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

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Abstract

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 HR 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 HR 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.

Keywords: Autonomic nervous system; Baroreflex; Blood pressure; Diabetes; Heart rate (variability); Neurotransmitters

1. Introduction

Dysfunction of the autonomic nervous system is a common complication in diabetes mellitus. Cardiovascular autonomic neuropathy (CAN) may carry an increased risk of mortality [1]. Since the introduction into clinical routine of cardiovascular tests based on changes in heart rate (HR) variability and blood pressure (BP) regulation, it has become evident that CAN may be frequently detected at early stages in asymptomatic diabetic patients [2–11]. Although effective glycemic control may prevent or delay CAN, total prevention of neuropathy is not achievable. Recent therapeutic approaches based on pathogenetic concepts of neuropathy are being tested in clinical trials in patients with CAN. The early detection of CAN is imperative for successful intervention. It appears therefore that a model of experimental diabetes could be useful to test whether reproducible alterations in cardiovascular regulation are observed at an early stage of diabetes, bearing in mind the second step could be the development of drugs preventing the occurrence of CAN. Streptozotocin (STZ) -induced diabetes has been extensively studied in

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rats and more recently in pigs [12–15]. In this latter species, 3 months of STZ-induced diabetes induced depressed left ventricular G protein–adenyl cyclase signal transduction, without apparent cardiac hypertrophy [14]. The aims of this study were to assess BP and HR variability in untreated pigs and at an early stage of experimental diabetes. The use of telemetric recordings allowed a longitudinal study of frequency-domain components of BP and HR variability using power spectral analysis.

2. Methods

2.1. Animals

Twelve sexually mature male Yucatan miniature pigs (*Sus scrofa)* from Charles River (Saint-Aubin, France) were studied. The pigs were 5 months old at the first recording session and their weight was 17.8±0.45 kg.

One month prior to the induction of diabetes, i.e. at 4 months old, the animals were equipped with a venous access port and with a telemetric BP transducer (TL 11 M2-D70-PCT from Data Sciences, Saint Paul, MN, USA). Animals were operated under isoflurane anaesthesia. For drug injections and blood withdrawals, a venous access consisting of a reservoir and a catheter (vascular access port, ref 2201.61, Vygon, Ecouen, France) was placed subcutaneously behind the right ear [16]. The catheter was inserted into the superficial jugular vein. The vascular access port was flushed three times a week with saline. For the BP transducer connection, the telemetry catheter tip was inserted retrogradely into the primary carotid artery. The catheter was secured at the point of entry to the vessel with a drop of tissue adhesive Histoacryl® (Braun, Paris, France). The telemetry transmitter body (diameter: 5 cm, height: 1 cm) was fixed subcutaneously behind the right ear. All animals received antibiotic treatment during the surgery and were treated twice a week for 1 month with an i.m. injection of lincomycine 0.1 mg/kg (Lincocine®, Pharmacia & Upjohn, Saint Quentin, France).

All experiments conformed to the relevant guidelines of the French Ministry of Agriculture for scientific experimentation on animals, and our laboratory and personnel are authorized to conduct such investigations according to the Ministry's Executive Order No. 00764. The investigation conforms with the *Guide for the Care and Use of Laboratory Animals* published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996).

2.2. Telemetry system

Prior to implanting the telemetry catheter, proper manufacturer calibration was verified by obtaining readings within ±3 mmHg of atmospheric zero pressure. Throughout the BP recording, the atmospheric pressure was automatically subtracted from the absolute BP value given by the transmitter to obtain BP values relative to atmospheric pressure. The receiver (RLA 1020, Data Sciences) was placed under the sling for measurement of cadiovascular variables. The telemetry signal was transformed back to a calibrated analogue signal using a Data Sciences UA10 universal adapter D/A converter. A MS-DOS-based computer contained a DQ100 processor card to sample and process data from a single transmitter source. This computer was loaded with a UA10 software program which was run continuously to achieve this task. The waveform sampling rate was set to 500 Hz.

