilators. Twenty-four hours after arterial catheter implantation, MBP was measured for 15 min before the bolus intra-arterial injection (0.1 ml phosphate-buffered saline of bradykinin (150–900 ng $kg^{-1}$), desArg$^9$-bradykinin (150–900 ng $kg^{-1}$), acetylcholine (30–600 ng $kg^{-1}$), prostaglandin $E_2$ (30–600 ng $kg^{-1}$) or sodium nitroprusside (3–18 μg $kg^{-1}$). After injection, the catheter was flushed with 0.1 ml phosphate-buffered saline. Each dose was administered in a random order and sufficient time was allowed (at least 10 min) for MBP to return to basal levels before subsequent injection. Each rat received only one compound. Groups consisted of 6 rats.

In a separate set of experiments, the effect of icatibant (100 μg $kg^{-1}$), a selective $B_2$ receptor antagonist, or Sar[D-Phe$^8$]des-Arg$^9$-bradykinin (100 μg $kg^{-1}$), a selective $B_1$ receptor antagonist, on the MBP changes induced by bradykinin or desArg$^9$-bradykinin were tested in sham-ovariectomized, ovariectomized vehicle-treated and ovariectomized oestrogen-treated rats. Rats received an intra-arterial bolus of antagonist or vehicle (saline) 5 min before the administration of bradykinin or desArg$^9$-bradykinin. Groups consisted of 4 rats.

### *Effects of sex hormones on bradykinin $B_2$ receptor expression in Wistar rats*

These experiments were performed in the Department of Biochemistry and Molecular Biology, University of South Carolina. Wistar rats (200–220 g) were purchased from Harlen Sprague-Dawley (Indianapolis, IN, U.S.A.). To compare the expression of bradykinin $B_2$ receptors between male and female rats, total RNA was extracted from kidney, adrenal gland, aorta, artery and heart (left ventricle) by use of guanidine-CsCl gradient centrifugation (Davis & Battey, 1986). To study the effects of sex hormones on the expression of the $B_2$ receptor, rats underwent ovariectomay or sham operation. Then, ovariectomized rats were randomly allocated to three groups given subcutaneous injections of vehicle, oestradiol benzoate (50 μg $kg^{-1}$) or progesterone (5 mg $kg^{-1}$) suspended in sesame oil. Sham operated rats received vehicle for the same duration of time. Injections were repeated every two days for two weeks. Total RNA was extracted from kidney, aorta, heart and uterus. The concentrations of RNA in the samples were determined by spectrophotometric measurements at 260 nm.

Total RNA of 500 ng was reverse transcribed in a 20 μl of $5 \times$ RT buffer, 10 μl 2 mM dNTP, 2 μl 100 mM dithiothreitol, 10 u m-MLV reverse transcriptase (Life Technologies Inc., Gaithersburg, MD, U.S.A.) and 10 pmol of the 3′ primer (5′-GTTCAAGGAGGTCCAGACGG-3′). The reaction mixture was incubated at 37°C for one hour to synthesize the first strand of CND. Polymerase chain reaction (PCR) was performed following reverse transcription (RT) in a 50 μl reaction volume by adding 5 μl of $10 \times$ PCR buffer, 2.5 u Taq DNA polymerase and 10 pmol of the 5′ primer (5′-GAACATCTTTGTCCTCAGCG-3′) to the reverse transcription products. PCR was run for 30 cycles in a thermal cycler with a denaturing phase of 1 min at 94°C, annealing phase of 2 min at 60°C and extension phase of 3 min at 72°C. RT–PCR products of 20 μl were subjected to Southern blot analysis by use of a nested oligonucleotide probe (5′-GTACTCCTTCATGGTCCGGAACACC-3′). The blotted membrane was washed twice in $6 \times$ SSC at 60°C and exposed to Kodak X-ray film at −80°C for one hour with an intensifying screen.

RT–PCR Southern blot analysis of rat cytoplasmic β-actin was performed simultaneously following the same protocol to normalize quality and quantity of these RNA samples. The DNA sequence of oligonucleotides are, 3′ primer (5′-TGGCATAGAGGTCTTTACGG-3′), 5′ primer (5′-GAACCCTAAGGCCAACCGTG-3′), and nested probe (5′-CGCACGATTTCCCTCTCAGC-3′). RT–PCR products of 1 μl were subjected to Southern blot analysis. The RT–PCR products of bradykinin $B_2$ receptor and β-actin were loaded on the same agarose gel in which β-actin products were loaded, 30 min ahead of the $B_2$ receptor samples. The band intensities were scanned by a densitometer and the ratios of bradykinin $B_2$ receptor to β-actin were calculated.

