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Reserpine induces vascular α_2-adrenergic supersensitivity and platelet α_2-adrenoceptor up-regulation in dog

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1 The aim of the present study was to investigate the influence of catecholamine levels on the regulation of α_2-adrenoceptor sensitivity in dogs.

2 Blood pressure and heart rate values at rest, plasma catecholamine levels, platelet and adipocyte α_2-adrenoceptors as well as the α_2-mediated cardiovascular responses to clonidine ($10\,\mu g\,kg^{-1}$ i.v., after α_1-, β-adrenoceptor plus muscarinic blockade) or noradrenaline (0.5, 1, 2 and $4\,\mu g\,kg^{-1}$ i.v. after α_1- and β-adrenoceptor blockade) were measured before and after reserpine treatment ($0.1\,mg\,kg^{-1}\,day^{-1}$ s.c. over 15 days).

3 Reserpine induced a significant decrease in resting systolic and diastolic blood pressures ($213 \pm 2/87 \pm 6$ mmHg before vs $158 \pm 5/59 \pm 3$ mmHg after treatment) as well as in heart rate (91 ± 2 beats min^{-1} before vs 76 ± 3 beats min^{-1} after treatment).

4 A 5 min tilt test performed under chloralose anesthesia, failed to modify blood pressure before treatment whereas it induced a significant fall in the same animals after the 15 day treatment. Plasma levels of noradrenaline significantly decreased (262 ± 58 vs $66 \pm 31\,pg\,ml^{-1}$) whereas plasma adrenaline levels were unchanged.

5 The α_2-mediated pressor responses to noradrenaline were significantly increased after reserpine. Clonidine induced a marked pressor effect (+72 and +45% in systolic and diastolic blood pressures respectively) after reserpine treatment. This effect was suppressed by administration of RX-821002, a new specific α_2-adrenoceptor antagonist.

6 Reserpine treatment significantly increased platelet α_2-adrenoceptor number (identified with [^{3}H]-yohimbine or [^{3}H]-RX821002) with no change in K_d values. α_2-Adrenoceptor number remained unchanged in adipocytes (identified with [^{3}H]-RX821002).

7 These results show that a 15 day treatment with reserpine induces a vascular α_2-adrenergic supersensitivity and an up-regulation in platelet α_2-adrenoceptors. In contrast, this phenomenon does not involve all the tissues since adipocyte α_2-adrenoceptors escape the effect of reserpine. We suggest that the levels of plasma noradrenaline play an important role in the regulation of the platelet and vascular α_2-adrenoceptors. In contrast, adipocyte α_2-adrenoceptors are not affected by changes in plasma noradrenaline levels.

Introduction

Adrenoceptors and receptor-mediated functions are reduced by chronic exposure to catecholamines and chronic administration of adrenoceptor agonists may be associated with a progressive decrease in the response of the effectors due to a down-regulation of the adrenoceptors (Brodde, 1983; Stiles *et al.*, 1984; Mahan *et al.*, 1987).

The consequences of depletion of endogenous stores of noradrenaline on adrenoceptor sensitivity have been largely investigated (for review see Fleming & Westfall, 1988). It has been demonstrated by Roscher *et al.* (1981) and Hawthorn & Broadley (1984) that the depletion of noradrenaline in cardiac tissue causes a supersensitivity to inotropic and chronotropic effects of β-adrenoceptor agonists in cardiac muscle from various species. Several reports have suggested that the supersensitivity phenomenon is specific, i.e. demonstrable only with β-adrenoceptor agonists (Broadley & Lumley, 1977; Hawthorn & Broadley, 1984). Indeed, several studies have demonstrated increased β-adrenoceptor density after chronic reserpine treatment (Tenner *et al.*, 1982; Latifpour & McNeill, 1984; Chess-Williams *et al.*, 1987).

In contrast, the existence of an α-adrenoceptor up-regulation is still under discussion; there are species and tissue differences in this regard (Latifpour & McNeill, 1984). Chess-Williams *et al.* (1987) failed to observe differences in the number of cardiac α-adrenoceptors after reserpine and Nasseri *et al.* (1985) found supersensitivity in vas deferens after reserpine with no change in α_2-adrenoceptors. In contrast, Watanabe *et al.* (1982), Bylund & Martinez (1980) and Fortin & Sundaresan (1989) observed a significantly increased number of α_2-adrenoceptors after reserpine in rat vas deferens, salivary glands and kidney cortex respectively. Fortin & Sundaresan (1989) also described an increased number of β- and α_1-adrenoceptors (but not angiotensin II receptors) in kidney cortex after reserpine treatment. Indeed, a clinical study from our group demonstrated the existence of vascular α-adrenergic supersensitivity to noradrenaline and up-regulation of platelet α_2-adrenoceptors in patients suffering from neurogenic orthostatic hypotension with low plasma levels of noradrenaline (Valet *et al.*, 1989).

These conflicting results led us to reinvestigate the consequences of such a sustained low plasma catecholamine level on the regulation of α_2-adrenergic sensitivity in dogs using reserpine as a catecholamine depleting agent. We have also tested whether the changes in platelet α_2-adrenoceptor numbers observed with reserpine are associated with increased vascular α_2-receptor-mediated function.

