O35

## $F_{2\alpha}$-isoprostane production is reduced by physiological concentrations of oestradiol in human endothelial cells in culture

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Free radical-generated $F_{2\alpha}$-isoprostanes are a group of compounds with vasoconstrictor properties and have been recognized as a stable, good biomarker for *in vivo* oxidative stress. Oestradiol (E2) has been proposed to exert antioxidant effects, a property related to the cardiovascular effects of oestrogens, but controversial results have been reported with the use of different doses and methods to study that antioxidant capacity. We investigated whether E2 exerts anti-oxidant actions modifying $F_{2\alpha}$-isoprostane production.

Cultured human umbilical vein endothelial cells were exposed to different physiological concentrations (0.1–10 nM) of E2 for different times of incubation, up to 48 h. In some experiments, anti-oestrogens (ICI 182780 or EM-652) or progestogens were added. Total (free plus esterified) $F_{2\alpha}$-isoprostanes were measured in culture medium after extraction with specific $F_{2\alpha}$-isoprostane affinity columns and assayed by using a commercial $F_{2\alpha}$-isoprostane EIA kit. Repeated-measures ANOVA was applied for comparisons of means, and then Student's unpaired *t* test was performed. $P$ values $< 0.05$ were considered significant.

Exposure to different E2 dosages for less than 8 h did not modify $F_{2\alpha}$-isoprostane production. Exposure to 1 nM E2 decreased $F_{2\alpha}$-isoprostane production only after 24 h, whereas the 10 nM E2-induced reduction was already significant after 16 h. The rest of the experiments were performed at 24 h of incubation with different compounds. E2 (1 and 10 nM) inhibited $F_{2\alpha}$-isoprostane production by 36 and 49 %, respectively ($P < 0.001$ *vs.* control, for both values). Exposure to anti-oestrogens alone (ICI 182780 or EM-652) slightly reduced $F_{2\alpha}$-isoprostanes ($P < 0.05$ *vs.* control), but much less than E2 ($P < 0.05$). ICI 182780 reversed the E2-induced reduction of $F_{2\alpha}$-isoprostane production ($P < 0.05$ *vs.* E2 values). Along with time course analysis, these results suggest that E2 effects were mediated through both oestrogen receptor-dependent and -independent mechanisms. The effect of two progestogens was also tested. Neither natural progesterone nor medroxyprogesterone acetate (a progestogen used in postmenopausal hormone therapy) changed $F_{2\alpha}$-isoprostane production at any of the tested concentrations (1, 10 and 100 nM). Combined exposure to E2 plus progesterone or medroxyprogesterone modified the endothelial cell production of $F_{2\alpha}$-isoprostanes in a different way: progesterone reversed the E2-induced reduction of $F_{2\alpha}$-isoprostane production while medroxyprogesterone did not.

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*All procedures accord with current local guidelines and the Declaration of Helsinki.*

O36

## Searching for new auto-antigens in renal vasculitis

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Vasculitis is an inflammatory disease affecting blood vessels with an autoimmunological origin. Autoimmunity is an immune reaction against the organism's own components produced by the existence of autoreacting clones which have not been destroyed during immuno-system maturation. Antibodies produced by this clones react against autoantigens. Some of these antibodies react against components of the blood vessel walls. These reactions produce damage in the vessel walls producing vasculitis. The clinical manifestations of vasculitis depend on the organ in which vessels are affected. The more commonly affected organs are lung and kidney. In the present work we have studied the immunoreactivity of the serum of a patient under no treatment, suffering from a vasculitis characterized by focal necrotizing glomerulonephritis with no immune deposits. We identified proteins that previously were unknown as autoantigens and that could be involved in the pathophysiology of this vasculitis.

With the immunoscreening method SEREX (serological identification of antigens by recombinant expression cloning), the patient's serum was used as a probe to screen a brain cDNA expression library. Five positive clones were found and four of these were purified and sequenced. By comparing the sequences with those in the databases by Blast we found that two clones were cDNA sequences with no counterpart in the databases. The other two clones encoded proteins of known functions but which are unknown as autoantigens. These proteins are jos 302 (HDAC5), jos 304 (TFC4), jos 307 and jos 313. The recombinant proteins of the four clones were probed as antigens by Western blot with different sera from patients affected with known vasculitis (i.e. LES) as well as sera from controls. Only the serum from the patient origin of this study recognized the recombinant proteins.