### *Drugs*

Bradykinin, desArg$^9$-bradykinin, acetylcholine, prostaglandin $E_2$, sodium nitroprusside, oestradiol benzoate and progesterone were purchased from Sigma Chemical Company (St. Louis, MI, U.S.A.). Icatibant and Sar[D-Phe$^8$]des-Arg$^9$-bradykinin were gifts from Hoechst AG (Frankfurt, Germany) and Prof. Domenico Regoli (University of Shrebrooke, Canada), respectively.

### *Statistical analysis*

All data are expressed as mean ± s.e.mean. Multivariate repeated-measures ANOVA was performed to test for interaction between time and grouping factor. Univariate ANOVA then was used between groups and over time. Differences within and between groups were determined by paired or unpaired Student's $t$ test, respectively, with the Bonferroni multiple-comparison adjustment.

## Results

### *Effect of ovariectomy and oestrogen replacement on the blood pressure response to bradykinin*

No difference was detected between groups regarding basal MBP levels (controls, $126 \pm 3$; ovariectomized rats given vehi-

cle, 121±3; ovariectomized rats given oestrogen, 123±4 mmHg, $P$=NS). As shown in Figures 1 and 2, and Table 1, ovariectomy reduced the magnitude of the vasodepressor response to bradykinin and revealed a secondary vasopressor effect. Oestrogen replacement restored the vasodepressor effect of bradykinin to normal and reduced the magnitude of the secondary vasopressor effect.

Figures 3 and 4 show the blood pressure responses to endothelium-dependent and endothelium-independent vasodilators. Ovariectomy reduced the vasodepressor responses to sodium nitroprusside, acetylcholine, desArg$^9$-bradykinin or prostaglandin $E_2$. Oestrogen replacement restored to normal the response to acetylcholine, desArg$^9$-bradykinin and prostaglandin $E_2$, but not that to sodium nitroprusside.

Pretreatment with icatibant, a selective $B_2$ receptor antagonist, completely abolished the vasodepressor and the secondary vasopressor response to bradykinin in sham operated rats as well as in ovariectomized rats with or without oestrogen replacement. The antagonist did not alter the vasodepressor response to desArg$^9$-bradykinin (data not shown).

Pretreatment with Sar[D-Phe$^8$]des-Arg$^9$-bradykinin, a selective $B_1$ receptor antagonist, prevented the vasodepressor response to desArg$^9$-bradykinin, whereas it did not alter the MBP changes induced by bradykinin (data not shown).

