

Publications de l'UMR 8604 (CNRS) (1999-2002)

2002

DAVID-DUFILHO M., BRUNET A., PRIVAT C., DEVYNCK M.A.

Quinacrine increases endothelial nitric oxide release: role of superoxide anion.

Eur. J. Pharmacol., 436 (3), 159-163, 2002

(Services cités : UMR 8604)

The effect of acute quinacrine treatment on agonist-induced nitric oxide (NO) release was investigated in cultured human endothelial cells using electrochemical monitoring of the in situ NO concentration. Quinacrine dose-dependently increased NO release with an apparent EC(50) of 0.2 [μ]M and a maximal effect at 1 [μ]M. Quinacrine did not modify the dependence of NO release on extracellular L-arginine. Acceleration or deceleration of O(2)([minus sign]) dismutation, which altered NO release in control cells, did not modify it in quinacrine-treated cells. Quinacrine did not modify NO amperometric signal or reaction with O(2)([minus sign]) produced by xanthine oxidation. In the presence of quinacrine, agonist-induced NO release became Mg(2+)-independent and could not be attributed to an inhibition of phospholipase A(2) activity. Quinacrine made NO release insensitive to Cu(2+) chelation. The present study demonstrates that acute treatment by low quinacrine concentrations increases endothelial NO release, possibly through an inhibition of O(2)([minus sign]) production.

STEPIEN O., ZHANG Y.Z., ZHU D.L., MARCHE P.

Dual mechanism of action of amlodipine in human vascular smooth muscle cells.

J. Hypertension, 20 (1), 95-102, 2002

(Services cités : UMR 8604)

Objectives It has been recently shown that calcium channel blockers (CCBs) could also control smooth muscle cell (SMC) growth/reactivity through mechanisms that were unrelated to their CCB property. Here, we investigated the effects of amlodipine and isradipine on Ca²⁺ movements and p42/p44 mitogen-activated protein kinase (ERK 1/2) activities, which are two early signalling events triggered by growth factors such as thrombin and basic fibroblast growth factor (bFGF). **Methods** In cultured human SMCs isolated from internal mammary arteries, Ca²⁺ movements and ERK 1/2 activation were studied by measurement of the intracellular Ca²⁺ concentration in Fura 2-labelled SMCs and by Western blots, respectively. **Results** In thrombin- and thapsigargin-stimulated SMCs, amlodipine and not isradipine dose-dependently reduced Ca²⁺ mobilization (i.e. Ca²⁺ release from internal stores); these dihydropyridines did not affect either Ca²⁺ influx or ERK 1/2 activation. In bFGF-stimulated SMCs, amlodipine and isradipine reduced both Ca²⁺ influx and ERK 1/2 activation without affecting Ca²⁺ mobilization. ERK 1/2 activation could also be directly stimulated by the L-type channel agonist Bay K 8644, demonstrating the involvement of voltage-gated Ca²⁺ influx in this process. Most of the observed effects described were obtained with approximately 10 nmol/l amlodipine/isradipine (i.e. concentrations close to the peak plasma level in treated patients). **Conclusions** In human SMCs, amlodipine can (i) specifically alter Ca²⁺ mobilization, likely by interacting with the sarcoplasmic reticulum and (ii) inhibit voltage-dependent Ca²⁺ influx and the resulting ERK 1/2 activation. It is likely that amlodipine exerts its growth-inhibitory potency by interfering with multiple branches of mitogenic signalling pathways. *J Hypertens* 20:95-102 (C) 2002 Lippincott

Williams Wilkins.

2001

BASSET A., BLANC J., MESSAS F., HAGEGE Y., ELGHOZI J.L.

Renin-angiotensin system contribution to cardiac hypertrophy in experimental hyperthyroidism: an echocardiographic study.

J. Cardiovasc. Pharmacol., 37 (2), 163-172, 2001

(Services cités : UMR 8604)

The objective of this study was to evaluate, using echocardiography, the involvement of the renin-angiotensin system (RAS) in left ventricular (LV) hypertrophy development in experimental hyperthyroidism. Thyrotoxicosis was produced by a daily intraperitoneal injection of L-thyroxine (T-4), 0.1 mg/kg per day for 15 days in Wistar rats. Control (euthyroid) rats received intraperitoneal daily injection of the thyroxine solvent. Two series of experiments were performed. In the first series, euthyroid (n = 10) and hyperthyroid (n = 14) rats were surgically prepared with a femoral artery catheter. After a 3-day recovery period, blood pressure and heart rate were measured and blood samples were collected in conscious and unrestrained rats. In the second series of experiment, measurement of LV geometry was realized with two-dimensional time-movement echocardiography on the 15th day of treatment in control conditions and after long-term treatment with the angiotensin II type 1 receptor antagonist valsartan (10 mg/kg per day for 15 days) in both euthyroid and hyperthyroid rats. The dose and duration of T-4 treatment was sufficient to induce a significant degree of hyperthyroidism with characteristic features including tachycardia, systolic hypertension, myocardial hypertrophy, hyperthermia, and weight loss. In addition, we measured an increase in free fractions of thyroid hormones, and a threefold increase in plasma lenin activity. Echocardiographic examinations in rats revealed a strong correlation between LV weight and echocardiographic LV mass. Hyperthyroid rats exhibited an increased LV mass with a marked increase in the LV end-diastolic posterior wall and septal thickness. Chronic treatment with valsartan prevented this concentric LV hypertrophy ($p < 0.01$), with full prevention of the LV posterior wall hypertrophy ($p < 0.001$) and decreased LV septal hypertrophy ($p < 0.05$). In conclusion, the cardiovascular alterations of hyperthyroidism were reproduced with thyroid hormone injections in rats. Activation of the RAS in hyperthyroid rats was accompanied by increased LV mass. Using valsartan, we demonstrated that the RAS impinged on the LV remodelling in our experimental hyperthyroidism model. A chronic treatment with an angiotensin II type I receptor antagonist prevented the development of the concentric LV hypertrophy associated with thyrotoxicosis. [References: 55]

BAUDRIE V., LAUDE D., CHAULOFF F., ELGHOZI J.L.

Genetic influences on cardiovascular responses to an acoustic startle stimulus in rats.

Clin. Exp. Pharmacol. Physiol., 28 (12), 1096-1099, 2001

(Services cités : UMR 8604)

1. The aim of the present study was to assess the cardiovascular differences among five inbred rat strains (n=16 per strain), including spontaneously hypertensive rats (SHR), Wistar Kyoto (WKY) rats, Wistar Furth (WF) rats, Fischer (F344) rats and Lewis (Lew) rats and the usual outbred Wistar (W) rat strain (n=25). 2. These strains were compared under resting conditions for blood pressure (BP) and heart rate (HR) levels and for their baroreceptor-HR reflex sensitivity. In addition, their responses to an acoustic startle stimulus were measured. 3. A consistent rise in BP was observed among the groups as a result of the noise stimulus. This rise in systolic BP (SBP) averaged (+/-SEM) 37 +/- 2 mmHg in the SHR and 34 +/- 4 mmHg in F344 rats, while the

response was only 23 +/- 3 mmHg in WKY rats. Pulse pressure (PP) was increased following noise in all groups. The delay for the BP response for all groups combined was 1.6 +/- 0.1 s. 4. Most animals had minimal HR variations, except F344 rats, responding with a 42 +/- 13 b.p.m. decrease 3.0 s after the stimulus (i.e. 1.3 s after the maximal 34 +/- 4 mmHg SBP rise). 5. The highest SBP (160 +/- 3 mmHg) and diastolic BP (104 +/- 3 mmHg) were observed in inbred SHR. Other groups were normotensive. Resting PP was elevated for SHR (56 +/- 2 mmHg) compared with the other groups (40 +/- 2 mmHg). The highest HR was found in F344 and WF rats, with 389 +/- 11 and 372 +/- 7 b.p.m., respectively. The lowest HR was observed in SHR and Lewis rats, with 335 +/- 7 and 323 +/- 7 b.p.m., respectively. The least sensitive baroreflex function was observed in SHR (0.8 +/- 0.1 b.p.m./mmHg) compared with the other strains (1.4 +/- 0.2 b.p.m./mmHg). 6. The present study confirms the importance of genetic factors on the cardiovascular responses of rats to a noise startle stimulus. Two inbred normotensive rat strains, namely F344 and WKY rats, which exhibit a substantial difference in pressor response to noise, may be used to unravel the mechanisms of sympathetic activation.