2.3. Breathing determination

Respiratory movements were detected by respiratory inductance plethysmography with a Respitrace® (Ardsley, NY, USA). This device uses elastic bands, which do not restrict breathing movements, wrapped around the rib cage to measure thoracic volume displacement during breathing. Analog signal from the rib cage was obtained simultaneously with the BP waveform.

2.4. Glycemia determination

Blood samples were collected via the vascular access port into heparinized tubes and glycemia was determined using an automated glucose analyser (E6666, Eppendorf, Koln, Germany).

2.5. Study protocol

All measurements were performed in conscious animals, kept in a sling during the time of the recordings.

For the first period, a baseline recording was performed for the analysis of HR and BP variability, followed by a baroreflex determination (see below). One week later, a second baseline recording was obtained, then atropine sulphate was administered (0.04 mg/kg i.v.) and after a BP and HR recording during muscarinic blockade, a second baroreflex determination was performed. After another week, a third baseline determination was obtained prior to propranolol administration (0.5 mg/kg i.v.). After a BP and HR recording during beta-adrenoceptor blockade was obtained, another baroreflex curve was obtained.

Five animals were kept as controls (C), while diabetes (D) was induced in seven pigs by two injections of STZ (55 mg/kg body weight i.v. initially and 50 mg/kg body weight i.v. 8 days later) in citrate buffer. Controls received two citrate buffer injections. Diabetic animals were treated intramuscularly twice a day with longer acting insulin (10–20 I.U. according to glycemia).

Each pig was studied before STZ (or vehicle) injections as described above and 3 and 6 months after STZ or

vehicle treatment. For the 3- and 6-month periods, recordings were limited to HR and BP baseline recordings. However, each pig was recorded on 3 separate days and the mean values for each animal were determined.

2.6. Data acquisition and processing

The details of data acquisition have previously been described [17]. An evenly spaced (equidistant) sampling of systolic BP (SBP), diastolic BP (DBP), pulse rate (taken as surrogate for HR) allowed a direct spectral analysis of a 128-s segment using a fast Fourier transform algorithm using the computer program PRX (Notocord Systems, Croissy sur Seine, France). Each spectral band corresponded to a harmonic of 1/128 Hz or 0.0078 Hz. The frequency of oscillation scale (abscissa) was analysed up to 0.5 Hz. The SBP, DBP and HR power spectra (ordinates) shown with the recordings have units of $mmHg^2$ or bpm^2. The modulus (square root of the power in mmHg or bpm) was used for calculations. The recording of breathing movements allowed a spectral analysis of the respiration. The respiratory modulation of BP and HR corresponded to the peak detected at the frequency of the respiration. The value of the peak at the respiration frequency and the values of the three consecutive higher and smaller frequencies (i.e. seven values corresponding to a total band width of 0.054 Hz) were integrated to obtain the respiratory component of the SBP, DBP, and HR spectra.

Spectral analysis of SBP, DBP and HR was calculated when respiration was quiet and stable, ensured by a marked peak in the spectral analysis of the respiration traces as shown in Fig. 1. Grunting was associated with unstable BP and HR and periods of grunts were discarded in order to limit the analysis to stationary periods.

Decriptive statistics of the distribution of the variables (SBP, DBP, HR) of the stationary periods of 128 s used for the spectral analysis were computed.

2.7. Pharmacological baroreflex determination

Changes in BP were induced in awake animals by graded infusion of the α-adrenoceptor agonist phenylephrine (3 μg/kg/min, 4.5 μg/kg/min, 6 μg/kg/min, during 20 min for each concentration). After the infusion a wash-out period was allowed for the restoration of HR and BP levels. Then, a graded infusion of the vasodilator sodium nitroprusside (1 μg/kg/min for 20 min and 2 μg/kg/min for another 20 min period) was performed.