Although we cannot determine the role of these proteins in the aetiopathology of this disease at this point, the results suggests that they could be used as markers in the diagnosis of subfamilies in immune diseases.

*All procedures accord with current local guidelines and the Declaration of Helsinki.*

O38

## Adenosine-induced dilatation of pressurized rat coronary resistance arteries with myogenic tone: role of nitric oxide

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Adenosine is a local regulator of physiological functions which has been shown to be a vasodilator (Olsson & Pearson, 1990). We have previously shown that adenosine produces a dilatation of pressurised rat coronary resistance arteries with myogenic tone (Lynch *et al.* 2002). The current study examines the role of the endothelium-derived nitric oxide (NO) in mediating the

adenosine-induced dilatation of pressurised rat coronary arteries with myogenic tone. Also the role of $K_{ATP}$ channels in adenosine-induced dilatation was investigated during NO inhibition.

Wistar rats were killed by cervical dislocation and septal coronary arteries were dissected. Arteries were pressurised to 60 mmHg and checked for leaks. The inner diameter and wall thickness was continually monitored using a video dimension analyzer. Once myogenic tone had stabilised the presence of a functional endothelium (FE) was assessed by a dilatory response to acetylcholine ($10^{-5}$ M) of greater than 20 % ($n = 5$). Vessels displaying a response of less than 20 % were deemed to be have impaired endothelial (IE) function ($n = 7$). After control responses, the dilatation to adenosine ($10^{-4}$ M) was assessed in the presence of the NO synthase inhibitor L-NAME ($10^{-5}$ M), followed by L-NAME and glibenclamide ($5 \times 10^{-6}$ M).

The mean ± S.E.M. diameter of vessels used was 221.0 ± 13.1 $\mu$m. The mean dilatation of vessels to acetylcholine was 27.2 ± 1.9 % in the FE group and 7.7 ± 1.8 % in the IE group. The mean ± S.E.M. increase in diameter of FE vessels in response to adenosine was 42.6 ± 9.7 $\mu$m. This dilatation was significantly reduced following incubation with L-NAME to 22.1 ± 7.3 $\mu$m ($P < 0.05$, Student's paired $t$ test). The mean increases in diameter of IE vessels in response to adenosine was 23.3 ± 3.7 $\mu$m. This dilatation was not significantly reduced following incubation with L-NAME (17.7 ± 5.5 $\mu$m ($P = 0.5$, paired $t$ test). The adenosine-induced dilatation of IE vessels was significantly reduced compared with FE vessels ($P < 0.05$, unpaired $t$ test). Addition of glibenclamide in the presence of L-NAME did not alter the adenosine-induced dilatation in either the FE or IE group.

The current study demonstrates that the adenosine-induced dilatation of pressurised rat coronary arteries with myogenic tone is partially dependent on endothelium-derived NO, the remaining dilatation is endothelium independent and insensitive to glibenclamide.

Lynch FM *et al.* (2002). *Pflugers Arch* **443**, S259.
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O39

## Effect of acute severe hypoxia on vascular tone of isolated, pressurised rat mesenteric small arteries

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We have previously shown that hypoxia inhibits isometric tension of depolarised or agonist-activated small rat mesenteric arteries (Otter & Austin, 1999). Small arteries mounted under isobaric conditions, however, may also develop intrinsic myogenic tone. In arteries from other vascular beds reduced oxygen tension has been shown to inhibit myogenic reactivity (Messina *et al.* 1992; Loutzenhiser & Parker, 1994), however its effects on myogenic tone of mesenteric arteries in unclear. In this study, therefore, we have investigated the influence of acute severe hypoxia on depolarisation- and agonist-induced contractions, and on myogenic tone, of mesenteric arteries examined under isobaric conditions.

Small mesenteric arteries (150–200 $\mu$m internal diameter) were isolated from male Wistar rats (humanely killed by cervical dislocation following stunning), cannulated and mounted on a pressure myograph. Vessels were pressurised to 60 mmHg and perfused with bicarbonate-buffered saline at 37 °C, gassed with 95 % air–5 % $CO_2$ ($P_{O_2}$ = 134.3 ± 6.4 mmHg (mean ± S.E.M.), $n = 18$; $n$ is the number of animals) and left to equilibriate for 90 min. Intraluminal diameter was continuously monitored and any constriction noted. Vessels were also stimulated with high $K^+$ solution (60 mM KCl isosmotically substituted for NaCl) or 10 mM phenylephrine (PE). Hypoxia was achieved by gassing with 95 % $N_2$–5 % $CO_2$ and determined as $P_{O_{2P}} < 10$ mmHg (time = 10.4 ± 1.2 min, $n = 18$).