DAVID-DUFILHO M., PRIVAT C., BURNET A., RICHARD M.J., DEVYNCK J., DEVYNCK M.A.

Transition metals and nitric oxide production in human endothelial cells.

C. R. Acad. Sci. Sér.III Sci. Vie, 324 (1), 13-21, 2001

(Services cités : UMR 8604)

The bioavailability of endothelial nitric oxide (NO) is regulated by transition metals but their mechanisms of action on NO synthesis and degradation are not clearly understood. Using differential pulse amperometry and NO microelectrodes, local NO concentration was measured at the surface of cultured human umbilical vein endothelial cells (HUVECs) stimulated by histamine or thrombin in the presence of transition metal chelators. The agonist-activated NO release required both extracellular Ca²⁺ and transition metals. In the presence of 1 mM external Ca²⁺, a low concentration of EGTA (5 μM) inhibited by 40 % the NO release from stimulated HUVECs. In the presence of extracellular L-arginine, the inhibitory effect of EGTA was even more marked and, in its absence, it was suppressed by adding exogenous superoxide dismutase. The decrease in NO release induced by the copper chelators, cuprizone and DETC, suggests that extracellular traces of Cu²⁺ could regulate NO availability. (C) 2001 Academie des sciences / Editions scientifiques et medicales Elsevier SAS. [References: 48]

DAVID-DUFILHO M., BRUNET A., PRIVAT C., DEVYNCK M.A.

Analysis of agonist-evoked nitric oxide release from human endothelial cells: role of superoxide anion.

Clin. Exp. Pharmacol. Physiol., 28 (12), 1015-1019, 2001

(Services cités : UMR 8604)

1. Dichlorofluorescein oxidation and electrochemical monitoring of in situ nitric oxide (NO) release from cultured human endothelial cells reveals that agonists such as thrombin and histamine simultaneously stimulate transient superoxide production. 2. The duration of small middle dotNO release was increased only in the simultaneous presence of extracellular L-arginine and exogenous superoxide dismutase. In contrast, the inhibition of membrane reduced nicotinamide adenine dinucleotide (phosphate) oxidases, the major source of small middle dotO₂- in endothelial cells, did not prolong small middle dotNO release, although extracellular L-arginine was also present. Comparison of these two experimental conditions suggested that H₂O₂ was involved in the extension of the small middle dotNO signal. 3. The present study

demonstrates that, in the absence of external L-arginine, small middle dotO₂- production does not constitute the major pathway controlling the duration of agonist-induced small middle dotNO signal. These results suggest that L-arginine and H₂O₂ act jointly to maintain nitric oxide synthase in an activated form.

ELGHOZI J.L., GIRARD A., RIBSTEIN J.

Baroreflex failure syndrome: an uncommon observation of an excessive blood pressure variability.

Rev. Méd. Interne, 22 (12), 1261-1268, 2001

(Services cités : UMR 8604)

Introduction. - The arterial baroreflex operates in physiological conditions. It induces sympathetic and vagal activity modulation resulting in arterial tone and heart rate changes. These appropriate responses limit blood pressure fluctuations and blood pressure is therefore regulated since the baroreflex constantly buffers the changes. Exegesis. - Bilateral carotid body tumor excision resulted in excessive fluctuations of blood pressure. Indices of spontaneous baroreflex activity were markedly altered in the patient described herein. Conclusion. - The excessive fluctuations of blood pressure due to the sinoaortic denervation demonstrate how powerful is this negative feedback control mechanism in control conditions. (C) 2001 Editions scientifiques et médicales Elsevier SAS. [References: 18]

GUEZENEC C.Y., LOUISY F., PORTIER H., LAUDE D., CHAPUIS B., PLESANT J.

Effects of aerobatics flight on oxygen consumption and heart rate control: influence on autonomic cardiovascular regulation during recovery.

Eur. J. Appl. Physiol. Occup. Physiol., 84 (6), 562-568, 2001

(Services cités : UMR 8604)

Oxygen consumption (VO₂) and blood pressure regulation were measured on five pilots during and after normal training aerobatics flights of a mean duration of 35 min. The acceleration vector along the longitudinal axis of the body (G_z) ranged from + 6.5 G_z to -3.5 G_z. VO₂ was continuously monitored by a miniature telemetric system (K2). Heart rate (f_c), the abdominal muscle electromyogram (EMG) and G_z levels were recorded synchronously on a magnetic tape recorder. A tilt test was performed pre- and post-flight to evaluate f_c and blood-pressure variability. The left forearm blood flow was measured by strain-gauge plethysmography. The mean VO₂ during flight was 1.2 lmin⁻¹, with a peak VO₂ of 2.1 lmin⁻¹. f_c ranged between 55 and 165 beats min⁻¹ and showed a progressive increase under the effect of + G_z, with a sudden fall during -G_z. The abdominal muscle EMG indicated the occurrence of muscle contraction under Ct load. Maximal responses were observed during the -G_z phase. Comparison between pre- and post-flight data showed lower post-flight systolic blood pressure with higher f_c. Before flight, upright tilt induced a significant increase in low/high frequency f_c, as assessed using spectral analysis. This change was suppressed after flight. In summary, these data show that aerobatics flight leads to enhanced energy expenditure, mainly because of increased skeletal muscle work. The post-flight tilt test showed that aerobatic flight favors parasympathetic drive and, consequently, modifies blood pressure regulation during recovery. This action may decrease + G_z tolerance to a second aerobatics flight performed shortly after the first.

[References: 31]

JAPUNDZIC-ZIGON N.

Effects of nonpeptide v-1a and v-2 antagonists on blood pressure fast oscillations in conscious

rats.

Clin. Exp. Hypertens., 23 (4), 277-292, 2001

(Services cités : UMR 8604)

This paper describes the effects of vasopressin nonpeptide selective V-1a (OPC-21268) and V-2 (OPC-31260) antagonists on fast blood pressure (BP) oscillations in conscious non-haemorrhaged and haemorrhaged rats. Equidistant sampling at 20 Hz allowed direct spectral analysis of BP on 30 overlapping 2048 point-time series. In non-haemorrhaged rats, V-1a antagonist (5 mg/kg; i.v) reduced BP and low-frequency (LF-BP) component while subsequent administration of V-2 antagonist (1 mg/kg; i.v) reversed these changes and enhanced the very low-frequency (VLF-BP) component. In haemorrhaged rats (5-15 ml/kg/min) V-2 antagonist pre-treatment enhanced the VLF-BP component during normotensive bleeding, while the V-1a antagonist pre-treatment modified BP variability after hypotensive haemorrhage by enhancing the HF-SBP component, The results suggest that under normotensive conditions vasopressin by the stimulation of both V-1a and V-2 receptors buffers BP variability in the VLF-BP frequency domain. In addition, under hypotensive conditions vasopressin, by the stimulation of V-1a receptors buffers the respiration-induced HF-BP oscillation. [References: 44]

LAMBERT G., LAMBERT E., FASSOT C., FRIBERG P., ELGHOZI J.L.

Subarachnoid haemorrhage-induced sympathoexcitation in rats is reversed by bosentan or sodium nitroprusside.

Clin. Exp. Pharmacol. Physiol., 28 (3), 200-205, 2001

(Services cités : UMR 8604)

1. The roles played by nitric oxide (NO) and endothelin (ET) in the genesis of sympathetic nervous activation following experimental subarachnoid haemorrhage was investigated using spectral analysis of blood pressure rhythms. 2. Subarachnoid haemorrhage was induced in conscious rats by injecting 0.3 mL homologous blood via a catheter placed along the surface of the brain and directed towards the circle of Willis, Three hours after the insult and after sympathetic activation was evident, animals received either an acute injection of the ET antagonist bosentan (5 mg/kg, i.v.; n = 7), an infusion of the NO donor sodium nitroprusside (SNP; 18 mug/h; n = 7) or no treatment (II = 7), 3. Three hours following the induction of subarachnoid haemorrhage, the mid-frequency components of systolic blood pressure were markedly elevated, indicating a pronounced sympathoexcitation, However, blood pressure and heart rate levels remained unchanged at this time. In the absence of treatment, the mid-frequency components of blood pressure remained elevated for a subsequent 2 h, Treatment with a nonhypotensive dose of SNP reversed the sympathoexcitation within 1 h, Treatment with bosentan was also effective in reducing the mid-frequency oscillations in blood pressure associated with subarachnoid haemorrhage. 4. Our results indicate that subarachnoid haemorrhage is associated with an acute activation of the sympathetic nervous system. The degree of sympathoexcitation can be reversed by the use of either bosentan or SNP. [References: 32]

PERNOLLET M.G., KUNES J., ZICHA J., DEVYNCK M.A.