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Methods

Animals and general procedure

Eight adult male beagle dogs (12–15 kg) received subcutaneous reserpine at the dose of 0.1 mg kg^{-1} daily for 15 days. This dose was selected according to preliminary experiments showing that it induces a marked decrease in plasma catecholamines without major behavioral disturbances nor weight loss (not shown). The duration of reserpine treatment (15 days) was chosen since (1) reserpine causes a complete emptying of adrenergic fibres after 24 h (Häggendahl & Dahlström, 1971) and (2) the turn-over of α_2-adrenoceptors is around 36 h (Taouis *et al.*, 1986; Paris *et al.*, 1987). α_2-Adrenergic sensitivity was assessed through physiological (blood pressure, heart rate, plasma catecholamine levels and head up tilt) and pharmacological (blood platelet [^{3}H]-yohimbine binding sites, adipocyte [^{3}H]-RX 821002 binding sites, blood pressure responses to clonidine and noradrenaline) tests performed at days 0 (controls: before reserpine) and 15 (treated: 24 h after the last injection of reserpine: end of treatment). Moreover, plasma catecholamine levels and platelet [^{3}H]-yohimbine binding sites were measured in conscious dogs on day 30 and 45, i.e. 15 and 30 days after the end of reserpine treatment. All the experiments and blood samplings were done at 08 h 00 min on 24 h-fasted animals.

Blood pressure and heart rate measurements in conscious dogs

Several days before any experiment, the dogs were trained to stand still for 1 to 2 h on a Pavlov table and accustomed to intravenous infusion, intra-arterial recording and venous blood sampling. Blood pressure was recorded by means of a catheter introduced into the abdominal aorta via the left femoral artery under local anaesthesia (with xylocaine) and connected to a Gould P23ID transducer on a Honeywell recorder. The heart rate was obtained by use of a heart period meter triggered by blood pressure.

α_2-Adrenoceptor-mediated blood pressure response

Before and 15 days after reserpine treatment, the effect of an acute intravenous injection of clonidine (10 μg kg^{-1}) was recorded 15 min after prazosin (0.1 mg kg^{-1}, i.v.) plus propranolol (1 mg kg^{-1}, i.v.) plus atropine (1 mg kg^{-1}, i.v.) in conscious dogs. Systolic and diastolic blood pressures and heart rate were measured 1, 5, 10 and 15 min after clonidine in conscious dogs. Under the same conditions, the effect of clonidine was studied 5 min after acute administration of RX-821002 (0.25 mg kg^{-1}, i.v.). In other experiments, before and 15 days after reserpine treatment, the acute pressor responses to various doses of noradrenaline (0.5, 1, 2 and 4 μg kg^{-1}) were studied 15 min after propranolol (1 mg kg^{-1}, i.v.) plus prazosin (0.1 mg kg^{-1}, i.v.). Each subsequent dose of noradrenaline was given at least 1 min after the return of blood pressure and heart rate to normal resting values.

Head up tilt (anaesthetized dogs)

The day after the noradrenaline test, each animal was placed on a tilting table, anaesthetized with α-chloralose (80 mg kg^{-1}, i.v.), intubated with a cuffed tracheal tube and spontaneously respired. It was supported under the shoulders in the supine position by straps. After measurement of resting blood pressures, heart rate and plasma catecholamines in this position, the tilting table was positioned at 80° for 5 min: blood pressure and heart rate were recorded every 30 s and plasma sampling for catecholamines taken at 1 and 5 min. A second head up tilt was performed under the same conditions (chloralose anaesthesia) at the end of the reserpine treatment (day 15).

Blood sampling and catecholamine assays

Fresh blood was collected (from a catheter introduced into the jugular vein 30 min beforehand in order to prevent any stress) on lithium heparin with 10 nM sodium metabisulphite, centrifuged at 2000 *g* for 10 min at 0°C. Plasma was stored at −80°C. Catecholamines were selectively isolated from the sample at 0°C, in darkness, by adsorption to activated alumina, then eluted with 0.1 M perchloric acid. Dihydroxybenzylamine was used as an internal standard to monitor recovery from this extraction step. Noradrenaline and adrenaline were assayed by a Waters high performance liquid chromatography apparatus using electrochemical (amperometric) detection: the working electrode potential was set at 0.65 V against a Ag/AgCl reference electrode. Catecholamines were separated on a C18 column (3.9 × 150 mm) at a constant flow rate of 1 ml min^{-1}. The electrochemical detector response was linear for concentrations ranging from 25 pg ml^{-1} to 100 ng ml^{-1}. Under these conditions the detection limit was 50 pg ml^{-1} (Valet *et al.*, 1988).

Platelet and adipocyte membrane preparation and binding procedure

The platelet membranes were prepared as follows: 20 ml of venous blood was collected over 2 ml of 0.16 M sodium citrate solution and immediately centrifuged at 160 *g* for 10 min at room temperature. The platelet rich plasma (PRP) was collected and the platelet membranes were prepared as previously described (Villeneuve *et al.*, 1985). The adipocyte membranes were prepared as follows: fat biopsies (omental adipose tissue) were taken immediately after the induction of general anaesthesia by pentobarbitone and isolated adipocytes prepared (according to Berlan & Lafontan, 1985, with minor modifications). Krebs-Ringer bicarbonate buffer containing bovine serum albumin (3.5%) (KRBA) and glucose (6 nM), pH 7.4 was used as previously described (Berlan & Lafontan, 1985). After collagenase digestion (1 mg ml^{-1} KRBA buffer), isolated fat cells were washed three times and the packed cells were washed four times in a hypotonic lysing medium to elicit total cell breakage and recover all fat cells ghosts.

The membranes from platelet or adipocytes were used for adrenoceptor determination by a radioligand binding technique as previously described by Berlan & Lafontan (1980). Total binding of [^{3}H]-yohimbine or [^{3}H]-RX-821002 was determined by incubating 100 μl aliquots of the resuspended membrane preparation (100–200 μg protein assayed by the method of Lowry) with the radioligand in a total volume of 400 μl or binding buffer. Specific binding was defined as the difference between total and non-specific binding determined in parallel with an excess (10 μM) of adrenaline. Incubations were carried out for 20 min at 25°C under constant shaking at 100 cycles per min. The samples were filtered through fibreglass filters (Whatman GF/C) placed on a Millipore manifold sampling unit. The filters were washed twice with 10 ml of cold binding buffer and the radioactivity trapped on the filter was counted in a Packard spectrometer with an efficiency of 35%. The number of sites (B_{max}) and K_d values were calculated with a computer-assisted analysis of binding at saturation and according to Scatchard plots.