SBP and HR curves were smoothed by a moving average of 30 s, in order to avoid fast fluctuations of SBP and HR. Then consecutive portions of 30 s of smoothed BP and HR records were averaged and used for the construction of the baroreflex curve. Phenylephrine in-

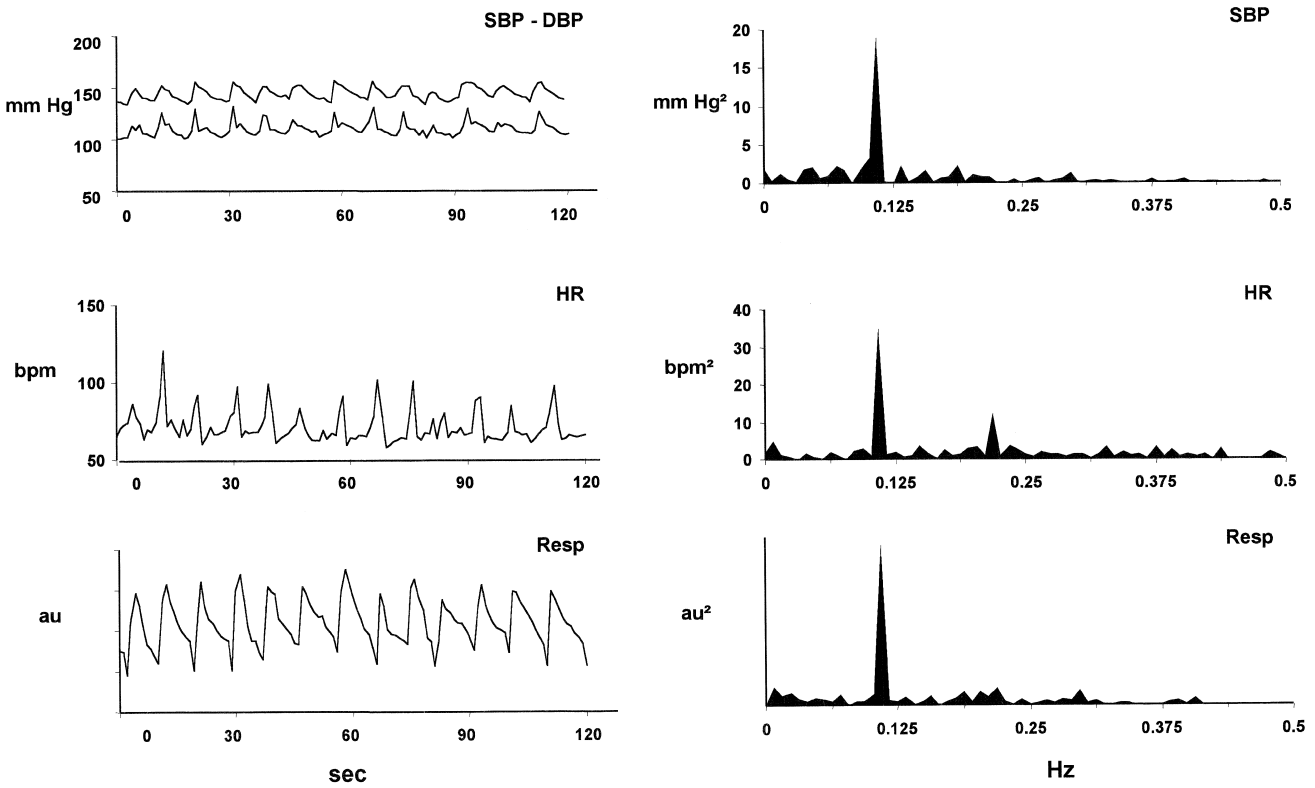

Fig. 1. Systolic and diastolic blood pressure (SBP, DBP), heart rate (HR) and respiratory movements (Resp, in arbitrary units, au) of a conscious untreated pig at rest over a 120-s period. Spectral power of the corresponding recordings (SBP, HR and Resp) are shown on the right to illustrate the synchronization of the corresponding oscillations with respiration.