In some arteries ($n = 5$), warming to 37 °C under normoxic conditions induced a constriction of luminal diameter from 166 ± 5 mm to 112 ± 8 mm ($n = 5$). In 4/5 arteries 10 min hypoxia reduced this temperature-dependent myogenic tone (mean dilatation was 37 ± 12 % ($n = 5$)). Addition of high $K^+$ solution or PE produced a rapid constriction (from 189 ± 10 mm to 64 ± 12 mm in high $K^+$ ($n = 5$) and 175 ± 9 mm to 70 ± 10 mm in PE ($n = 8$)). The change in diameter was similar in response to both agents (125.0 ± 5.7 mm ($n = 5$) for high $K^+$ compared with 110.3 ± 7.4 mm ($n = 8$) for PE). In PE-constricted tissues a 5 min hypoxia caused a 67.0 ± 8.6 % dilatation which was significantly greater than the dilatation observed to hypoxia in arteries constricted by high $K^+$ (15.7 ± 2.2 %; $P < 0.05$, Student's unpaired $t$ test).

Thus, temperature-dependent myogenic tone was reduced by acute hypoxia in pressurised rat mesenteric small arteries. Furthermore, hypoxia resulted in a greater dilatation of PE-pre-constricted arteries compared to arteries pre-constricted by high $K^+$ solution.

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O40

## Hypoxia-induced production of oxygen free radicals in anaesthetised rats

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Hypoxia is a common feature of many diseases of respiratory and cardiovascular origin (Aber & Bayley 1963). This is often accompanied by tissue damage and oedema formation. Circumstantial evidence has implicated the involvement of leukocytes (Mian & Marshall, 1993; Sanidas *et al.* 2001) and oxygen free radicals in the genesis of this cellular damage (Granger *et al.* 1989). The primary aim of this study was to determine the effects of whole body hypoxia on the production of oxygen free radicals in whole blood.

Sixty male Wistar rats (250–400g) were anaesthetised with a mixture of Hypnorm–Hypnovel 2.7 ml $kg^{-1}$ and exposed to 3 min of spontaneously breathing either 6 % $O_2$ ($n = 15$), 12 % $O_2$ ($n = 15$) or 21 %$O_2$ ($n = 30$), in a purpose built environmental chamber maintained at 38 °C. One millilitre of blood was extracted by cardiothoracic puncture from the left ventricle into heparinised vacutainer (final concentration of heparin 10 U $ml^{-1}$). The rats were then humanely killed using Pentobarbitone at 0.8 ml $kg^{-1}$, directly injected into the heart.

The chemiluminescent response of 10 ml blood was obtained by adding 90 ml isoluminol and 10 ml of buffered saline (pH 7.4) into flat bottomed, anti-reflective test tubes and following the chemilumunescent response every min for 10 min in a luminescence detector (LB 9509 Junior). The peak chemiluminescent response was noted in relative light units (RLU). Exposure of rats to 21 % $O_2$ resulted in a basal level of chemiluminescence of 271 ± 12.3 RLU (mean ± S.D.). The intensity of this luminescence was significantly increased by exposure to 6 % $O_2$ (695 ± 20.2 RLU)* and 12 % $O_2$ (559 ± 16.7 RLU)*. (*$P < 0.05$, Student's unpaired *t* test.)

This is the first reported study of an increase in the production of oxygen free radicals in blood, from animals exposed to whole body hypoxia. These findings have implications as to the role of oxygen free radicals in mediating cell damage during hypoxic episodes.

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## O41

## High-energy diet induces obesity and markedly alters metabolic and vascular function in rats

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Obesity is associated with increased cardiovascular mortality and morbidity. It causes endothelial dysfunction, which is a marker for coronary-heart disease (Schachinger *et al.* 2000) and the main cause of death in obese subjects (Suwaidi *et al.* 2000). Yet surprisingly little work has been done to investigate how it damages the arteries. Here we have studied the role of an energy-rich diet on metabolic changes and arterial contractility.