Cyclic nucleotides in hypertriglyceridemic thrombin and nitric platelets of genetically and hypertensive rats oxide responses are unrelated to plasma triglyceride levels.

Thromb. Res., 104 (1), 29-37, 2001

(Services cités : UMR 8604)

Prague hereditary hypertriglyceridemic (HTG) rats constitute a genetic model of hypertension

associated with hyperlipidemia and insulin resistance. Various cell alterations, including changes in membrane dynamics, ion transport, and decreased platelet responses to thrombin have been observed in this strain. As hypertriglyceridemia appears to be associated with reduced endothelium-dependent vasodilation and platelet aggregation, we examined whether triglycerides could modulate cell responsiveness through changes in cyclic nucleotides in platelets of HTG rats. From the age of 6 weeks, these hypertensive animals were subjected for 10 weeks to interventions that modified circulating triglycerides levels (2.17 +/- 0.09 mmol/l), leading to their reduction (gemfibrozil treatment, 0.87 +/- 0.05 mmol/l) or elevation (high fructose intake, 3.23 +/- 0.07 mmol/l). Basal cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP) contents were 15% and 48% lower in isolated platelets of HTG rats than in those of Lewis controls. cAMP level was further reduced in HTG rats subjected to high fructose intake. Irrespective of their plasma triglyceride levels, the thrombin-induced increase in platelet cGMP levels present in Lewis rats was absent in platelets of HTG rats. In contrast, no strain- or treatment-related differences were observed in the magnitude or kinetics of cGMP response to exogenous nitric oxide (NO). NO-induced cGMP and cAMP changes were associated in an opposite manner with trimethylamino-diphenylhexatriene (TMA-DPH) anisotropy, a biophysical parameter that reflects the microviscosity of the outer part of the cell membrane. Our results indicate that the attenuation of platelet responsiveness to thrombin in HTG rats represents a strain difference that cannot merely be due to a difference in plasma triglyceride levels. Platelet hyporesponsiveness to agonists such as thrombin in HTG rats cannot be explained by a change in levels of inhibitory cyclic nucleotides, since they were actually found to be low and not high. (C) 2001 Elsevier Science Ltd. All rights reserved. [References: 42]

PORTIER H., LOUISY F., LAUDE D., BERTHELOT M., GUEZENNEC C.Y.

Intense endurance training on heart rate and blood pressure variability in runners.

Med. Sci. Sport Exercise, 33 (7), 1120-1125, 2001

(Services cités : UMR 8604)

Physical training with incomplete recovery times can produce significant fatigue. A study of cardiovascular responses showed that there is a sympathetic and a parasympathetic form of fatigue. Purpose: The purpose of this experimentation was to measure the effects of intense endurance training on autonomic balance through a spectral analysis study of the heart rate (HR) and systolic blood pressure (SBP). Methods: Eight elite runners were tested twice: after a relative rest period (RRP) of 3 wk and after an 12-wk intense training period (ITP) for endurance. At the end of each phase, the subjects were tested by means of a VO₂max test and a tilt-table test. Results: The resting heart rate (HR) variability was lower (P < 0.001) in the intensive training phase. Likewise, there were differences in the low-frequency (0.04-0.150 Hz; LF) and high-frequency (0.150-0.500 Hz; HF) components and the LF/HF ratio of the HR spectral analysis. The LF spectral power was significantly lower in the supine position (P < 0.05) during ITP. Upright tilting was accompanied by a 22.6% reduction in HF values during the rest period, whereas in ITP the HF spectral power rose by 31.2% (P < 0.01) during tilt, characterizing a greater parasympathetic system control. Sympathetic control represented by the LF/HF ratio regressed markedly (P < 0.01) in response to the tilt test in ITP. Conclusions: The spectral analysis of SEP in the high frequencies shows that the changes in cardiac parameters are coupled with a decrease in sympathetic vasomotor control (-18%) and a reduction in diastolic pressure (-3.2%) in the response to the tilt test at the end of ITP. Spectral analysis could be a means of demonstrating impairment of autonomic balance for the purpose of detecting a state of fatigue that could result in overtraining. [References: 37]

2000

BASSET A., BLANC J., ELGHOZI J.L.

Contribution of the renin-angiotensin system to short-term arterial pressure variability in hyperthyroid rats.

Arch. Mal. Coeur Vaisseaux, 93 (8), 905-910, 2000

(Services cités : UMR 8604)

Objectives: To produce a chronic thyrotoxicosis model in rat, and to evaluate, using spectral analysis, the involvement of the renin-angiotensin system (RAS) in short-term variability of blood pressure (BP) in experimental hyperthyroidism. Design and methods: Thyrotoxicosis was produced by a daily intraperitoneal (i.p.) injection of L-thyroxine (T4: 0.1 mg/kg for 15 days) in Wistar rats. Control (euthyroid) rats received i.p. daily injection of the thyroxine solvent. Two series of experiments were performed in conscious and unrestrained rats. In the first series, 10 euthyroid and 14 hyperthyroid rats were surgically prepared with a femoral artery catheter to measure BP and heart rate (HR) and to collect blood samples on the last day of treatment. In the second series of experiments (n=12 in each group), on the fifteenth day of treatment, BP and HR were recorded by telemetry in control conditions and after a specific blockade of the RAS by the angiotensin type 1 receptors antagonist: valsartan (10 mg/kg, i.p.). BP recordings were analysed by the Fast Fourier Transform on consecutive 204.8-s stationary periods. Results: The dose and duration of T4 treatment was sufficient to induce a significant degree of hyperthyroidism with characteristic features including: tachycardia, systolic hypertension, myocardial hypertrophy, hyperthermia, and weight loss. In addition, we measured an increase in free fractions of thyroid hormones, and a 3 fold-increase of plasma renin activity. Hyperthyroidism modified systolic BP (SBP) variability profiles. An amplification of low frequency (LF) oscillations (2.37±0.12 mmHg vs 1.78±0.11 mmHg, p<0.01) was observed after T4 treatment. In hyperthyroid rats, valsartan diminished the slow fluctuations of SEP (p<0.001) and increased the mid-frequency oscillations (2.44±0.20 mmHg vs 1.32±0.18 mmHg, p<0.001). Conclusion: The cardiovascular alterations of hyperthyroidism are reproduced with thyroid hormone injections in rats. Activation of the RAS in hyperthyroid rats was accompanied by increased SEP variability in the LF range. Using the angiotensin type I receptors antagonist, valsartan, we demonstrated that the RAS impinged on the LF oscillations of the SEP in our experimental hyperthyroidism model. [References: 10]

BLANC J., LAMBERT G., ELGHOZI J.L.

Contribution of the renin-angiotensin system to the short-term blood pressure variability in the conscious rat.