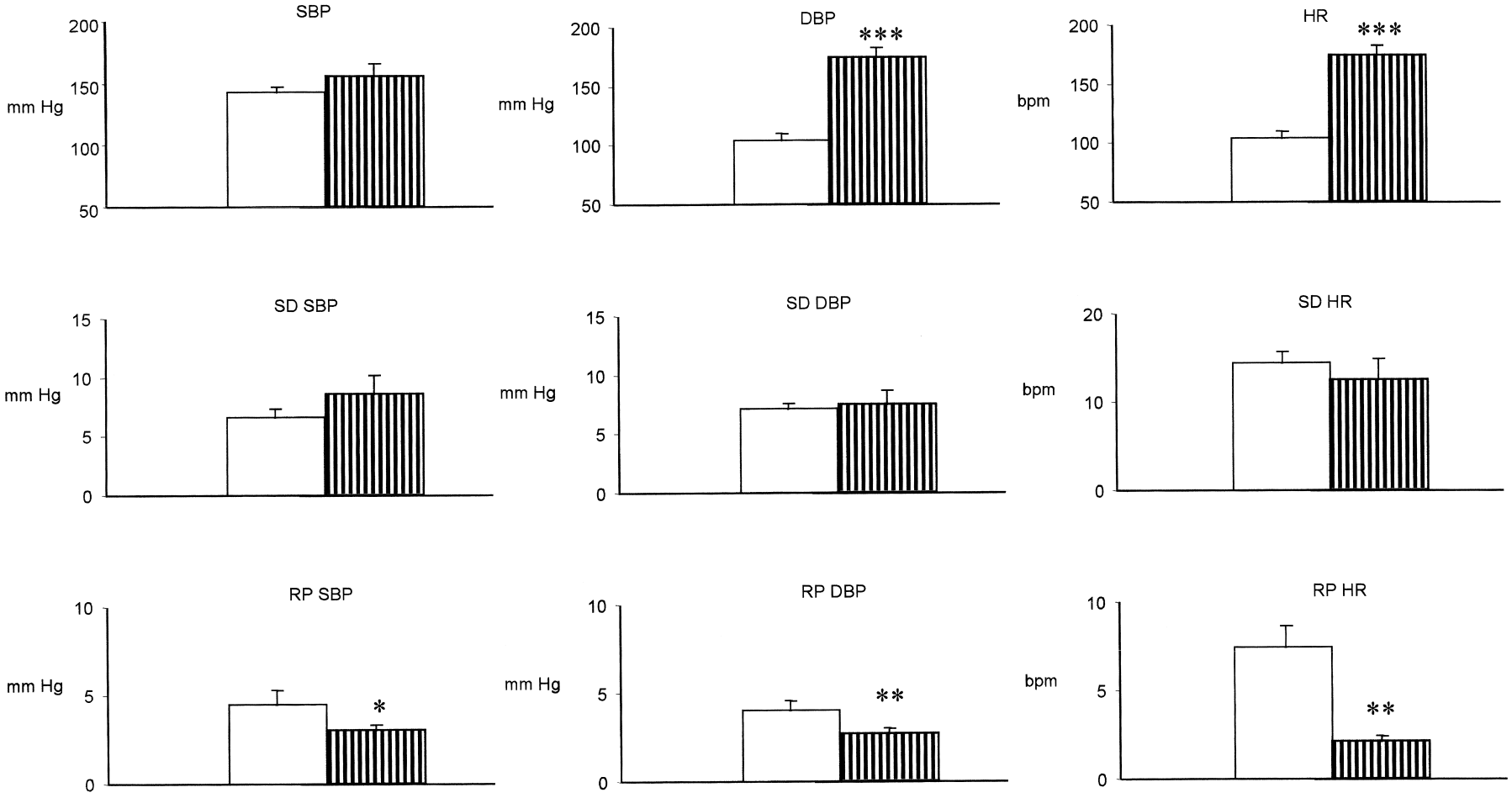

Fig. 2. Effect of atropine on the average levels of systolic and diastolic blood pressure (SBP and DBP) and heart rate (HR), on the standard deviation (S.D.) or overall variability of SBP, DBP and HR distribution and on the respiratory component (RP, modulus) of SBP, DBP and HR obtained from the spectral analysis of the corresponding time series. *$P<0.05$, **$P<0.01$, ***$P<0.001$ for the comparison between atropine (hatched columns) and untreated (white) values ($n=12$). Vertical bars represent S.E.M.