Data analysis

Each experiment was performed on the eight animals. Values are means ± s.e.mean. Statistical analysis was made using paired *t* test and differences considered significant when P was less than 0.05.

Drugs and chemicals

[^{3}H]-yohimbine (90 Ci mmol^{-1}) was obtained from Amersham (Amersham, U.K.) and [^{3}H]-RX 821002 (2-(2-methoxy,

1,4 benzodioxan-2yl)-2-imidazoline) was a gift from Reckitt and Colman Labs (Kingston-upon-Hull, U.K.). (−)-Noradrenaline bitartrate (Levophed, sterile commercial ampoule) came from Winthrop (Clichy, France), clonidine (Catapressan, sterile commercial ampoule) came from Boehringer Ingelheim (F.R.G.), reserpine (Serpasil, sterile commercial ampoule, reserpine dissolved in acetic acid, propylene glycol and water) and phentolamine mesylate from Ciba Geigy (Basel, Switzerland), prazosin (dissolved in 0.9% NaCl) from Pfizer (Orsay, France), (±)-propranolol (Avlocardyl, sterile commercial ampoule, propranolol dissolved in 0.9% NaCl) from Sigma (France).

Results

Effect of chronic reserpine treatment on resting blood pressure, heart rate, plasma catecholamine levels

Blood pressure, heart rate and plasma catecholamine levels were measured in conscious animals before and after 15 days with reserpine treatment (0.1 mg kg^{-1} daily, s.c.). The treatment induced significant decreases in plasma noradrenaline (75%) (without any modification in plasma adrenaline levels), in systolic (26%) and diastolic (32%) blood pressures and heart rate (16%) (Table 1). It should be mentioned that before reserpine the mean value of systolic (but not diastolic) blood pressure was slightly elevated although the animals were quiet (see the values of adrenaline). We have no explanation for this unexpected observation.

Effect of chronic reserpine treatment on cardiovascular and plasma catecholamine responses to tilting

A 5 min tilt was performed under chloralose anaesthesia before and after treatment. Before reserpine, tilting failed to modify systolic and diastolic blood pressures and induced a significant increase (25% after 1 and 5 min) in heart rate (Figure 1). Fifteen days after reserpine treatment, resting blood pressures and heart rate (measured in lying position in anaesthetized animals) were significantly ($P < 0.05$) lower than those before reserpine (as in conscious animals). Under these conditions, 1 min tilting significantly ($P < 0.05$) decreased systolic (39%) and diastolic (37%) blood pressures and increased heart rate (56%). Similar variations were observed after 5 min, i.e. at the end of the test.

Before reserpine, tilting induced an early significant ($P < 0.05$) increase in plasma noradrenaline (98% after 1 min) as well as adrenaline (210% after 1 min) levels which was sustained during the whole test (133% and 638% after 2 min for noradrenaline and adrenaline respectively). In treated animals, tilting failed to modify plasma noradrenaline levels. However, a significant ($P < 0.05$) increase (315%) in plasma adrenaline level was observed at the end of the test (5 min).

Table 1 Effect of a 15-day reserpine treatment (0.1 mg kg^{-1} daily) on cardiovascular parameters and plasma catecholamine levels in 8 conscious dogs

	Controls	*Treated*
Systolic blood pressure (mmHg)	213 ± 2	158 ± 5*
Diastolic blood pressure (mmHg)	87 ± 6	59 ± 2*
Heart rate (beats min^{-1})	91 ± 2	76 ± 3*
Plasma noradrenaline (pg ml^{-1})	262 ± 58	66 ± 31*
Plasma adrenaline (pg ml^{-1})	133 ± 24	163 ± 32

The measurements were made the day before the beginning of reserpine treatment (controls) and the day after the last injection of reserpine (treated). Values are the mean ± s.e.mean.
* Significantly different ($P < 0.05$) compared to the values measured in controls.

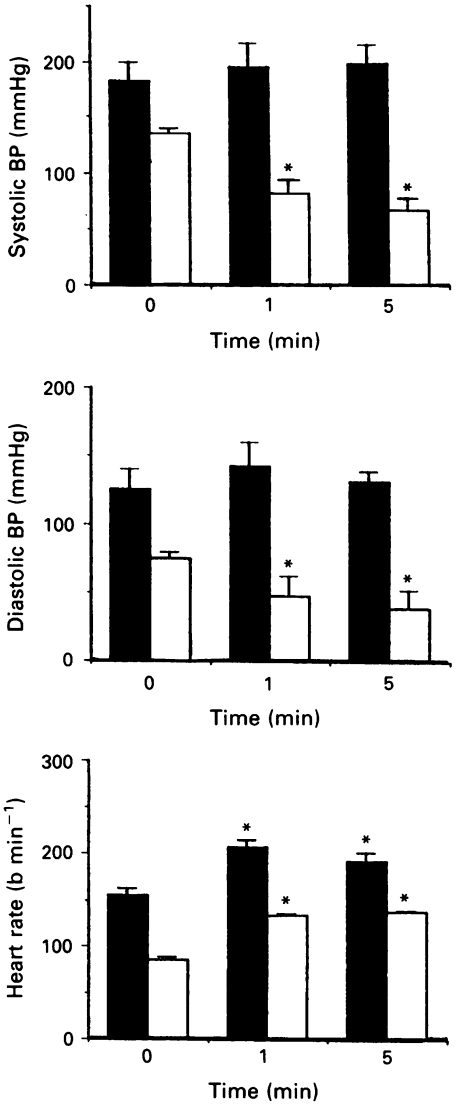

Figure 1 Effect of a 5 min head up tilt on systolic and diastolic blood pressures (BP in mmHg) and heart rate (beats min^{-1}) in 8 chloralose-anaesthetized dogs before (solid columns) and after 15-day treatment with reserpine (0.1 mg kg^{-1}, daily) (open columns). The values were measured in the lying position (time 0) and 1 and 5 min after the beginning of the tilt test. Statistical evaluations were made using Student's t test for paired comparison when the values at times 1 and 5 min (i.e. in standing position) were compared with corresponding values at time 0 (i.e. in lying position). * $P < 0.05$. Mean values are given with s.e.mean shown by vertical bars.