Arch. Mal. Coeur Vaisseaux, 93 (8), 1019-1022, 2000

(Services cités : UMR 8604)

This study was designed to assay, using spectral analysis, the influence of the renin-angiotensin system activation on the blood pressure variability. Rats were surgically prepared with a supra-renal catheter inserted via the left carotid artery to perform local infusions and with a femoral artery catheter to measure blood pressure (BP) and heart rate (HR). The beta-adrenoceptors stimulation by isoprenaline was used to increase the plasma renin activity (PRA). A first group (n=8) was infused with isoprenaline (0, 0.003, 10, 100, 300 ng/kg/min) at a rate of 20 µL/min. A second group (n=8) received a bolus of the angiotensin II (AII) AT1 receptor-antagonist valsartan (2 mg/kg/mL, i.a.) prior to isoprenaline infusions. Five groups were used for blood sampling (one group infused with one concentration of isoprenaline) to assay PRA and

catecholamines (CA). BP recordings were analysed using the fast Fourier transforms (FFT) on 2048 points time series (204.8 s). Isoprenaline from the concentration of 10 ng/kg/min increased PRA with a maximum effect of 8.5 fold with the highest concentration (300 ng/kg/min, $p < 0.05$); CA were not modified. Isoprenaline amplified the low-frequency (LF: 0.02-0.20 Hz) component of the systolic BP (SBP) variability (10 ng/kg/min : 4.16 ± 0.62 mmHg(2) versus : 2.90 ± 0.44 mmHg(2) for control value, $p < 0.05$) even if It did not modify BP and HR levels, Isoprenaline lowered BP and had a tachycardic effect at concentrations greater than or equal to 100 ng/kg/mL (at 100 ng/kg/mL : SEP = 115 ± 3 mmHg, HR = 464 ± 5 bpm, versus control : SEP = 128 ± 3 mmHg, HR = 351 ± 7 bpm, $p < 0.05$). Valsartan modified neither BP levels nor BP variability but exerted a tachycardic effect ($+25$ bpm, $p < 0.001$). Valsartan prevented the amplification of the LF oscillations of SEP induced by isoprenaline (10 ng/kg/min : 2.53 ± 0.38 mmHg(2) versus : 2.20 ± 0.25 mmHg(2) for control value (valsartan, ns). We conclude that a moderate endogenous production of renin increases SEP variability in the LF range in the conscious rat. This effect which does not affect BP and HR levels is mediated by All AT1 receptors and does not involve the sympathetic nervous system. [References: 9]

BLANC J., LAMBERT G., ELGHOZI J.L.

Endogenous renin and related short-term blood pressure variability in the conscious rat.

Eur. J. Pharmacol., 394 (2-3), 311-320, 2000

(Services cités : UMR 8604)

This study was designed to investigate, by use of spectral analysis, the blood pressure variability changes induced in the conscious rat by activation of plasmatic renin activity. Rats were surgically prepared with a supra-renal catheter inserted via the left carotid artery to perform the infusions, and with a femoral artery catheter to measure blood pressure and heart rate. Secretion of renin was induced using beta-adrenoceptor stimulation produced by isoprenaline. A first group ($n=8$) was infused with isoprenaline: 0.003, 10, 100 and 300 ng/kg/min, at a rate of 20 microl/min. A second group ($n=8$) was given a bolus injection of the angiotensin AT(1) receptor antagonist, valsartan (2 mg/kg, i.a.), prior to isoprenaline infusions. The lack of effect of infusion per se was checked in additional animals ($n=8$) infused with saline only (20 microl/min). Five other groups of animals were prepared with arterial catheters as mentioned previously. Each group received one concentration of infused isoprenaline and samples of blood were collected for further determinations of plasma renin activity and catecholamine concentrations. Blood pressure recordings were analysed using the fast Fourier transform on 2048 points time series (204.8 s). Isoprenaline increased plasma renin activity and did not modify plasma catecholamine concentrations. The low-frequency (0.02-0.2 Hz) component of the systolic blood pressure variability was amplified by isoprenaline (10 ng/kg/min isoprenaline: 4.16 ± 0.62 mm Hg(2) vs. 2.90 ± 0.44 mm Hg(2) for control value, $P < 0.05$), a concentration that did not alter either blood pressure or heart rate levels. Isoprenaline lowered blood pressure and increased heart rate, starting at concentrations of 100 ng/kg/min. Valsartan, whose principal effect was generation of tachycardia ($+25$ bpm) modified neither blood pressure levels nor blood pressure variability. Valsartan prevented the amplification of the low-frequency oscillations of systolic blood pressure induced by isoprenaline (10 ng/kg/min isoprenaline: 2.53 ± 0.38 mm Hg(2) vs. 2.20 ± 0.25 mm Hg(2) for control value (valsartan, ns). We conclude that a moderate increase of plasma renin activity enhanced systolic blood pressure variability in the low-frequency range, without affecting blood pressure and heart rate levels.

CONSTANT I., LAUDE D., ELGHOZI J.L., MURAT I.

Assessment of autonomic cardiovascular changes associated with recovery from anaesthesia in children: a study using spectral analysis of blood pressure and heart rate variability.

Paediatr. Anaesth., 10 (6), 653-660, 2000

(Services cités : UMR 8604)

Recovery from anaesthesia is associated with large changes in cardiovascular autonomic activity, which are poorly documented in children. This study was undertaken to investigate the cardiovascular autonomic activity in anaesthetized and recovering children, using a noninvasive approach based on spectral analysis of heart rate (HR) and blood pressure (BP) variability. Ten children (aged 5-13 years) undergoing major surgery were studied. Continuous HR and BP were recorded using a noninvasive device during deep anaesthesia and recovery. Spectral analysis was used to determine the main oscillatory components of HR and BP signals. For each power spectrum, the frequency components were identified as follows (i): the low frequency (LF) component (0.04-0.14 Hz) both parasympathetically and sympathetically mediated for HR and corresponding to vasomotor sympathetic modulation for BP; and (ii) the high frequency (HF) component (0.2-0.6 Hz) parasympathetically mediated for HR, and reflecting mechanical influence of ventilation on cardiac output for BP. In addition, the LF : HF ratio for HR, reflecting the cardiac sympathovagal balance, was calculated. Under deep anaesthesia, HR variability and BP variability were very low and mainly due to mechanical influence of intermittent positive pressure ventilation. Conversely, the recovery period was associated with a marked increase of HR and BP overall variability. Compared to anaesthesia, spectral analysis of HR and BP revealed that the LF component of BP and HR spectra increased 40-fold during recovery; the LF : HF ratio of HR was also increased during recovery (0.1 +/- 0.1 versus 1.3 +/- 1.2, P = 0.008). The results of this study demonstrate that the recovery period is associated with an increase of cardiovascular sympathetic drive in children after major surgery. [References: 32]

ELGHOZI J.L., HERPIN D.

The autonomic nervous system and hypertension: quid novi ?

Arch. Mal. Coeur Vaisseaux, 93 (11 Suppl S), 1381-1386, 2000

(Services cités : UMR 8604)

Recent publications concerning the role of the autonomic nervous system in hypertension have demonstrated the nature of spontaneous oscillations of the blood pressure. The contribution of sympathetic stimulations indicates the baroreflex nature of certain rhythms, which opens a perspective of understanding the relations between increased sympathetic activity and hypertension. The sympathetic nervous system, angiotensin II and aldosterone are related, and antihypertensive therapy may therefore target one or the other, providing that the regulating functions are maintained. These concepts are developed in a recent series of articles which are presented schematically in this update. [References: 12]

KUNES J., DEVYNCK M.A., ZICHA J.

Chronic changes in plasma triglyceride levels do modify platelet membrane microviscosity in rats.

Life Sci., 67 (8), 959-967, 2000

(Services cités : UMR 8604)

Lipid metabolism disorders were proposed to mediate numerous cell membrane alterations in various forms of hypertension. Elevated plasma triglycerides were found to be associated with changes in membrane structure and function related to altered microviscosity in particular domains of the cell membrane. The aim of our study was to determine if an abnormal triglyceride

metabolism might play a causal role in these alterations of membrane dynamics. Using genetically hypertensive rats of the Prague hereditary hypertriglyceridemic (HTG) strain we investigated whether the elevation of circulating triglycerides induced by high fructose intake and/or their lowering by chronic gemfibrozil treatment (for 10 weeks starting at the age of 6 weeks) are followed by reciprocal changes in membrane microviscosity. Two different fluorescent probes exploring either the outer membrane leaflet (TMA-DPH anisotropy) or the membrane lipid core (DPH anisotropy) were used in platelets of HTG rats. DPH (diphenylhexatriene) fluorescence anisotropy was decreased in platelets of fructose-treated HTG animals with highly elevated plasma triglyceride levels, whereas it was increased in gemfibrozil-treated HTG rats in which triglyceride levels were almost normalized. On the contrary, TMA-DPH (trimethylamino-diphenylhexatriene) anisotropy was not substantially altered in platelets from HTG rats by the above modifications of circulating triglycerides. No changes of plasma cholesterol or blood pressure were associated with the triglyceride-dependent modifications of membrane core microviscosity. Our interventional study demonstrates a major causal role of circulating triglycerides in the control of the microviscosity of membrane lipid core. (C) 2000 Elsevier Science Inc. All rights reserved. [References: 38]

LAMBERT E., LAMBERT G., FASSOT C., FRIBERG P., ELGHOZI J.L.