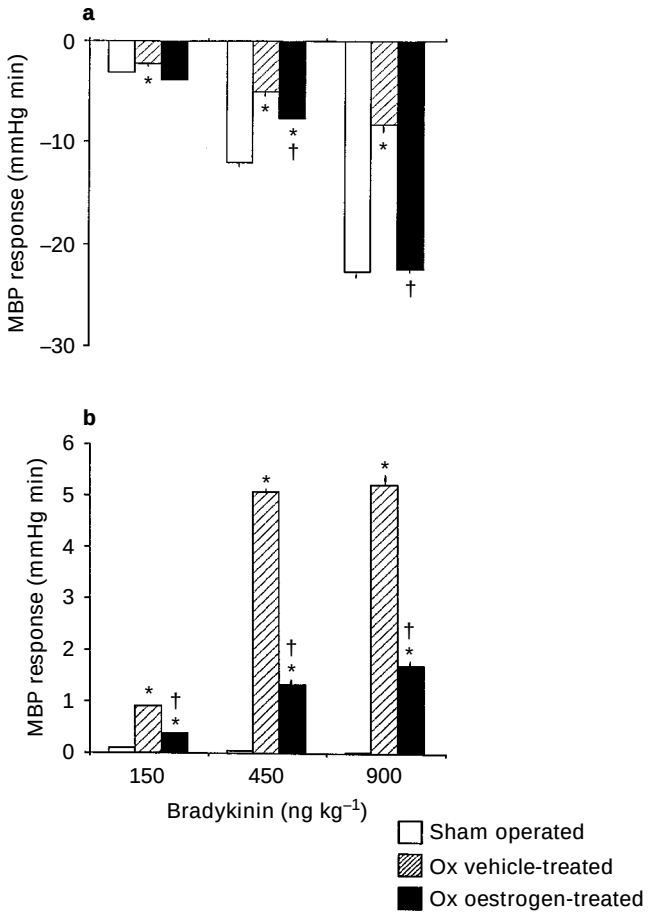


**Figure 1** Effect of ovariectomy and oestrogen replacement on the vasodepressor response to bradykinin. The blood pressure responses to bolus intra-arterial injection of graded doses of bradykinin in sham-operated (open columns), ovariectomized, vehicle-treated (hatched columns), and ovariectomized, oestrogen-treated (solid columns) female rats are shown. Each group consisted of 6 rats. Responses are expressed as the area under the curve. Bradykinin induced a vasodepressor response (a), followed by a vasopressor effect (b). Ovariectomy reduced the vasodepressor and enhanced the vasopressor response. The vasodepressor effect was reversed by oestrogen replacement. *$P$<0.05 versus sham-operated, †$P$<0.05 versus ovariectomized, vehicle-treated rats.

### *Sex dimorphism of the rat bradykinin $B_2$ receptor expression*

The expression of bradykinin $B_2$ receptors in the kidney, adrenal gland, aorta, heart and left ventricle were compared between male and female rats by RT–PCR Southern blot analysis (Figure 5). Females were found to have higher expression levels than male rats in the kidney, adrenal gland, aorta, artery and left ventricle.

### *Effects of sex hormones on rat bradykinin $B_2$ receptor expression*

As shown in Table 2 and Figure 6, the expression of rat bradykinin $B_2$ receptors in aorta decreased after ovariectomy (lane 2), and was restored to normal by oestrogen (lane 3), but not by progesterone (lane 4). The expression of $B_2$ receptors in the

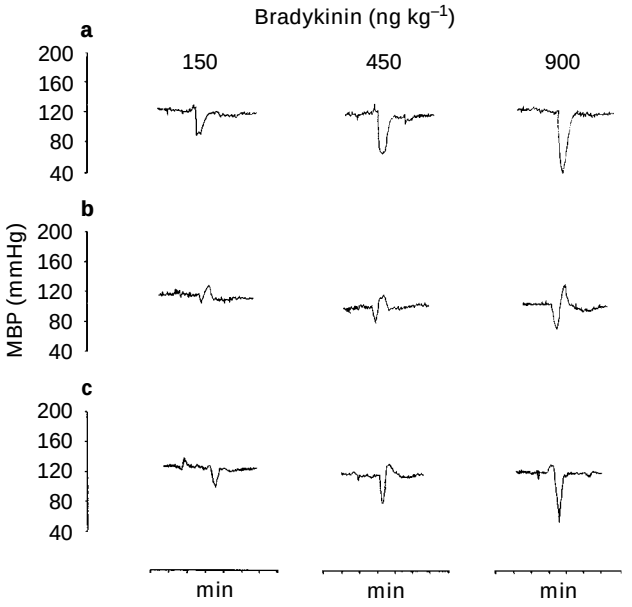


**Figure 2** Blood pressure response to bradykinin. Traces represent typical responses of mean blood pressure (MBP) to bolus intra-arterial injection of graded doses of bradykinin in sham-operated (a), ovariectomized, vehicle-treated (b) and ovariectomized, oestrogen-treated (c) female rats. The vasodepressor response was reduced in the ovariectomized, vehicle-treated rat and it was followed by a secondary increase in MBP. Vasodepressor responses to bradykinin were restored by oestrogen replacement in the ovariectomized rat indicated by traces in (c).