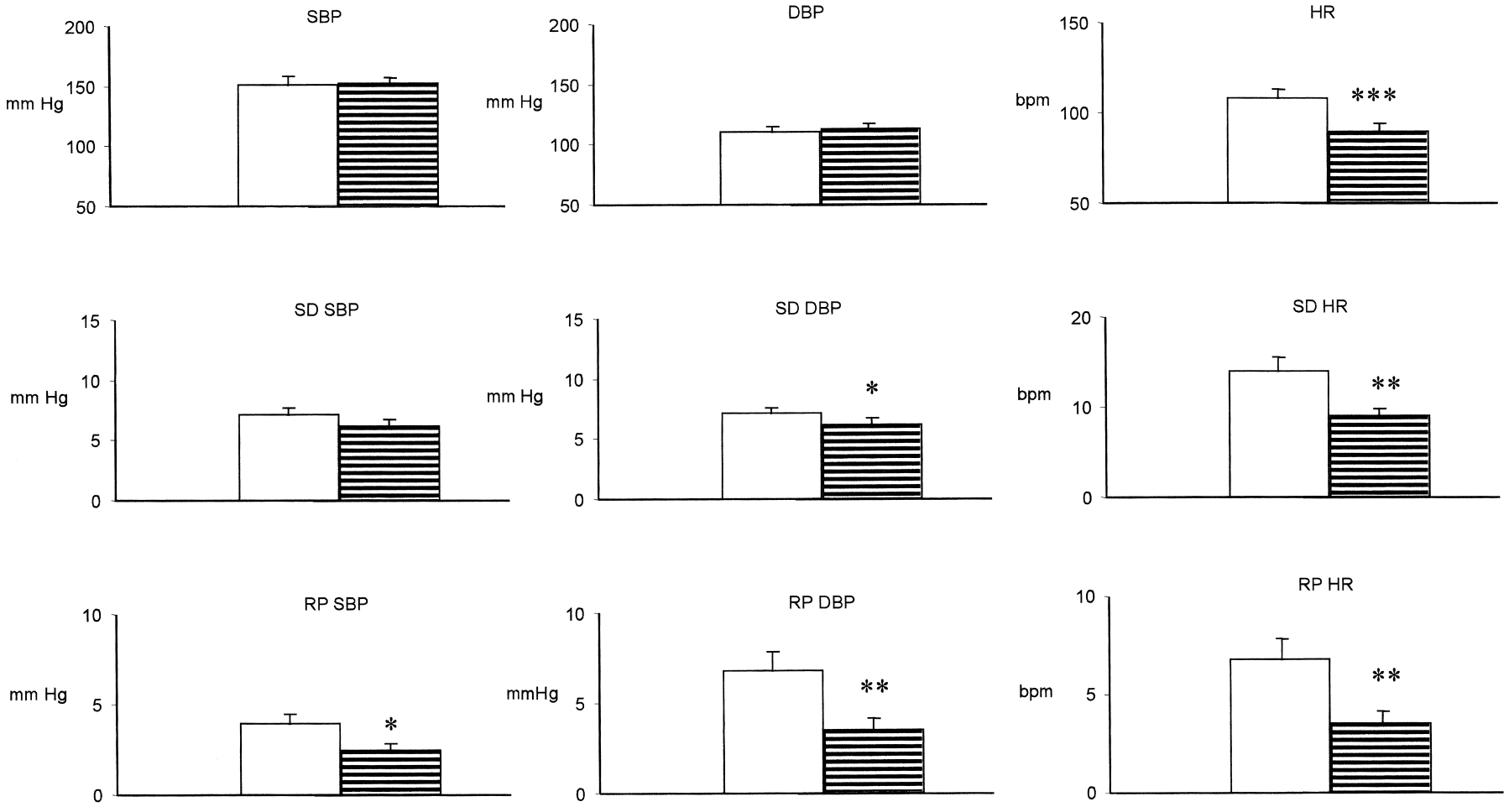

Fig. 3. Effect of propranolol on the cardiovascular variables. *$P<0.05$, **$P<0.01$, ***$P<0.001$ for the comparison between propranolol (hatched columns) and untreated (white) values ($n=12$).

Table 1
Baseline averages values, standard deviation (S.D.) and respiratory peak (RP) of systolic blood pressure (SBP), diastolic blood pressure (DBP) and heart rate (HR) of the 12 pigs (n=12, mean±SEM)

SBP (mm Hg)	S.D. SBP (mm Hg)	RP SBP (mm Hg)	DBP (mm Hg)	S.D. DBP (mm Hg)	RP DBP (mm Hg)	HR (bpm)	S.D. HR (bpm)	RP HR (bpm)
147.6±4.9	7.7±0.6	4.7±0.7	115.5±4.2	7.8±0.4	4.1±0.4	109.5±5.1	15.9±1.2	7.8±1.1

duced an increase in BP together with a decrease in HR. Conversely, sodium nitroprusside induced a decrease in BP together with an increase in HR. The correlation between BP and HR led to the construction of the sigmoidal baroreflex curve [18,19] and provided the estimation of the upper plateau, the lower plateau and the maximal gain of the curve.

Three barocurves were obtained: during resting conditions, following atropine and following propranolol.

2.8. Cross-spectral analysis

The transfer function analysis was utilized to assess the relationship between spontaneous SBP and HR (derived from the pulse interval) fluctuations in the frequency domain. The transfer function provides information about coherence, gain and phase relation of both signals in the frequency range considered [20–22]. Cross-spectral analysis between SBP and HR were performed during resting conditions, following atropine and propanolol administration, and after 3 and 6 months following STZ (or vehicle) administration during resting conditions.