Subarachnoid hemorrhage induced sympathoexcitation arises due to changes in endothelin and/or nitric oxide activity.

Cardiovasc. Res., *45* (4), 1046-1053, 2000

(Services cités : UMR 8604)

Objective: The demonstration of the effectiveness of endothelin antagonists and nitric oxide donors in managing vasospasm following subarachnoid hemorrhage is encouraging. Whether such drugs can modify the sympathoexcitation that accompanies this condition remains unknown and was the basis for the present report. Methods: Subarachnoid hemorrhage was induced in conscious rats by injecting blood via a catheter placed along the surface of the brain and directed towards the circle of Willis. We combined measurements of arterial plasma catecholamines with the spectral analysis of blood pressure variability in order to examine sympathetic nervous activation following subarachnoid hemorrhage. Experiments were performed in untreated animals and in rats following pretreatment with either bosentan or sodium nitroprusside. Results: Indicative of a pronounced sympathoexcitation, the 0.2-0.6 Hz frequency components of blood pressure were markedly elevated following subarachnoid hemorrhage (2.5 ± 0.5 vs. 8.9 ± 2.6 mmHg(2), $P < 0.01$). parallel changes in plasma norepinephrine concentration were observed (1.0 ± 0.2 vs. 2.4 ± 0.4 nmol/l, $P < 0.01$). The subarachnoid injection of saline did not modify blood pressure variability or plasma norepinephrine concentrations. Pretreatment with either bosentan or sodium nitroprusside completely prevented the subarachnoid hemorrhage induced sympathoexcitation. Conclusions: Experimental subarachnoid hemorrhage is associated with a pronounced activation of the sympathetic nervous system. It would appear that this sympathoexcitation has its roots ensconced in either the release of endothelin or an impairment in nitric oxide mediated vasodilation. (C) 2000 Elsevier Science B,V. All rights reserved. [References: 36]

LE QUAN SANG K.H., LE FEUVRE C., BRUNET A., PHAM T.D., METZGER J.P., VACHERON A., DEVYNCK M.A.

Influence of sin-1 on platelet Ca^{2+} handling in patients with suspected coronary artery disease: ex vivo and in vitro studies.

Thromb. Haemost., 83 (5), 752-758, 2000

(Services cités : UMR 8604, Cardiologie Adulte, URC)

The 3-morpholinopyridone (SIN-1) generates both nitric oxide (NO) and superoxide anion (O₂⁻). It elicits dose-dependent vasodilation in vivo, in spite of the opposite effects of its breakdown products on vascular tone and platelet aggregation. This study was designed to investigate the influence of intravenous SIN-1 injection on platelet Ca²⁺ handling in patients undergoing coronary angiography. SIN-1 administration reduced cytosolic [Ca²⁺] in unstimulated platelets by decreasing Ca²⁺ influx. It attenuated Ca²⁺ mobilization from internal stores evoked by thrombin or thapsigargin. In vitro studies were used as an approach to investigate how simultaneous productions of NO and O₂⁻ from SIN-1 modify thrombin- or thapsigargin-induced platelet Ca²⁺ mobilization. Superoxide dismutase, the O₂⁻ scavenger, enhanced the capacity of SIN-1 to inhibit Ca²⁺ mobilization but catalase had no effect. This suggests that the effects of SIN-1 on platelet Ca²⁺ handling resemble those of NO, but are modulated by simultaneous O₂⁻ release, independently of H₂O₂ formation. [References: 50]

MARCHE P.

Amlodipine and the mechanisms of vascular hypertrophy.

Drugs, 59 (Special Issue 2), 1-7, 2000

(Services cités : UMR 8604)

In several cardiovascular diseases, including hypertension, atherosclerosis and restenosis, an uncontrolled proliferation of smooth muscle cells from the arterial wall participates in the vascular hypertrophy that is often observed to be associated with these diseases. In this article, the mechanisms of smooth muscle cell activation and proliferation are briefly reviewed, focusing on the predominant role played by Ca²⁺ ions and the voltage-dependent Ca²⁺ channels in cellular Ca²⁺ homeostasis. In addition, the major alterations in pathways involved in smooth muscle cell activation and proliferation that have been observed in the cardiovascular diseases under discussion are reviewed. The effects of amlodipine on the molecular/cellular pathways discussed above are then presented. Finally, we discuss recent experimental results obtained with amlodipine, which make it an excellent candidate for the treatment of those cardiovascular diseases. [References: 40]

MARCHE P., STEPIEN O.

Calcium antagonists and vascular smooth muscle cell reactivity.

Z. Kardiol., 89 Suppl 2 140-144, 2000

(Services cités : UMR 8604)

OBJECTIVES: To determine the mechanisms whereby calcium channel blockers (CCBs) control the reactivity of vascular smooth muscle cells (VSMCs). **BACKGROUND:** Although CCBs are known to play an important role in the calcium homeostasis of VSMCs, they are suspected to exert additional effects in this cell type. Thus, the possibility that CCBs could affect VSMC growth/proliferation through a mechanism distinct from the inhibition of calcium channels was investigated. **METHODS:** VSMCs were isolated from rat aortae and cultured. The influence of nifedipine and amlodipine on basic fibroblast growth factor (bFGF)-stimulated DNA synthesis and proliferation was studied by measuring bFGF-induced BrdU incorporation into VSMCs and cell counts, respectively. The influence of amlodipine (and of isradipine) on the mobilization of intracellular Ca²⁺ stores was determined by studying the fluorescence of thapsigargin-stimulated VSMCs pre-labeled with the fluoroprobe Fura-2. **RESULTS:** Both nifedipine and amlodipine inhibited bFGF-induced VSMC growth/proliferation. In the case of nifedipine but not in that of

amlodipine, this inhibitory effect could be accounted for by the L-type Ca(2+)-channel antagonist property of the drug. On the other hand, amlodipine but not isradipine, diltiazem, and verapamil, did inhibit thapsigargin-induced Ca²⁺ mobilization. CONCLUSIONS: These findings suggest that in addition to its L-type Ca(2+)-channel antagonist property, amlodipine also exerts a "thapsigargin-like" activity which, together with its particular antioxidant property, might participate in its antiatherogenic potency.

MESANGEAU D., LAUDE D., ELGHOZI J.L.

Early detection of cardiovascular autonomic neuropathy in diabetic pigs using blood pressure and heart rate variability.

Cardiovasc. Res., 45 (4), 889-899, 2000

(Services cités : UMR 8604)

Cardiac autonomic neuropathy is a common complication in insulin dependent diabetes mellitus. Nevertheless, little is known about when this impairment occurs during the time course of the disease. Analysis of blood pressure (BP) and heart rate (HR) variability could be used to detect early signs of autonomic alteration. To test this proposal, twelve sexually mature male Yucatan miniature pigs were equipped with an arterial catheter for telemetric BP analysis, and with a venous access. BP and HR were recorded together with respiratory movements while the animals were resting in a sling. After the first recording session performed when the pigs were 5 months old, streptozotocin (STZ) was used to induce diabetes in seven pigs, while the five others were controls. BP and HR were measured 3 and 6 months after the onset of diabetes and at a similar age in the controls. BP and HR oscillated at the respiratory range (0.19 Hz), Spectral analysis showed this respiratory component was the main determinant of the short-term variability of BP and HR, Atropine increased HR and BP and markedly diminished the respiratory sinus arrhythmia. Propranolol diminished KR and the respiratory peak of HR. A reduced respiratory oscillation of BP paralleled the diminution of the respiratory peak of HR. Baroreceptor-HR reflex was estimated using injections of phenylephrine and nitroprusside, and by cross-spectral analysis between BP and HR. Atropine shifted the curve to higher values, while propranolol reduced the level of the upper plateau. Atropine decreased both the coherence and gain of the cross-spectral analysis. STZ injection resulted in a type 1 diabetes. At 3 months, diabetic pigs exhibited low levels of BP and a reduced overall variability of HR and BP. Spectral analysis indicated the respiratory sinus arrhythmia was markedly reduced. In addition, the sensitivity of the baroreceptor-HR reflex was reduced. At a latter stage of diabetes these alterations were marked and the level of the resting HR was increased. These data demonstrate the dual (vagal and sympathetic) control of HR in pigs and the dominant role of respiration in the genesis of HR and BP fluctuations. The spectral and cross-spectral analysis of BP and HR were altered after 3 months of diabetes and could be proposed as early detectors of cardiac autonomic neuropathy. (C) 2000 Elsevier Science B.V. All rights reserved. [References: 31]

STEPIEN O., MARCHE P.