It is noticeable that plasma noradrenaline (but not adrenaline) levels measured in the lying position in reserpinized anaesthetized animals were much lower than those measured in the same reserpinized unanaesthetized dogs (15 ± 5 and 66 ± 31 respectively, $P < 0.05$).

Effect of chronic reserpine treatment on the cardiovascular responses to clonidine and noradrenaline

In order to explore the consequences of chronic reserpine treatment on vascular α_2-adrenoceptor-mediated responses, the effect of intravenous administration of clonidine (10 μg kg^{-1}) on systolic and diastolic blood pressures were studied after α_1- (prazosin) plus β- (propranolol) adrenoceptor plus muscarinic (atropine) receptor blockade. Blood pressure (and heart rate: not shown) values were similar in control and reserpine-treated dogs after this adrenoceptor plus muscarinic receptor blockade (Table 2). In control dogs, clonidine failed to modify blood pressure during the 15 min following its

Table 2 Influence of the α_2-adrenoceptor antagonist RX-821002 ($0.25\,mg\,kg^{-1}$, i.v.) on the effect of clonidine ($10\,\mu g\,kg^{-1}$, i.v.) on peak pressor effects (in mmHg) in control and 15-day reserpine treated conscious dogs ($0.1\,mg\,kg^{-1}$, daily)

	Control		*Treated*	
	Systolic	*Diastolic*	*Systolic*	*Diastolic*
A	216 ± 8	77 ± 3	156 ± 8	56 ± 6
B	127 ± 15[a]	80 ± 1	144 ± 6	90 ± 6
C	153 ± 15	100 ± 7	248 ± 21[b]	145 ± 9[b]
D	144 ± 9	87 ± 8	166 ± 12	97 ± 17
E	150 ± 5	95 ± 6	170 ± 13	110 ± 8

In each animal ($n = 8$) blood pressures (mmHg) were measured in the following order: (A) Basal blood pressure (after 30 min rest); (B) 15 min after propranolol ($1\,mg\,kg^{-1}$, i.v.) + prazosin ($0.2\,mg\,kg^{-1}$, i.v.) + atropine ($1\,mg\,kg^{-1}$, i.v.); (C) 1 min after clonidine; (D) 15 min after RX-821002; (E) 1 min after clonidine. Values are the mean ± s.e.mean.
[a] Significantly different ($P < 0.02$) compared with A values.
[b] Significantly different ($P < 0.02$) compared with B values.

administration although a slight (but not significant) tendency to increase and then a slight (but not significant) decrease were observed during the first 5 min and at the 15th min respectively (Figure 2). In reserpine-treated animals, clonidine induced a significant pressor response (+72% for systolic blood pressure and +61% for diastolic blood pressure) (Figure 2) without any modification in heart rate. The pressor effect of clonidine was abolished by previous administration of $0.25\,mg\,kg^{-1}$ i.v. RX 821002, an α_2-adrenoceptor antagonist (Table 2).

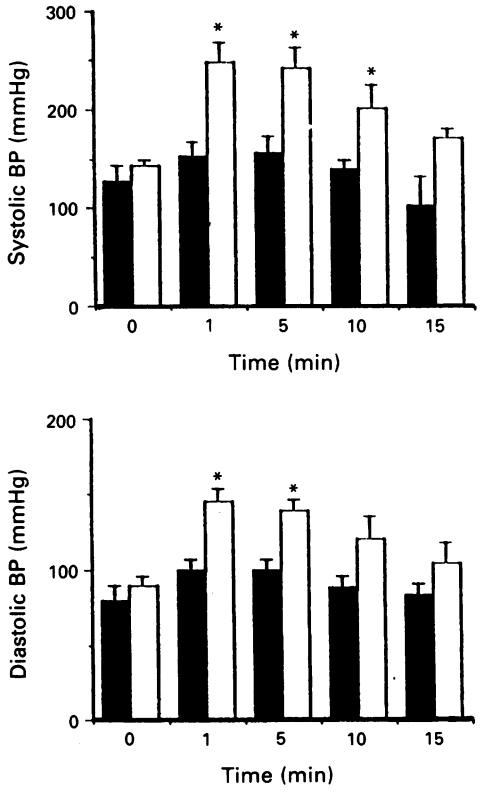

Figure 2 Effect of clonidine ($10\,\mu g\,kg^{-1}$, i.v. as a bolus) on systolic and diastolic blood pressures (mmHg) in 8 conscious dogs before (solid columns) and after (open columns) reserpine treatment ($0.1\,mg\,kg^{-1}$, daily for 15 days). The values were measured after prazosin ($0.1\,mg\,kg^{-1}$, i.v.) plus propranolol ($1\,mg\,kg^{-1}$, i.v.) plus atropine ($1\,mg\,kg^{-1}$, i.v.). Mean values are given with s.e.mean shown by vertical bars. * $P < 0.05$ when values obtained after reserpine are compared with those measured before reserpine in the same animals.