Male Wistar rats were either fed laboratory chow throughout (control; $n = 10$); or given a fat-enriched, glucose-enriched diet (Naderali *et al.* 2001) for 16 weeks (diet-fed; $n = 23$). After 16 weeks, animals were killed by $CO_2$ inhalation followed by exsangunation. Trunk blood was collected for later analysis of insulin, glucose, leptin, non-esterified fatty acids (NEFA) and triglycerides. For contractile studies, third-order mesenteric resistance arteries were dissected out and mounted (in duplicate) on a computerised myograph based on Mulvany's principle.

At the end of the experiment, body weight (647.2 ± 19.0 *vs.* 574.5 ± 13.0 g), epididymal (19.1 ± 1.1 *vs.* 12.5 ± 1.2 g) and perirenal (22.3 ± 0.9 *vs.* 14.3 ± 1.2 g) fat pad masses and heart weights (1.8 ± 0.2 *vs.* 1.5 ± 0.1 g), but not gastrocnemius muscle (3.2 ± 0.1 *vs.* 3.1 ± 0.1 g) mass, were significantly ($P < 0.001$, Student's paired *t* test) higher in diet-fed rats than chow-fed controls. Terminal plasma levels of glucose (8.11 ± 0.26 *vs.* 7.61 ± 0.31 mM) and insulin (8.47 ± 0.91 *vs.* 8.33 ± 0.63 mU $ml^{-1}$) were comparable between the two groups, while diet-fed rats had significantly raised plasma NEFA (0.59±0.06 *vs.* 0.41±0.05 mM) and triglyceride (0.45 ± 0.07 *vs.* 0.22 ± 0.2 mM) and leptin (44.9 ± 2.7 *vs.* 22.4 ± 3.9 ng $ml^{-1}$) levels (by >100 %; $P < 0.0001$ paired *t* test). Myograph studies of mesenteric arteries showed that vasoconstrictor responses to noradrenaline (0.5–6 $\mu$M) and KCl (10–125 mM) were comparable between two groups indicating that diet and dietary-induced obesity had no effects on arterial contractility. However, both endothelium-dependent and -independent vasorelaxation responses were markedly attenuated in diet-fed rats. Vasorelaxation responses to carbamylcholine (10 mM to 100 $\mu$M) were significantly (> 16 %; $P < 0.01$ ANOVA) attenuated in the diet-fed group suggesting endothelial dysfunction. Sodium nitroprusside-induced vasorelaxation (10 nM to 100 $\mu$M) was also significantly (> 10 %; $P < 0.05$ ANOVA) decreased in the diet-fed group indicating abnormalities in vascular smooth muscle cells.

In conclusion, feeding rats with an energy-rich diet causes obesity and marked arterial dysfunction associated with it, an effect that may be mediated by raised leptin, NEFA and/or triglyceride levels.

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## O42

## Relationship between elastin organisation and structural and mechanical abnormalities in resistance arteries from spontaneously hypertensive rats

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Hypertension is associated with alterations in vascular structure and mechanics. In resistance arteries these modifications have been mainly attributed to changes in collagen. Elastin, an important determinant of vascular elasticity, has been less studied. The aim of the present work has been to study the role of elastin in the structural and mechanical abnormalities in resistance arteries from hypertensive rats.

Structural and mechanical characteristics of mesenteric resistance (MRA) and middle cerebral (MCA) arteries from adult spontaneously hypertensive (SHR, $n = 10$) and normotensive Wistar Kyoto rats (WKY, $n = 7$) were studied with pressure myography. Rats were killed with 50 mg $kg^{-1}$ pentobarbital I.P. following EU guidelines. Briefly, arterial segments were pressurised at a range of pressures (10–120 mm Hg) in 20 mmHg steps and internal and external diameters were measured in active (physiological salt solution, PSS) and passive (0 calcium PSS) conditions. From these measurements incremental distensibility–pressure and stress–strain curves were calculated to determine arterial distensibility and elasticity, respectively. Thereafter, arteries were pressure-fixed at 70 mmHg in 4 % paraformaldehyde and elastin content and 3-D structure was analysed with fluorescent confocal microscopy (Leica TCS SP2) and image analysis software (Metamorph) using the autofluorescent properties of elastin ($E_x$ 488 nm/$E_m$ 515 nm).