**Table 1** Effects of ovariectomy and oestrogen replacement on blood pressure responses to bradykinin

| *Operation* | *Treatment* | *Bradykinin* (ng kg$^{-1}$) | *Vaso-depressor response* | *Vasopressor response* |
|---|---|---|---|---|
| Sham | Vehicle | 50 | −29±2 | 3±1 |
| | | 150 | −37±3 | 2±1 |
| | | 300 | −47±5 | 1±1 |
| Ovariectomy | Vehicle | 50 | −12±5* | 10±4* |
| | | 150 | −16±5* | 19±6* |
| | | 300 | −15±7* | 23±7* |
| Ovariectomy | Oestrogen | 50 | −24±1$^{\S}$ | 5±3$^{\S}$ |
| | | 150 | −28±2$^{\S}$ | 10±3*$^{\S}$ |
| | | 300 | −42±2$^{\S}$ | 9±4*$^{\S}$ |

Values are mean±s.e.mean and represent absolute changes in mean blood pressure at the peak of the initial vasodepressor and subsequent vasopressor response to bradykinin. Each group consisted of 6 rats. *$P$<0.05 versus sham operated rats given vehicle, $^{\S}P$<0.05 versus ovariectomized rats given vehicle.

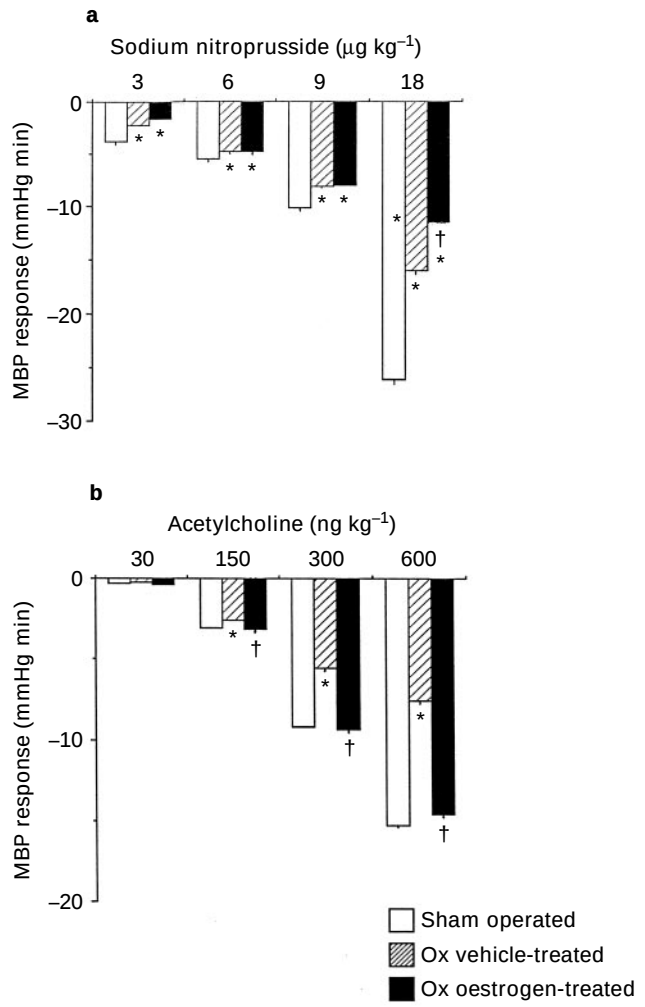


**Figure 3** Effect of ovariectomy and oestrogen replacement on the vasodepressor response to sodium nitroprusside and acetylcholine. The blood pressure responses to bolus intra-arterial injections of graded doses of sodium nitroprusside (a) and acetylcholine (b) in sham-operated (open columns), ovariectomized, vehicle-treated (hatched columns) and ovariectomized, oestrogen-treated (solid columns) female rats are shown. Each group consisted of 6 rats. Responses are expressed as the area under the curve. Ovariectomy reduced the vasodepressor responses to these agents. Oestrogen replacement restored the vasodepressor response to acetylcholine but not that to sodium nitroprusside. $*P<0.05$ versus sham-operated; $\dagger P<0.05$ versus ovariectomized, vehicle-treated rats.