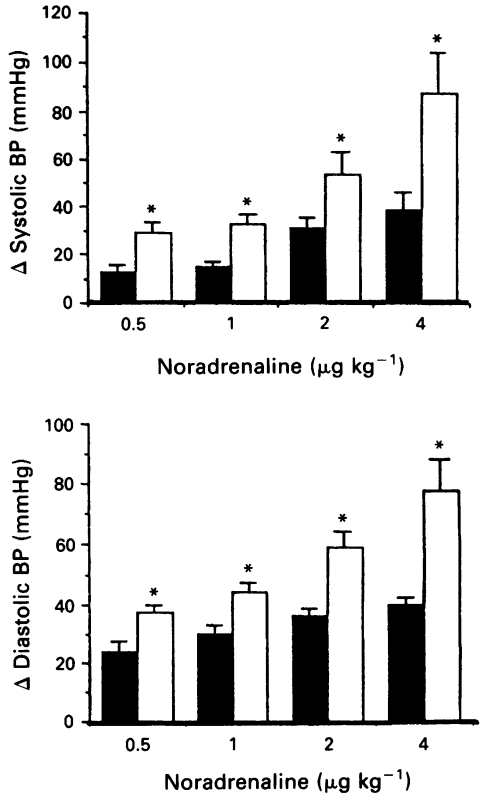

Figure 3 Pressor responses to noradrenaline (0.5, 1, 2 and $4\,\mu g\,kg^{-1}$, i.v. as bolus) in 4 conscious dogs before (solid columns) and after (open columns) reserpine treatment ($0.1\,mg\,kg^{-1}$ daily for 15 days). The values represent the maximal increase in systolic and diastolic blood pressures (BP in mmHg) after noradrenaline injection measured after prazosin ($0.1\,mg\,kg^{-1}$, i.v.) plus propranolol ($1\,mg\,kg^{-1}$, i.v.). Mean values are given with s.e.mean shown by vertical bars. * $P < 0.05$ when values obtained after reserpine are compared with those measured before reserpine in the same animals.

The effect of noradrenaline (0.5, 1, 2 and $4\,\mu g\,kg^{-1}$) was studied 15 min after β- and α_1-blockade in conscious animals. Under these conditions, resting values of systolic and diastolic blood pressures and heart rate were respectively 163 ± 2, 70 ± 6 mmHg and 98 ± 3 beats min^{-1} before reserpine and 131 ± 6 ($P < 0.01$), 48 ± 2 ($P < 0.02$) mmHg and 82 ± 10 beats min^{-1} (NS) after reserpine treatment. Noradrenaline induced a dose-dependent increase in systolic and diastolic blood pressure before as well as after reserpine (Figure 3). However, the magnitude of this pressor response was significantly ($P < 0.05$) higher in reserpinized animals.

Effect of chronic reserpine treatment on platelet and adipocyte α_2-adrenoceptors

Reserpine treatment significantly ($P < 0.02$) increased the number of platelet α_2-adrenoceptors labelled with [^{3}H]-RX821002 or with [^{3}H]-yohimbine (153 ± 21 fmol mg^{-1} protein after the 15-day treatment vs 101 ± 14 fmol mg^{-1} protein before reserpine, $n = 4$, without any significant modification in K_D values: 2.06 ± 0.43 vs 1.97 ± 0.20) (Table 3 and Figure 4).

For labelling α-adrenoceptors in adipocytes, [^{3}H]-RX 821002 was used since this compound is a better radioligand for α_2-adrenoceptor sites in this tissue when compared with other classical α_2-adrenoceptor radioligands (Carpene *et al.*, 1989), especially when the α_2-binding sites are low, which is the case in normal non-obese dogs (Taouis *et al.*, 1987) as in this study. Competition studies by yohimbine, (−)- and (+)-adrenaline and prazosin against [^{3}H]-RX 821002 on adipocyte membranes were made in order to validate the

Table 3 Effect of a 15-day reserpine treatment (0.1 mg kg^{-1} daily) on platelet and adipose tissue α_2-adrenoceptors obtained from 8 dogs

	Controls		*Treated*	
	B_{max} (fmol mg^{-1} protein)	K_D (nmol l^{-1})	B_{max} (fmol mg^{-1} protein)	K_D (nmol l^{-1})
Platelet	136 ± 16	3.80 ± 1.03	314 ± 40**	2.43 ± 0.74
Adipocyte	98 ± 29	1.62 ± 0.20	128 ± 15	1.71 ± 0.52

The maximal number (B_{max}) of [^{3}H]-yohimbine (platelet) or [^{3}H]-RX 821002 (adipose tissue) binding sites was determined by Scatchard analysis. K_D is the equilibrium dissociation constant (−1/slope of Scatchard plot). Values are the mean ± s.e.mean.
** Significantly different ($P < 0.02$) compared to the values measured in controls.

α_2-adrenoceptor selectivity of the radioligand. The order of potency was: yohimbine > (−)-adrenaline ≫ (+)-adrenaline = prazosin (Table 4 and Figure 5). Reserpine treatment did not modify [^{3}H]-RX 821002 binding on fat cell membranes (Table 3). Fifteen days after the end of reserpine treatment, platelet [^{3}H]-yohimbine binding site number remained significantly elevated (and plasma noradrenaline levels low). Normal values were measured 30 days after the end of reserpine treatment (plasma noradrenaline was in the normal range) (Figure 6). In contrast, there was no change in plasma adrenaline levels

Table 4 Inhibition of [^{3}H]-RX 821002 binding by yohimbine, (−)- or (+)-adrenaline and prazosin on adipocyte membranes from control untreated dogs