Amlodipine inhibits thapsigargin-sensitive c-alpha(2+) stores in thrombin-stimulated vascular smooth muscle cells.

Amer. J. Physiol. - Heart Circ. Physiol., 279 (3), H1220-H1227, 2000

(Services cités : UMR 8604)

Ca²⁺ channel blockers, such as amlodipine, inhibit vascular smooth muscle cell (VSMC) growth through interactions with targets other than L-type Ca²⁺ channels. The effects of amlodipine on Ca²⁺ movements in thrombin- and thapsigargin-stimulated VSMCs were therefore investigated

by determining the variations of intracellular free Ca^{2+} concentration in fura 2-loaded cultured VSMCs. Results indicated that 10-1,000 nM amlodipine inhibited 1) thrombin- induced Ca^{2+} mobilization from a thapsigargin-sensitive pool and 2) thapsigargin-induced Ca^{2+} responses, including Ca^{2+} mobilization from internal stores and store-operated Ca^{2+} entry. These effects of amlodipine do not involve L-type Ca^{2+} channels and could not be reproduced with 100 nM isradipine, diltiazem, or verapamil. The inhibition by amlodipine of Ca^{2+} mobilization appears therefore to be a specific property of the drug, in addition to its Ca^{2+} channel-blocking property. It is suggested that amlodipine acts in this capacity by interacting with Ca^{2+} -ATPases of the sarcoplasmic reticulum, thus modulating the enzyme activity. This mechanism might participate in the inhibitory effect of amlodipine on VSMC growth. [References: 43]

1999

BLANC J., PONCHON P., LAUDE D., ELGHOZI J.L., JOVER B.

Blood pressure variability in established L-NAME hypertension in rats.

J. Hypertension, 17 (11), 1527-1534, 1999

(Services cités : UMR 8604)

Methods Blood pressure variability was evaluated in conscious Wistar control rats and rats with established L-NAME hypertension (20 mg/kg pr 24 h, 4 weeks).

CONSTANT I., LAUDE D., MURAT I., ELGHOZI J.T.

Pulse rate variability is not a surrogate for heart rate variability.

Clin. Sci., 97 (4), 391-397, 1999

(Services cités : UMR 8604)

To investigate the differences between heart rate (HR) variability and pulse rate (PR) variability, short-term variability of finger pulse wave and ECG signals were studied in 10 children with a fixed ventricular pacemaker rhythm (80 beats/min). Ten healthy children in sinus rhythm served as a reference population. Distal PR and HR were measured continuously using a Finapres device and an ECG respectively. Power spectra for HR and PR were calculated in both the supine and orthostatic positions. In paced subjects, PR spectra exhibited the characteristic respiratory peak, although the HR spectra were flat. Similarly, in healthy children the respiratory fluctuations were more pronounced when calculated from the finger pulse wave signal compared with the ECG signal. The overestimation of HR respiratory fluctuation resulting from distal PR measurement was more pronounced in the standing position; however, this postural effect was demonstrated only in healthy subjects. We observed mechanical respiratory modulation of distal PR independent of classical HR modulations. Our results suggest a mechanical respiratory influence via cardiac output and aortic transmural pressure changes on pulse wave velocity. We conclude that respiratory PR variability does not precisely reflect respiratory HR variability in standing healthy subjects and in patients with low HR variability. Consequently, HR modulation should be studied using the ECG signal rather than the distal pulse wave signal. However, when ECG recording is not available, the distal pulse wave is an acceptable alternative. [References: 23]

CONSTANT I., LAUDE D., ELGHOZI J.L., MURAT I.

Assessment of short-term blood pressure variability in anesthetized children: A comparative study between intraarterial and finger blood pressure.

J. Clin. Monitor. Comput., 15 (3), 205-214, 1999

(Services cités : UMR 8604)

Objective. Continuous blood pressure (BP) measurement provides instantaneous information on

hemodynamic status, and allows for assessment of sympathetic modulation of vasomotor tone using spectral analysis. As an alternative to intraarterial blood pressure (IABP) measurement, the Finapres, a photoplethysmographic device, allows for non-invasive continuous measurement of finger blood pressure (FBP). This study was designed to evaluate the accuracy of spectral measurements of FBP variability in children during anesthesia and recovery. For this purpose, reliability of BP measurement and short-term BP variability assessed by FBP were calculated and compared with IABP. Methods. Finger blood pressure was compared with IABP from the ipsilateral radial artery, in 14 children undergoing major surgery. Sixty-seven simultaneous recordings of both signals were performed during anesthesia and 32 during recovery period. The accuracy of the FBP was determined by measuring its bias and precision according to the Bland and Altman method. To assess the ability of the FBP to follow short term BP variability, bias of total spectral power and bias of the 3 main spectral components (LF, MF, HF) were calculated. Transfer functions between invasive and non-invasive signals were calculated. Results. The average bias of SBP measurement was 3.8 ± 7.4 mmHg during anesthesia and 2.2 ± 6.7 mmHg during recovery. During anesthesia overall variability and spectral components of FBP and IABP were similar with both techniques; while during recovery, a selective amplification of the low frequencies (< 0.15 Hz) of FBP was observed. Frequency response analysis of the pressure waveform, showed a high coherence between both signal with a gain of 0.96 ± 0.52 mmHg FBP/mmHg IABP under anesthesia, and of 0.74 mmHg FBP/mmHg IABP during recovery. Conclusions. The differences evidenced between FBP and IABP spectral profiles might result from specific physiological properties of digital arteries, which are sympathetic effectors. This study supports the use of FBP in children to assess non-invasively the vascular sympathetic component of the autonomic nervous system during anesthesia and recovery. [References: 38]

ELGHOZI J.L., PONCHON P.

Contribution of catecholamines, angiotensin and vasopressin to the low-frequency fluctuations of blood pressure in rats.

Stud. Health Technol. Informat., 60PG 159-168, 1999

(Services cités : UMR 8604)

FASSOT C., LAMBERT G., GAUDET-LAMBERT E., FRIBERG P., ELGHOZI J.L.

Beneficial effect of renin-angiotensin system for maintaining blood pressure control following subarachnoid haemorrhage.

Brain Res. Bull., 50 (2), 127-132, 1999

(Services cités : UMR 8604)

Subarachnoid haemorrhage is a serious condition often accompanied by delayed cerebral ischaemia. Earlier reports have provided evidence suggesting a role for angiotensin II in the development of cerebral vasospasm following subarachnoid bleeding. We sought to examine the influence of angiotensin II blockade with losartan on blood pressure and survival in animals following experimental subarachnoid haemorrhage, induced in conscious rats by injecting homologous blood via a catheter placed along the surface of the brain. We combined measurements of plasma renin activity with blood pressure recording in order to examine renin-angiotensin system activation following experimental subarachnoid haemorrhage. Following subarachnoid injury an approximately three-fold increase in plasma renin activity occurred (3.4 ± 1.0 vs. 10.1 ± 1.8 ng angiotensin I produced/ml/h, $p < 0.01$). In animals treated with losartan (20 mg/kg) prior to the induction of subarachnoid haemorrhage blood pressure fell dramatically following the cerebral injury (124 ± 5 vs. 94 ± 7 mmHg, $p < 0.001$), whereas blood pressure

remained unchanged in control animals. Survival was markedly reduced in those animals treated with losartan. Given the pronounced decrease in blood pressure and impaired survival following subarachnoid haemorrhage in animals treated with losartan, it would appear that the acute activation of the renin-angiotensin system following this insult is in fact a desirable, compensatory response.

GAGNET C., DEVYNCK M.A., SIMON A., LEVENSON J.