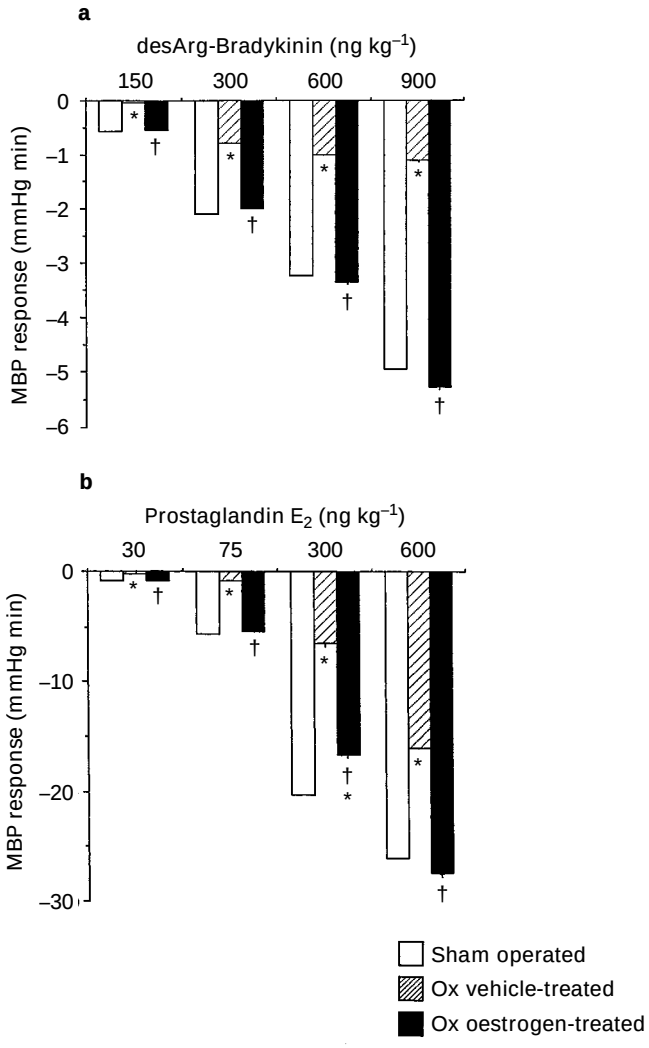


**Figure 4** Effect of ovariectomy and oestrogen replacement on the vasodepressor response to desArg$^9$-bradykinin and prostaglandin $E_2$. The blood pressure responses to bolus intra-arterial injections of graded doses of desArg$^9$-bradykinin (a) and prostaglandin $E_2$ (b) in sham-operated (open columns), ovariectomized, vehicle-treated (hatched columns), and ovariectomized, oestrogen-treated (solid columns) female rats are shown. Each group consisted of 6 rats. Responses are expressed as the area under the curve. Ovariectomy reduced the vasodepressor responses to these agents. The vasodepressor response was restored to normal by oestrogen replacement. $*P<0.05$ versus sham-operated; $\dagger P<0.05$ versus ovariectomized, vehicle-treated rats.

kidney decreased after ovariectomy (lane 2), and was restored by oestrogen (lane 3) and progesterone (lane 4). Neither ovariectomy nor sex hormone replacement had effects on the expression of $B_2$ receptors in the heart and uterus. The expression level of $\beta$-actin was not different between the samples.

## Discussion

Oestrogen, a member of the steroid hormone family, exerts its biological effects by interacting with intracellular receptors in tissues relevant to sexual function, such as breast, ovaries and uterus. Radioligand binding, immunoblot and mRNA analyses indicate that tissues, not classically defined as oestrogen targets, including vascular endothelial cells, also express oestrogen receptors (Venkov *et al*., 1996; Kim-Schulze *et al*., 1996). Binding of oestrogen to its receptor leads to transcriptional regulation of gene expression. There is evidence that sex hormones contribute to the transcriptional regulation of genes encoding for components of the kallikrein-kinin system. Consensus regulatory sequences for oestrogen and progesterone are contained in human pancreatic/renal kallikrein gene (Murray *et al*., 1990) and immunoreactive kallikrein and kallikrein mRNA levels are decreased by ovariectomy, with these alterations corrected by treatment with oestrogen (Madeddu *et al*., 1991). Upregulation in the expression of kininogen gene by oestrogen has been shown in the rat liver (Chen *et al*., 1992). In addition, oestrogen may enhance the activity of kinins generated by the enzymatic action of kallikrein on kininogen by affecting the activity of kininase II, a kinin degrading enzyme (Proudler *et al*., 1995).