When compared to WKY rats, MRAs from SHR showed: (i) a significantly smaller lumen diameter in active (at 60 mmHg: WKY = 295 ± 15; SHR = 250 ± 11 $\mu$m, $P < 0.05$, 2-way ANOVA) and passive conditions (at 60 mmHg: WKY = 313 ± 8; SHR = 260 ± 7 $\mu$m, $P < 0.05$, 2-way ANOVA). This was observed at all pressures tested; (ii) significantly decreased

distensibility at low pressures (at 20 mmHg: WKY = 1.46 ± 0.06; SHR = 1.00 ± 0.04 % $mmHg^{-1}$, $P < 0.01$, 2-way ANOVA); (iii) a leftward shift of the stress–strain curve with a significantly increased $\beta$ value (WKY = 3.9 ± 0.1; SHR = 4.5 ± 0.2; $P < 0.05$, Student's unpaired *t* test). MCAs from SHR showed a significant reduction of lumen in active (at 60 mmHg: WKY = 206 ± 9; SHR = 171 ± 18 $\mu$m, $P < 0.05$, 2-way ANOVA), but not in passive conditions (at 60 mmHg: WKY = 236 ± 7; SHR = 230 ± 8 $\mu$m, n.s., 2-way ANOVA), and no difference in the mechanical parameters studied ($\beta$ value: WKY = 4.7 ± 0.4; SHR = 5.5 ± 0.6, n.s., Student's unpaired *t* test).

With respect to elastin organisation, MRA showed a a network of elastin fibres in the adventitia and a well defined internal elastic lamina (IEL). In MRAs from SHR, 3-D structure of IEL was altered, with a significant increase in the relative area occupied by elastin (SHR = 0.83 ± 0.02; WKY = 0.5 ± 0.04; Student's unpaired *t* test, $P < 0.01$) and smaller fenestra. However, total elastin content was similar between strains due to the reduced luminal surface area of SHR MRAs (SHR = 0.76 ± 0.02 $mm^2$; WKY = 0.91 ± 0.05 $mm^2$; $P < 0.05$). Elastin distribution in the adventitia was not different between strains. In MCA only a thin IEL was visible and elastin content and structure was similar between strains.

In conclusions, (1) the inward remodelling observed in MRAs from SHR is accompanied by a re-organisation of elastin in the IEL. This alteration can be responsible for the reduced elasticity observed at low pressures. (2) MCAs from SHR did not show alterations in structure, mechanics or elastin organisation. The reduction in MCA internal diameter in active conditions is due to an increase in the intrinsic tone of the artery.

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*All procedures accord with current National and local guidelines.*

O42b

## Tonic release of epoxyeicosatrienoic acids modulates activity of intraparenchymal arterioles in brain slices

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Cerebral blood flow is regulated locally to match the continuously changing metabolic demands of the active neuropil. Recent studies indicate that astrocytes may act as an intermediary stage in the transmission of vasodilator signals from neurones to cerebral microvessels. Epoxyeicosatrienoic acids (EETs), produced by breakdown of arachidonic acid in astrocytic membranes, have been shown to dilate isolated cerebral blood vessels (Alkayed *et al.* 1996*a*). Studies *in vivo* have indicated that tonic release of EETs may provide a level of dilator tone within the cerebral circulation (Alkayed *et al.* 1996*b*). However, this finding is equivocal (Bhardwal *et al.* 2000) and it is possible that anaesthesia is a confounding factor. We have therefore used a brain slice preparation in which responsiveness of individual intraparenchymal arterioles can be studied *in situ* in the neuropil in the absence of anaesthesia.

Coronal slices of cerebral cortex 350 $\mu$m thick were prepared from urethane-anaesthetised (1.5 g $Kg^{-1}$ 20 % solution) 150–250 g male Wistar rats. Slices were maintained at 31–33 °C in artificial cerebrospinal fluid containing (mM): NaCl, 125.8; KCl, 3.1; $MgSO_4$, 1.3; $CaCl_2$, 2.4; $NaHCO_3$, 6.0; D-glucose 10.0, gassed with 95 % $O_2$–5 % $CO_2$ to pH 7.35–7.45. Images of cerebral arterioles 9.3 ± 0.4 $\mu$m (mean ± S.E.M.) internal diameter (ID), 30–70 $\mu$m below the surface of the slice, were captured using a CCD camera and Openlab image analysis software (Improvision Ltd). A low level of rhythmic contractile activity (vasomotion, 2.45 ± 0.7 $min^{-1}$) was observed.