Competing drug	EC_{50} (nmol l^{-1})	K_i (nmol l^{-1})	*n Hill*
Yohimbine	6.88 ± 0.87	1.71 ± 0.22	0.93 ± 0.02
(−)-Adrenaline	977 ± 308	243 ± 77	0.53 ± 0.03
(+)-Adrenaline	9669 ± 2871	2409 ± 751	0.43 ± 0.02
Prazosin	10122 ± 767	2524 ± 372	0.94 ± 0.12

K_i: equilibrium dissociation constant (apparent dissociation constant was calculated from the equation: $EC_{50}/(1 + ([^3H]\text{ ligand})/K_D \text{ ligand})$. EC_{50}: concentration of competitive agent causing 50% inhibition of specific [^{3}H]-RX 821002 binding. K_D ligand: K_D values obtained by Scatchard analysis derived from saturation curves. Fat cell membranes were incubated with 4 nM of [^{3}H]-RX 821002. Each value is the mean ± s.e.mean of 3 separate experiments performed in duplicate.

Table 5 Inhibition of [^{3}H]-yohimbine binding by UK-14,304 on platelet or adipocyte membranes from control and 15 day reserpine-treated dogs (0.1 mg kg^{-1}, daily)

	K_{iH} (nmol l^{-1})	K_{iL} (nmol l^{-1})	% *RH*
		Platelets	
Control	1.77 ± 0.32	78.1 ± 3.4	44 ± 3.4
Treated	1.97 ± 0.81	270 ± 59.5*	54 ± 2.4
		Adipocytes	
Control	2.51 ± 1.30	2179 ± 880	59.2 ± 8.1
Treated	0.78 ± 0.60	2453 ± 1468	50.5 ± 3.2

Data were analysed by probit transformation and the Hill coefficients determined. The inhibition curves were fitted by two-site inhibition models and values were obtained using TWOSITEINHIB (Barlow, 1983). K_{iH} and K_{iL} which represent the inhibition constants at the high and low affinity state receptors respectively were calculated from the equation: $K_i = EC_{50}/(1 + ([^3H\text{-yohimbine}]/K_d)$, where EC_{50} is the concentration of UK-14,304 displacing 50% of bound radioligand and K_d is the dissociation constant of the radioligand determined by Scatchard analysis. The proportion of receptors in the high affinity state (RH) is given as percentage of total binding sites. Values are the mean ± s.e.mean.
* Significantly different ($P < 0.05$) compared to the values measured in controls.

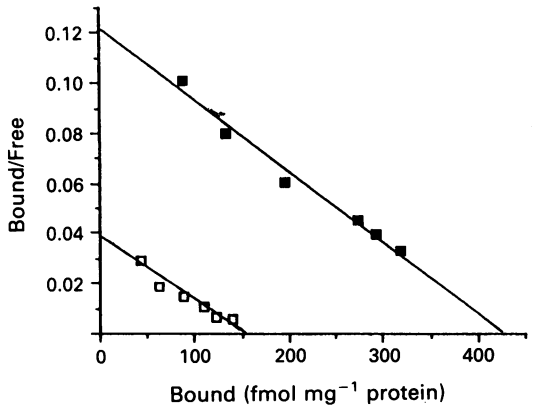

Figure 4 An example of [^{3}H]-yohimbine binding to dog platelet membrane in the same dog before (□) and after (■) reserpine treatment (0.1 mg kg^{-1} daily for 15 days). The figure represents the Scatchard plot derived from the saturation binding curve. The equilibrium dissociation constant ($K_D = -1$/slope) and the maximal number of binding sites (B_{max} = intersection of the curve with the abscissa scale) were calculated by regression analysis.

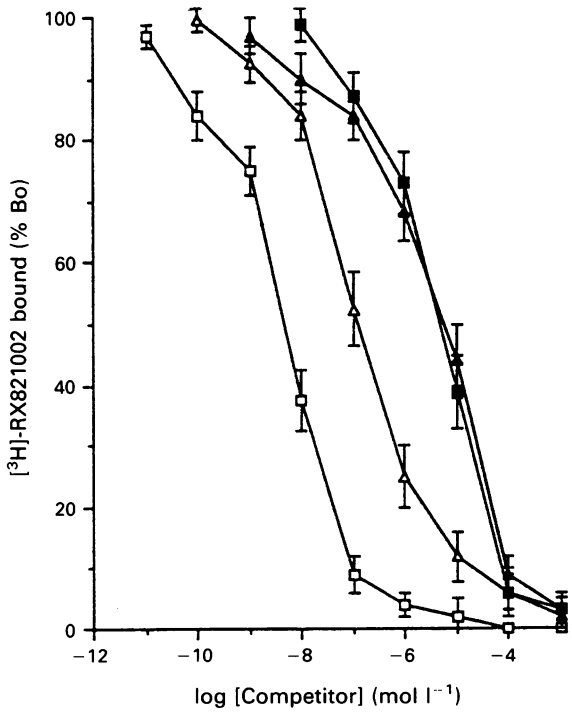

Figure 5 Inhibition of [^{3}H]-RX 821002 binding to dog fat cell membranes by various concentrations of (□) yohimbine, (△) (−)-adrenaline, (▲) (+)-adrenaline and (■) prazosin. The data are expressed as a percentage of the specific binding in absence of competitor (B_0). Each point represents the mean of three separate experiments performed in duplicate; s.e.mean shown by vertical bars. The inhibition-curves were fitted by a one-site model. The parameters obtained from the analysis of the data are presented in Table 5.