A sexual dimorphism in the cardiovascular response to blockade of bradykinin $B_2$ receptors is indicated by increased blood pressure levels during adult age in female rats given icatibant since the first days of life (Madeddu *et al*., 1996). We speculated that, in addition to kallikrein and kininogen, the $B_2$ receptor gene represents a possible target for the action of oestrogen. As far as we know, the present study is the first to demonstrate that a sexual dimorphism exists in $B_2$ receptor expression, mRNA levels being higher in the kidney, adrenal gland, aorta, artery and left ventricle of female rats. In addi-

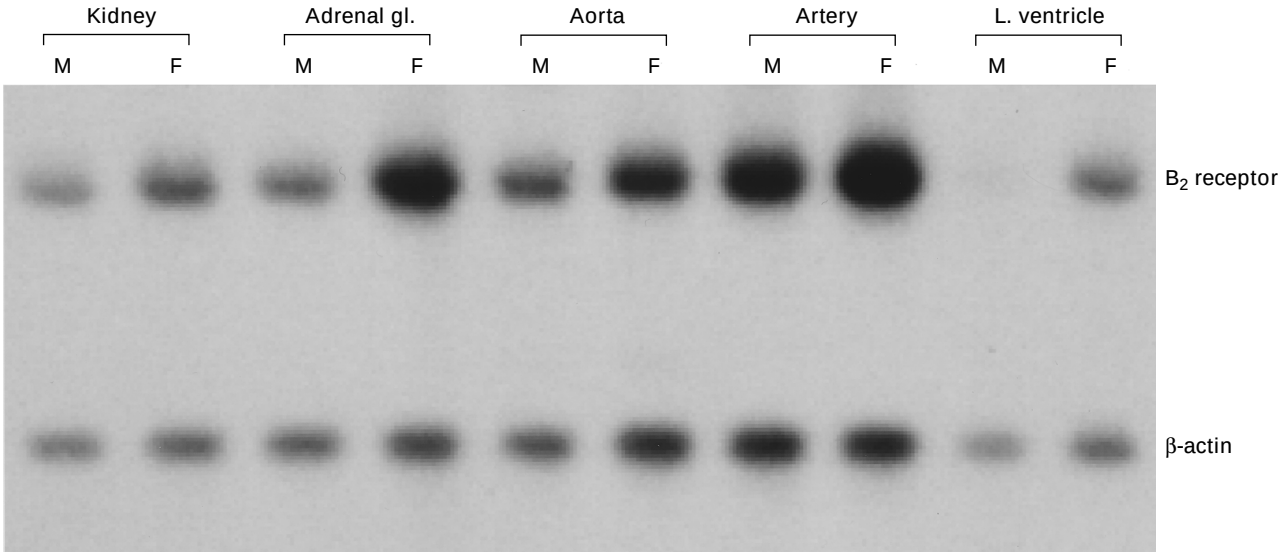


**Figure 5** Sex dimorphism of rat bradykinin $B_2$ receptor expression. Total RNAof 500 ng from each tissue was used for RT–PCR Southern blot analysis of rat bradykinin $B_2$ receptors. RT–PCR Southern blot of rat $\beta$-actin was performed simultaneously with the same amounts of total RNA. The $B_2$ receptor expression is shown on the top and the rat $\beta$-actin expression is shown on the bottom. Female rats appeared to have higher expression levels of bradykinin $B_2$ receptor than male rats in the kidney, adrenal gland, aorta artery and left ventricles.

tion, $B_2$ receptor mRNA levels were decreased by ovariectomy in the kidney and aorta, with these alterations corrected by treatment with oestrogen. Specificity of these observations is confirmed by the finding that neither ovariectomy nor hormone replacement caused alterations in $\beta$-actin mRNA levels. In the aorta, the effect of eostrogen was selective, since progesterone failed to correct the decrease in $B_2$ receptor expression produced by ovariectomy. The expression of $B_2$ receptors in the heart was not affected by ovariectomy or hormone replacement, thus suggesting that the sexual dimorphism detected in this organ is not related to oestrogen and progesterone.

We found that oestrogen replacement did not alter the basal blood pressure of ovariectomized rats. Epidaemiological studies such as the 'Postmenopausal Oestrogen/Progestinic Interventions Trial' showed no effect of chronic treatment on blood pressure (The Writing Group for PEPI, 1995). It is likely that the haemodynamic effect of oestrogen is compensated by a series counterregulatory responses capable of maintaining systemic blood pressure unaltered. However, the influence of oestrogen on vascular tone could be unmasked in stimulated conditions, as suggested by the finding that ovariectomy reduces the vasodepressor response to exogenous bradykinin, with these alterations corrected by oestrogen replacement. Oestrogen-related changes in $B_2$ receptor expression in the vasculature could have contributed to the alteration of systemic blood pressure responses to exogenous bradykinin. However, our observations on receptor gene expression were limited to the aorta, thus we cannot say if changes also occur in resistance arterioles. Another possibility is that oestrogen restores the vasodepressor effect of bradykinin by decreasing the activity of kininase II (Proudler *et al*., 1995).