Influence of hypercholesterolemia and endothelin-3 pre-treatment on the effects of shear forces on platelet aggregation and cyclic GMP content.

Atherosclerosis, 143 (1), 91-97, 1999

(Services cités : UMR 8604)

Shear forces induce platelet aggregation and stimulate the endothelial production of anti-aggregatory factors. Among them, endothelin-3 (ET-3) has been reported to reduce aggregation and to increase platelet cyclic GMP (cGMP) content. Since hypercholesterolemia modifies both platelet aggregability and endothelial function, we compared in 14 hypercholesterolemic and 15 normocholesterolemic subjects the influences of shear forces (240 and 650 s(-1)) on platelet aggregation and cGMP content, and their modulation by ET-3. Spontaneous maximal aggregation occurred earlier and at a greater extent in hypercholesterolemic than in normocholesterolemic subjects (63 +/- 2 vs 46 +/- 6% P < 0.01). Pre-treatment with ET-3 abolished the shear-induced facilitation of maximal aggregation in platelets of normocholesterolemic (from 70 +/- 2 to 52 +/- 2% at 240 s(-1) and from 73 +/- 1 to 59 +/- 2% at 650 s(-1); P < 0.05) and hypercholesterolemic (from 78 +/- 3 to 64 +/- 2 at 240 s(-1) and from 78 +/- 2 to 66 +/- 3 at 650 s(-1); P < 0.05) subjects. cGMP content did not significantly differ between normocholesterolemic and hypercholesterolemic subjects (6.1 +/- 0.5 vs 6.9 +/- 0.7 pmol/10(9) platelets). It was reduced in platelets submitted to shear forces (P < 0.05). This shear-dependent reduction was suppressed by ET-3 pre-treatment. These results demonstrate that shear forces enhance platelet aggregation and diminish their cGMP content. ET-3 reduces the pro-aggregating effects of shear, suggesting a rise in cGMP content as a dynamic associated mechanism. (C) 1999 Elsevier Science Ireland Ltd. All rights reserved. [References: 42]

GIRARD A.

Variabilité de la pression artérielle du sujet âgé.

Ann. Cardiol. Angéiol., 48 (7), 495-499, 1999

(Services cités : UMR 8604)

In the elderly, the difference in blood pressure (BP) between the day and the night is less marked than in young subjects, but it is also more unstable and more sensitive to changes in position, meals, and physical activity. A deficient cardiac baroreflex appears to be involved in this phenomenon. Mayer's oscillation, usually considered to reflect sympathetic vascular activation, is not amplified by standing, but generates slower oscillations, at lower frequencies. The sensitivity of the cardiac baroreflex is inversely correlated with age, but, after the age of 60 years, the correlation with the level of systolic blood pressure (SBP) is greater than with age and accentuation of SBP is the main determinant in problems of postural hypotension. Hypertension and alterations in the afferent pathway of the baroreflex, adrenergic receptors, structure of the arterial wall, and intravascular volume appear to participate in the changes of blood pressure variability observed in the elderly.

HOLAND S., GIRARD A., MEYER-BISCH C., ELGHOZI J.L.

Cardiovascular responses to a acoustic startle stimulus in man.

Arch. Mal. Coeur Vaisseaux, 92 (8), 1127-1131, 1999

(Services cités : UMR 8604)

OBJECTIVE: To describe the effects of an auditory startle stimulus on blood pressure (BP) and heart rate (HR) in man. Three sound levels were tested. **DESIGN AND METHODS:** Twelve normotensive volunteers were studied in supine position. Polygraphic recordings were obtained for finger BP, R-R interval using an electrocardiograph, respiratory movements using a thoracoabdominal belt and for electrooculomyogram using adhesive electrodes. A background noise of 55 dB was administered through headphones and the acoustic startle was generated using 3 synthesized white noises of 95, 110 or 120 dB administered at 5-min intervals during the tele-expiratory phase in a randomized order. Noise duration was fixed to 150 msec. The sham stimulation (0 dB, event marker) was compared to the 3 levels of noise (one way ANOVA with repeated measures followed by multiple comparisons). Confidence intervals (95%) were calculated for BP and HR using the 30 sec period preceding each stimulation to obtain individual significance of the responses for the 30 sec following each stimulation. **RESULTS:** A biphasic cardiovascular profile was observed following noise stimulation. The early response (0-10 sec) observed after the immediate motor contraction (blink) combined BP and HR increases. The average systolic BP rise was 15.9 +/- 2.6 mmHg (peak at 4.8 sec) and the average HR increase was 11.9 +/- 1.6 bpm (peak at 2.8 sec) for the 110 dB noise. These effects were highly significant compared to the sham response ($P < 0.001$). This 110 dB intensity determined 44% of significant systolic BP values and 25% significant HR values during this early period. Similar profiles were obtained with 95 and 120 dB with a lesser amplitude. The delayed response (10-30 sec) combined moderate BP and HR decreases. **CONCLUSION:** This is the first description of the BP response to an acute loud noise in man. The early (within 10 sec) BP and HR rises may depend upon the autonomic component of the startle reflex. The reproducibility of this cardiovascular profile obtained with a 110 dB white noise makes this test applicable to the clinical trials of antihypertensive drugs.

IOUZALEN L., STEPIEN O., MARCHE P.

Effects of BAY 10-6734 (Embusartan), a new angiotensin II type I receptor antagonist, on vascular smooth muscle cell growth.

J. Pharmacol. Exp. Therap., 289 (1), 181-187, 1999

(Services cités : UMR 8604)

Angiotensin II (AII), an important hypertrophic factor in the cardiovascular system, exerts most of its known effects in vivo through the AII receptor type 1 (AT(1)) subclass of AII receptors. These receptors are also responsible for the growth-related effects of AII in cultured vascular smooth muscle cells (VSMCs). We presently investigated the effects of BAY 10-6734 (Embusartan), a new orally active AT(1) antagonist, on VSMC growth and proliferation of cultured VSMCs isolated from the aortae of Wistar Kyoto rats and spontaneously hypertensive rats. BAY 10-6734 and losartan (considered as AT(1) receptor antagonist of reference), as well as their respective active metabolites, were studied for their inhibition of: 1) [I-125]AII binding to its receptors, 2) AII-induced DNA and protein synthesis (by measuring the incorporation of 5-bromo-2'-deoxyuridine and [H-3]L-leucine, respectively), and 3) AII-induced variations in intracellular Ca²⁺ concentration, using cells labeled with Fura-2. All of the tested compounds inhibited the aforementioned parameters in a concentration-dependent manner. Half-maximal inhibitory concentration values indicated that BAY 10-6734 was significantly more potent than losartan and that spontaneously hypertensive rat-derived VSMCs were more sensitive than Wistar

Kyoto rat-derived ones. Neither BAY 10-6734 nor losartan affected the intracellular Ca²⁺ concentration of unstimulated VSMCs but both compounds inhibited both AII-induced Ca²⁺ mobilization from internal stores and Ca²⁺ influx. Neither compound affected arginine-vasopressin-, basic fibroblast growth factor-, or serum-induced DNA and protein synthesis. BAY 10-6734 appears therefore as a potent and specific new inhibitor of AII-induced growth-related events in VSMCs. [References: 40]

LARFARS G., LANTOINE F., DEVYNCK M.A., PALMBLAD J., GYLLENHAMMAR H.
Activation of nitric oxide release and oxidative metabolism by leukotrienes B-4, C-4, and D-4 in human polymorphonuclear leukocytes.