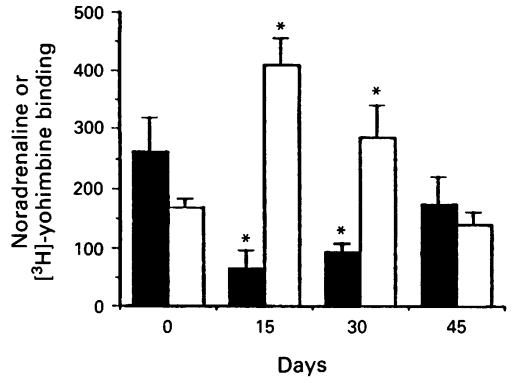

Figure 6 Time course of plasma noradrenaline levels (pg ml^{-1}; solid columns) and platelet [^{3}H]-yohimbine binding sites (fmol mg^{-1} protein; open columns) in 8 reserpine-treated dogs (0.1 mg kg^{-1} daily for 15 days, i.e. from day 1 to day 15). The values were measured before (day 0), at the end (day 15) and 15 (day 30) and 30 (day 45) days after the end of reserpine treatment. Mean values are given with s.e.mean shown by vertical bars. * $P < 0.05$ when compared with pretreatment values (day 0).

(133 ± 24, 163 ± 32, 140 ± 18 and 153 ± 46 pg ml^{-1} plasma before reserpine, at the end of reserpine treatment, 15 and 30 days after the end of reserpine treatment respectively).

α_2-Adrenoceptors exist in two affinity states in the membrane, the high affinity state (RH) coupled to adenylate cyclase, and the low affinity state (RL) uncoupled. The two affinity states can be delineated on membranes by the competition of UK-14304 (an α_2-adrenoceptor agonist) against [^{3}H]-yohimbine. The computer-aided non-linear regression analysis of the competition curves (using the 'twositeinhib' model as described by Barlow, 1983) showed that the proportion of the α_2-adrenoceptors in the high affinity state is the same before and after reserpine treatment in platelet or in adipocyte membranes (Table 5).

Discussion

The purpose of the present study was to determine whether administration of reserpine (recognized as an effective agent chronically to interrupt or reduce adrenergic neurotransmission) results in a modification of α_2-adrenergic sensitivity in the dog; i.e. if noradrenaline depletion from sympathetic nerve endings for a prolonged period of time produces an up-regulation of platelet α_2-adrenoceptors and α_2-adrenergic supersensitivity in vascular tissue. We selected a schedule of treatment with reserpine leading to a marked reduction in plasma noradrenaline levels and resting blood pressure or heart rate (Table 1).

The development of vascular supersensitivity to noradrenaline after pretreatment with reserpine has been previously demonstrated in the cat's cardiovascular system by Fleming & Trendelenburg (1961). In fact, it was never clearly shown that this supersensitivity was linked to changes in number (or affinity) of vascular α-adrenoceptors (Fleming & Westfall, 1988). Both α_2- and α_1-adrenoceptors are present on certain blood vessels (Timmermans & Van Zwieten, 1981). The present results in dogs demonstrate the existence of a supersensitivity of vascular α_2-adrenoceptors after reserpine since: (1) the pressor response to noradrenaline (after α_1- plus β-adrenoceptor blockade) was increased in reserpine-treated dogs, and (2) clonidine, which failed to change blood pressure significantly in untreated animals (probably because it was administered after α_1, β-adrenoceptor plus muscarinic blockade) elicited under the same conditions, a marked pressor response after reserpine. These results suggest that a low level of plasma noradrenaline may be responsible for the vascular α_2-supersensitivity. Since direct measurement of α_2-adrenoceptors by binding on vessels remains difficult and has led to controversial data (Watanabe *et al.*, 1982; Nasseri *et al.*, 1985), we investigated α_2-adrenoceptors on more accessible tissue (blood platelets and adipose tissue) for which the binding to α_2-adrenoceptors is well established.

Platelet α_2-adrenoceptors showed a significant increase after a 15 day reserpine treatment. The adaptive up-regulation of platelet α_2-adrenoceptors appears to be a long-lasting phenomenon, reversible and closely related to the plasma noradrenaline level (Figure 6). So, the low plasma noradrenaline level seems to be responsible for the platelet α_2-adrenoceptor up-regulation. A direct effect of reserpine on platelet α_2-adrenoceptors can be discounted since we verified that it failed to compete with [^{3}H]-yohimbine binding on platelet membranes (not shown). Fortin & Sundaresan (1989) also suggested that the α_2-adrenoceptor up-regulation that they observed in rat kidney cortex after reserpine treatment is linked to the low level of circulating catecholamines since surgical renal denervation only failed to modify the α_2-adrenoceptor number. Moreover, Langer (1981) suggested that in the dog, vascular α_2-adrenoceptors (which are mainly extrasynaptic as the platelet α_2-adrenoceptors) are stimulated by circulating noradrenaline but not by the endogenously released neurotransmitter and Bobik & Anderson (1983) demonstrated that surgical denervation of arteries in the dog was not followed by any appreciable rise in [^{3}H]-yohimbine binding sites. Taken together, these results could reasonably suggest that in dogs, vascular α_2-adrenoceptors are regulated by circulating noradrenaline.

The numbers of both platelet α_2-adrenoceptors in low and high affinity states were increased after development of up regulation (the proportion remained constant: Table 5) and the absolute increment of the receptors in the high affinity state (effective receptors in the transduction of the neurotransmitter message) can explain the increased response of the tissues to α_2-agonists.

It has long been maintained that changes in receptors after reserpine are not the sole mechanism underlying supersensitivity in smooth muscle (Fleming *et al.*, 1973). In fact, we cannot exclude the occurrence of a non-specific phenomenon in the vascular responses to noradrenaline (or clonidine) although the number of α_2-adrenoceptors increased in most of the studies with different tissues (Bylund & Martinez, 1980; Watanabe *et al.*, 1982; Fortin & Sundaresan, 1989), including the present one.