Bjornstad-Ostensen & Berg (1994) have shown that the blood pressure response to bradykinin is characterized by a first period in which the increase in circulating kinins leads to predominance of vasodilatation, followed by a second phase of recovery where hypertensive reflex mechanisms, in this case adrenergic activation, take over while plasma kinins are being removed. Ovariectomy not only reduced the initial vasodepressor effect of bradykinin, but it revealed a secondary vasopressor action of the peptide. Various mechanisms could have contributed to the latter effect, including unbalanced prevalence of adrenergic activation. Contribution of the $B_1$ receptor in the secondary hypertensive phase has to be considered since surgical manipulation and implantation of arterial catheters might have induced the expression of this receptor subtype, which is not expressed under normal conditions in the rat. However, a prevalent vasodepressor effect was observed with desArg$^9$-bradykinin, a peptide with higher affinity for the $B_1$ receptor compared with bradykinin (Regoli & Barabe, 1980), and this hypotensive effect was prevented by Sar[D-Phe$^8$]des-Arg$^9$-bradykinin, a selective $B_1$ antagonist. In addition, both the vasodepressor and vasopressor responses to bradykinin were prevented by pretreatment with icatibant, a selective $B_2$ receptor antagonist, but they were unaffected by Sar[D-Phe$^8$]des-Arg$^9$-bradykinin. Taken together, these ex-

**Table 2** Effects of oestrogen and progesterone on bradykinin $B_2$ receptor expression in Wistar rats

| *Tissue* | *Operation* | *Hormone replacement* | *Relative $B_2$ mRNA level* |
|---|---|---|---|
| Aorta | Sham | Vehicle | 1.000 |
| | Ovariectomy | Vehicle | $0.765 \pm 0.037$* |
| | Ovariectomy | Oestrogen | $0.987 \pm 0.020^{\S}$ |
| | Ovariectomy | Progesterone | $0.790 \pm 0.016$* |
| Kidney | Sham | Vehicle | 1.000 |
| | Ovariectomy | Vehicle | $0.466 \pm 0.021$* |
| | Ovariectomy | Oestrogen | $1.162 \pm 0.062^{\S}$ |
| | Ovariectomy | Progesterone | $1.077 \pm 0.166^{\S}$ |
| Heart | Sham | Vehicle | 1.000 |
| | Ovariectomy | Vehicle | $1.081 \pm 0.172$ |
| | Ovariectomy | Oestrogen | $1.007 \pm 0.075$ |
| | Ovariectomy | Progesterone | $0.879 \pm 0.106$ |
| Uterus | Sham | Vehicle | 1.000 |
| | Ovariectomy | Vehicle | $1.032 \pm 0.016$ |
| | Ovariectomy | Oestrogen | $1.024 \pm 0.006$ |
| | Ovariectomy | Progesterone | $0.976 \pm 0.067$ |

Values are mean $\pm$ s.e.mean. Each group consisted of 3 rats. The intensities of the bands in Southern blot were determined by a densitometer. The expression of $B_2$ receptors in the aorta and kidney decreased after ovariectomy and was restored to normal by replacement with oestradiol. Progesterone restored the expression of $B_2$ receptors in the kidney but not in the aorta of ovariectomized rats. Neither ovariectomy nor sex hormones affected the $B_2$ receptor mRNA levels in the heart and uterus. The expression level of β-actin did not differ between groups. *$P<0.05$ versus sham + vehicle; $^{\S}P<0.05$ versus ovariectomy + vehicle.