Addition of miconazole, a cytochrome P450 epoxygenase inhibitor (20 $\mu$M for 5 min), reduced ID by 17.9 ± 2.9 % and increased vasomotion (3.9 ± 1.3 to 7.8 ± 1.5 contractions $min^{-1}$, $n = 8$). In four further vessels already preconstricted and showing vasomotion in the presence of a thromboxane $A_2$ agonist, U46619 (75 nM), addition of AMPA (1 $\mu$M for 5 min) to stimulate astrocytes and neurones, produced dilatation (ID increased from 11.8 ± 28 $\mu$m to 13.1 ± 2.8 $\mu$m) and a decrease in vasomotion (7.9 ± 1.4 to 2.3 ± 1.0 $min^{-1}$). In the presence of 20 $\mu$M miconazole, each vessel showed a further small constriction (decrease in ID from 11.4 ± 3.0 $\mu$m to 9.6 ± 2.9 $\mu$m) and increased vasomotion (8.7 ± 1.5 to 10.2 ± 2.0 $min^{-1}$) but AMPA still produced dilatation (ID increased to 11.7 ± 2.6 $\mu$m) and inhibited vasomotion to 0.8 ± 0.5 $min^{-1}$.

The results suggest that tonic release of cyctochrome P450 metabolites of arachidonic acid may participate in regulating basal cerebrovascular tone. However, these substances do not appear to be involved in mediating AMPA-evoked dilatation.

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P71

## Endogenous levels of ovarian hormones modulate the activity of $\beta$-adrenoceptors in isolated rat aorta and tail arteries

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The aim of this study was to assess whether the physiological status of ovarian hormones modifies the function of vascular $\beta$-adrenoceptors, as has been previously reported in ageing and hypertension.

We have analysed vascular function of aorta and tail arteries from 15-week-old female Wistar rats, in oestrus phase and age-matched ovariectomized prepubertal (OVXp), with isometric tension recording. Rats were killed by $CO_2$ inhalation. To study muscular and endothelial $\beta$-adrenoceptors, concentration–response curves to isoprenaline were performed in aortic segments with endothelium. Presynaptic $\beta$-adrenoceptors were studied in tail arteries without endothelium by means of electrical field stimulation (EFS, 200 mA, 0.2 ms, 0.5–8 Hz, for 15 s, at 2 min interval) in the presence of $\alpha_2$-adrenoceptor blockers. In tail artery segments, endothelium removal was achieved by gentle rubbing of the intimal surface with a stainless steel wire. The presence of functional endothelium was assessed by a relaxation with acetylcholine (0.01–10 $\mu$M) in precontracted arteries greater than 70 %. The absence of relaxation confirmed that endothelium had been removed.

In aortic segments precontracted with noradrenaline (NA, 0.1 $\mu$M), isoprenaline (0.01–100 $\mu$M) elicited relaxations that were significantly decreased in OVXp rats. Isoprenaline-induced relaxations were significantly diminished by propranolol (1 $\mu$M, a $\beta$-adrenoceptor antagonist) and L-NAME (100 $\mu$M, an

inhibitor of nitric oxide synthase). L-NAME blockade was significantly larger in estrus than OVXp rats. Cholera toxin (0.003–1 $\mu$g ml$^{-1}$, an activator of the stimulatory G-protein, $G_s$) caused relaxations in aorta that were significantly decreased by deprivation of ovarian hormones.

In tail arteries, isoproterenol (0.01–100 $\mu$M) did not induce relaxation in precontracted segments either from oestrus nor from OVXp rats. NA (0.001–10 $\mu$M) induced contractions that were not modified by deprivation of ovarian hormones. In this artery, EFS induced contractile responses in the presence of yohimbine (1 $\mu$M, an $\alpha_2$-adrenoceptor antagonist). Isoprenaline (1 $\mu$M) enhanced these EFS-induced contractions, the increase being significantly larger in arteries from OVXp rats.

These results suggest that the physiological status of ovarian hormones: (1) improves the muscular and endothelial $\beta$-adrenoceptor mediated relaxation, through increases in both nitric oxide and the activity of arterial $G_s$, and (2) decreases the presynaptic release of noradrenaline by means of a $\beta$-adrenoceptor-mediated mechanism.

All of the surgical and experimental procedures used in these studies followed the directions of the European Community Guidelines (Real Decreto 223/88, March 14, Spain). Supported by Grants from DGCYT (PM 97/0011) and Ministerio de Ciencia y Tecnología (BFI 2001–0638)

*All procedures accord with current National and local guidelines.*