The increase in platelet α_2-adrenoceptors after reserpine treatment also agrees with the data from Bylund & Martinez (1980). Watanabe *et al.* (1982) and Fortin & Sundaresan (1989). However, these authors only investigated one tissue each in rat (salivary glands, vas deferens and kidney cortex respectively) and, as far as we know, no information is available concerning different tissues from the same animals after reserpine. Thus, it was of interest to compare the evolution of α_2-adrenoceptors located on two different tissues i.e. platelets and adipose tissue (Taouis *et al.*, 1987).

Surprisingly, we observed no modification in adipocyte α_2-adrenoceptor number after reserpine. This discrepancy cannot be attributed to the nature of the ligand used for labelling the α_2-adrenoceptors in this tissue since [^{3}H]-RX 821002 is considered to be a better radioligand than [^{3}H]-yohimbine for labelling this population of α-adrenoceptors (Langin *et al.*, 1989), especially in rat and hamster adipose tissue (Carpene *et al.*, 1989; Saulnier-Blache *et al.*, 1989): the non-specific binding for [^{3}H]-RX 821002 is very low (10%) when compared with [^{3}H]-yohimbine (40–60%), thus allowing a better identification of α_2-adrenoceptors when their number is low. Moreover, the total number of α_2-adrenoceptors identified by [^{3}H]-RX 821002 on fat cells is quite similar to that previously obtained with [^{3}H]-yohimbine (Taouis *et al.*, 1989). This compound also exhibits specific α_2-selectivity in dog adipose tissue (Table 4 and Figure 5). Moreover, we also found that

reserpine treatment failed to change the percentage of α_2-adrenoceptors in high and low affinity states.

We can propose one explanation for this lack of up-regulation of fat cell α_2-adrenoceptors: adrenaline (when compared to noradrenaline) is the major stimulant of the adipocyte α_2-adrenoceptors (Lafontan & Berlan, 1982). In fact, adrenaline levels did not change after reserpine. Moreover, such a difference in regulation of α_2-adrenoceptors by agonists (noradrenaline or adrenaline) between tissues has been previously described in rabbits by Deighton *et al.* (1988a,b). Recently, Crampes *et al.* (1989) also found that platelet α_2-adrenoceptors increased after moderate hypocaloric diet in obese humans without any modification in α_2-adrenoceptor responsiveness in adipose tissue. So, it seems that fat cell α_2-adrenoceptor number is not regulated by endogenous noradrenaline but rather by other factors such as the development of fat cell size and fat deposit (Carpene *et al.*, 1983; Marcus *et al.*, 1987; Taouis *et al.*, 1987). Although, according to Deighton *et al.* (1988a,b), extrapolations of platelet binding data to other tissues containing similar populations of α_2-adrenoceptors should be exercised with caution, the present data suggest that platelet (and not adipocyte) α_2-adrenoceptors can be a suitable index for investigating the vascular α_2-adrenergic sensitivity. This hypothesis is also supported by recent data from Daly *et al.* (1988) suggesting a functional subdivision in α_2-adrenoceptors. These authors, using various substituted benzazepines, suggest that platelet α_2-adrenoceptors are similar to those in brain and saphenous vein but different from those located at the presynaptic level on sympathetic nerve endings. This conclusion also agrees with our observations in Parkinsonian patients suffering from orthostatic hypotension who exhibit low noradrenaline (but not adrenaline) plasma levels, increased numbers of platelet α_2-adrenoceptors and vascular supersensitive responses to noradrenaline (Valet *et al.*, 1989). Thus, we suggest that circulating noradrenaline is the major regulatory agent of platelet α_2-adrenoceptor number and vascular α_2-adrenergic sensitivity.

Another point of discussion could be the difference between the results of Nasseri *et al.* (1985) and ours. These authors, using [^{3}H]-rauwolscine on rat caudal artery, established that there were no measurable changes in ligand binding to α_2-adrenoceptors after reserpine administration. They concluded that changes in α-adrenoceptors are not involved in post-junctional supersensitivity of smooth muscle caused by chronic reserpine treatment. Our results fit better with those of Watanabe *et al.* (1982) who observed an increment in rat vas deferens postsynaptic α_2-adrenoceptors after reserpine. These conflicting data are probably related to the sensitivity of the methods available for quantifying α_2-adrenoceptor binding sites in vascular tissue.

Other results deserve some comment. Tilting is a well-established method for evaluating the mechanisms of adaptation to orthostatism (Linde & Hjemdahl, 1982; Schatz, 1986). In control dogs, the adaptation of the sympathetic nervous system was clearly shown during tilting by the increase in plasma noradrenaline and adrenaline levels. After reserpine treatment, the response pattern seen during tilting bears striking differences: tilting failed to increase plasma noradrenaline levels and arterial blood pressure fell during the whole manoeuvre (Figure 1). Plasma adrenaline levels were not reduced by reserpine treatment confirming the fact that the adrenal medulla is more resistant than sympathetic nerve endings to catecholamine depletion by reserpine (see Rand & Jurevics, 1977). However, during tilting, plasma adrenaline levels only increased after 5 min (in contrast to control dogs) suggesting that reserpine alters the orthostatism-induced release of adrenaline from the adrenal medulla. The tilting manoeuvre is therefore an accurate procedure with which to validate the noradrenergic depleting effect of reserpine. Finally, it can be concluded that reserpinized dogs are appropriate models of orthostatic hypotension.

In conclusion, the present data demonstrate that reserpine treatment induces a platelet α_2-adrenoceptor up-regulation and a vascular α_2-adrenergic supersensitivity to administered noradrenaline (or clonidine) in dog. However, this α_2-adrenoceptor regulation does not involve all the tissues investigated in this study since in contrast to platelets, adipocytes escape the effect of reserpine.

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