Blood, 93 (4), 1399-1405, 1999

(Services cités : UMR 8604)

Because arachidonate metabolites are potent mediators of inflammation, we have studied the effects of leukotriene B-4 (LTB₄) and the cysteinyl leukotrienes C-4 and D-4 (LTC₄ and LTD₄) on the release of nitric oxide (NO), in vitro, by human polymorphonuclear granulocytes (PMN). Two independent and highly sensitive real-time methods were used for these studies, ie, the NO-dependent oxidation of oxyhemoglobin (HbO₂) to methemoglobin and a NO-sensitive microelectrode. When activated with LTB₄, LTC₄, or LTD₄, but not with other lipoxygenase products such as 5S-HETE, 5-oxo-EETE or 5S,12S-diHETE, PMN produced NO in a stimulus- and concentration-dependent manner. The rank order of potency was LTB₄ = LTC₄ > LTD₄, corresponding to 232 +/- 50 pmol of NO/10(6) PMN for 100 nmol/L LTB₄ after 30 minutes. The kinetic properties of the responses were similar for all three leukotrienes with a maximum response at 13 +/- 3 minutes. Cysteinyl leukotriene and LTB₄ antagonists inhibited the agonist-induced NO production by 70%, and treatment with Bordetella pertussis toxin, or chelation of cytosolic Ca²⁺, [Ca²⁺]_i, also efficiently inhibited this response. In contrast, treatment of PMN with cytochalasin B (5 µg/mL) enhanced the LTB₄-induced NO formation by 86%. Thus, this is the first demonstration that the cysteinyl leukotrienes LTC₄ and LTD₄, as well as LTB₄ activate NO release from human PMN by surface receptor, G-protein and [Ca²⁺]_i-dependent mechanisms. This effect differs from activation of the nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, for which only LTB₄ is an activator. (C) 1999 by The American Society of Hematology. [References: 38]

LARFARS G., LANTOINE F., DEVYNCK M.A., GYLLENHAMMAR H.

Electrochemical detection of nitric oxide production in human polymorphonuclear neutrophil leukocytes.

Scand. J. Clin. Lab. Invest., 59 (5), 361-368, 1999

(Services cités : UMR 8604)

The detection of nitric oxide (NO) release by human polymorphonuclear neutrophil leukocytes (PMNs) presents several difficulties, mainly due to concomitant production of O₂⁻ and H₂O₂, which could interfere with the measurements. A Nafion and nickel porphyrin-coated microelectrode was used to measure NO production in PMNs in vitro. It allowed detection of 6.3 +/- 1.9 nM NO in a PMN-containing system and was unaffected by added chemicals. Addition of the chemotactic oligopeptide f-met-leu-phe (fMLP; 100 nM) induced a NO release which reached a value of 71 +/- 30 pmol NO/10(6) PMN x ml(-1) 5 min after stimulation in the presence of SOD (150 U/ml). If SOD was omitted, the corresponding value was 36 +/- 20 pmol NO/10(6) PMN x ml(-1). Presence or absence of catalase did not alter the amount of NO measured. Addition of the NO-synthase inhibitor NG-monomethyl-L-arginine (LNMMA; 1 mM) reduced

the current by 82 +/- 20%. These results agree with the rate of NO production in human PMNs when measured spectrophotometrically using the NO-dependent oxidation of oxyhaemoglobin to methaemoglobin. The NO production in human PMN was dependent on fMLP concentrations, but independent of cell-concentrations of 0.5-3.5 x 10⁶/ml. This paper shows that an electrochemical method, e.g. Nafion and porphyrin-coated microelectrode, is suitable for studies of NO release from stimulated human PMNs. [References: 31]

MILLANVOYE-VAN BRUSSEL E., DAVID-DUFILHO M., PHAM T.D., IOUZALEN L., DEVYNCK M.A.

Regulation of arachidonic acid release by calcium influx in human endothelial cells.

J. Vasc. Res., **36** (3), 235-244, 1999

(Services cités : UMR 8604)

In response to stimuli, endothelial cells release arachidonic acid, a lipid precursor of various vasoactive substances. We have investigated the relationships between cytosolic Ca²⁺ movements and arachidonic acid release in human umbilical vein endothelial cells. Histamine, a receptor-dependent agonist, and thapsigargin, a specific inhibitor of sarco-/endoplasmic Ca²⁺ pumps, time- and dose-dependently increased the release of [1-C-14]-arachidonic acid. This release was inhibited by AACOCF₃, a selective inhibitor of cytosolic phospholipase A₂ (PLA₂). In the absence of Ca²⁺ influx, arachidonic acid release was suppressed in both histamine- and thapsigargin-stimulated cells, despite marked elevations of cytosolic Ca²⁺ concentration ([Ca²⁺]_i). In the presence of Ca²⁺ influx, arachidonic acid release was reduced in cells treated with BAPTA, an intracellular Ca²⁺ buffer, or with SK&F 96365, a receptor-operated Ca²⁺ channel blocker. Arachidonic acid release was analyzed as a function of the two successive phases of Ca²⁺ response to stimulation: Ca²⁺ peak and plateau phase, reflecting Ca²⁺ mobilization from internal stores and Ca²⁺ influx, respectively. The amount of arachidonic acid released was directly related to [Ca²⁺]_i values measured at the influx phase with a 80 nM [Ca²⁺]_i threshold, similar to that reported for PLA₂ translocation. This suggests that Ca²⁺ entry from the extracellular space is essential for activating cytosolic PLA₂ in human endothelial cells. [References: 47]

PRIVAT C., STEPIEN O., DAVID-DUFILHO M., BRUNET A., BEDIQUI F., MARCHE P., DEVYNCK J., DEVYNCK M.A.

Superoxide release from interleukin-1B-stimulated human vascular cells: in situ electrochemical measurement.

Free Radical Biol. & Med., **27** (5-6), 554-559, 1999

(Services cités : UMR 8604)

Release of superoxide anion by cultured vascular cells was investigated with the use of selective microelectrodes. Local concentration of superoxide anion (O₂⁻) was followed by differential pulse amperometry on a carbon microfiber at 0.1 V/SCE. The oxidation current allows O₂⁻ detection in the 10⁻⁸ M concentration range without interference of the other major oxygen species. Interleukin-1 beta-stimulated O₂⁻ release that progressively increased to reach local concentrations at the cell membrane level of 76 +/- 11 nM 40-60 min after stimulation in human cord vein endothelial cells, and 131 +/- 18 nM 1-2 h after stimulation in internal mammary artery smooth muscle cells. In the two types of cells, the O₂⁻ oxidation signal was suppressed in the presence of superoxide dismutase. Spontaneous O₂⁻ release from unstimulated cells was undetectable. These results demonstrate that selective microelectrodes allow direct and real-time monitoring of local O₂⁻ released from vascular endothelial as well as from smooth muscle

cells submitted to an inflammatory stimulus. (C) 1999 Elsevier Science Inc. [References: 40]

ZICHA J., KUNES J., DEVYNCK M.A.

Abnormalities of membrane function and lipid metabolism in hypertension - A review.

Amer. J. Hypertens., 12 (3), 315-331, 1999

(Services cités : UMR 8604)

Hypertension, which is characterized by multiple alterations in the structure and function of the cell membrane, is often associated with important metabolic abnormalities including those concerning lipid metabolism. Dyslipidemia accompanying essential hypertension consists of elevated plasma triglycerides, low HDL cholesterol, and increased levels of atherogenic LDL cholesterol particles. The altered membrane microviscosity seen in hypertensive subjects reflects the changes of membrane lipid composition resulting from intensive exchange between circulating and membrane lipids, as well as from abnormal cellular lipid synthesis and metabolism. The changes of membrane microviscosity are known to modulate the activity of proteins involved in ion transport, signal transduction, cell Ca²⁺ handling, intracellular pH regulation, etc. Alterations in plasma or membrane lipids are indeed closely associated with ion transport abnormalities as well as with impaired control of cytosolic Ca²⁺ and pH in various forms of hypertension in both humans and rats. Such lipid-dependent modifications of membrane properties in cells participating in the cardiovascular regulation might be a part of pathogenetic mechanisms responsible for chronic blood pressure elevation. Thus nutritional and pharmacologic interventions aiming to normalize abnormal lipid metabolism could be useful for amelioration of membrane abnormalities and lowering of high blood pressure. Future studies of functional membrane alterations in hypertension or dyslipidemia will therefore require the detailed determination of membrane lipid composition and the measurement of microviscosity in particular membrane domains. *Am J Hypertens* 1999; 12:315-331 (C) 1999 American Journal of Hypertension, Ltd. [References: 217]

ZICHA J., SANG K.H.L.Q., KUNES J., DEVYNCK M.A.

Membrane microviscosity, blood pressure and cytosolic pH in Dahl rats: the influence of plasma lipids.

J. Hypertension, 17 (6), 785-792, 1999

(Services cités : UMR 8604)

Objective To determine the relationships between blood pressure, membrane microviscosity, plasma lipids and cytosolic pH in Dahl rats susceptible or resistant to salt hypertension.