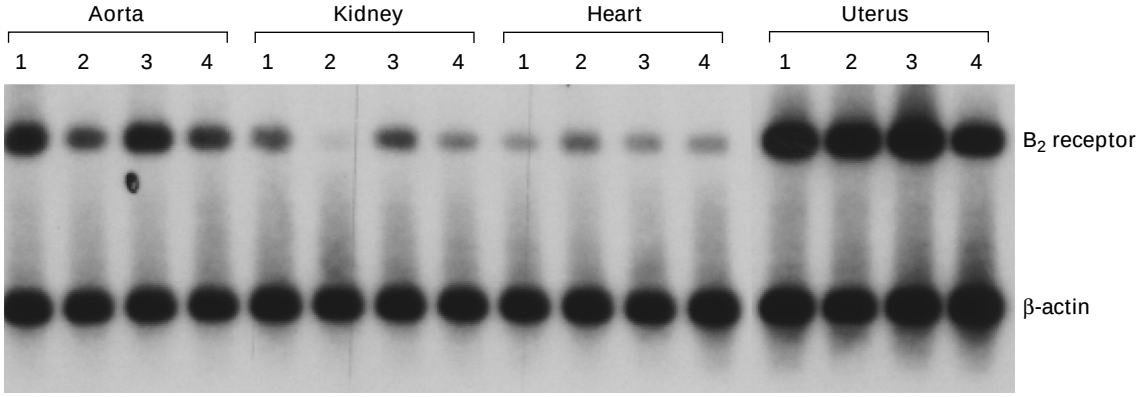


**Figure 6** Effects of sex hormones on rat bradykinin $B_2$ receptor expression. Total RNA of 500 ng from each tissue was used for RT–PCR Southern blot analysis of the rat bradykinin $B_2$ receptor. RT–PCR Southern blot of rat $\beta$-actin was performed simultaneously with the same amounts of total RNA. Lane 1, vehicle in sham-operated rat; lane 2, vehicle in ovariectomized rat; lane 3, oestradial benzoate in ovariectomized rat; lane 4, progesterone in ovariectomized rat.

periments suggest that the vasodepressor response to desArg$^9$-bradykinin is mediated by the $B_1$ receptor, while the haemodynamic responses to bradykinin (including the exacerbated hypertensive effect in ovariectomized rats) are mediated by activation of the $B_2$ receptor.

Recent evidence indicates that menopause is associated with endothelial dysfunction (Taddei *et al*., 1996), as suggested by a steeper age-related decline in acetylcholine-induced vasodilatation in women after 44 years of age. However, the mechanism by which menopause produces this effect remains unclear. Possible participation of oestrogen is suggested by the demonstration that long-term administration of this hormone upregulates the transcription of nitric oxide synthase (Weiner *et al*., 1994). Our finding that the vasodepressor responses to bradykinin, desArg$^9$-bradykinin and acetylcholine were restored to normal by hormone replacement supports the hypothesis that oestrogen modulates endothelium-dependent vasodilatation in female rats. Selectivity of the effect of oestrogen was shown by its inability to restore the vasodperessor response to sodium nitroprusside, an endothelium-independent vasodilator, to normal.

It is well documented that activation of G-protein coupled endothelial $B_2$ receptors by bradykinin promotes, via stimulation of phosphpolipase $A_2$ and C, an enhanced cytosolic calcium concentration and the formation of prostaglandins and nitric oxide. Thus, both pathways may be implicated in the alterations of $B_2$-dependent vasodilatation in ovariectomized rats. Our studies also indicated that the vasodilator potency of prostaglandin $E_2$ is diminished by ovariectomy and restored to normal by oestrogen replacement. These results suggest that prostaglandin-mediated vasodilatation can be modulated by oestrogen. However, the evaluation of the mechanisms underlying this interaction is beyond the aim of the present study and deserves further research.

Endothelial dysfunction associated with menopause might account for increased cardiovascular morbidity and mortality in postmenopausal women. Relevant to this aspect is also the observation that oestrogen replacement therapy reduces coronary events and improves endothelial function. Recent evidence indicates that endogenous kinins exert cardioprotection and are instrumental for the beneficial effect of ACE inhibitors in myocardial ischaemia and hypertrophy (Parratt, 1994; Linz *et al*., 1995). It is tempting to speculate that at least part of the protective action of oestrogen is mediated by kinins released at the level of the myocardium and/or coronary vasculature, or both (Cherry *et al*., 1982). In this context, it is noteworthy that $B_2$ receptor expression was altered by ovariectomy and restored to normal by oestrogen replacement in the aorta, whereas no effect was observed in the heart.

In conclusion, oestrogen modulates bradykinin $B_2$ receptor gene expression and blood pressure responses to endothelium-dependent vasodilators. The beneficial effects of oestrogen replacement might be mediated, at least in part, by activation of the kallikrein-kinin system.

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