2.9. Drugs

Atropine sulphate was purchased from Aguettant (Lyon, France), propranolol from Zeneca (Cergy, France), longer acting insulin Endopancrine Zinc Protamine® from Organon (Puteaux, France), lincocine (Pharmacia & Upjohn), phenylephrine hydrochloride, sodium nitroprusside and streptozotocin from Sigma (L'isle d'Abeau, France).

2.10. Statistics

The results are presented as mean±S.E.M. Baseline levels of cardiovascular variables, baroreflex indices and the effects of autonomic blockers (atropine or propranolol) were assessed on the entire cohort of 12 animals. Effects of autonomic blockers on cardiovascular variables and baroreflex indices were compared using a paired t-test. The parametric Pearson's test was used to analyze the linear relationship between cross-spectral and pharmacological estimates of baroreflex sensitivity.

The effects of STZ and vehicle on glycemia were compared using a two-way analysis of variance (ANOVA). The effects of STZ or vehicle on cardiovascular variables were compared using a two-way ANOVA for repeated measures followed by an unpaired t-test for the comparison of control and diabetic animals, at 3 and 6 months. All comparisons were considered significant when $P<0.05$.

3. Results

3.1. Resting cardiovascular variables

Baseline cardiovascular levels are summarized in Table 1. Cyclic fluctuations of a 5–8 s period were observed in all animals. The spectral analysis of SBP, DBP and HR exhibited a mostly unique peak synchronised with respiration as shown in the example in Fig. 1. The average respiratory rhythm was 0.185±0.018 Hz, corresponding to an average 5.4-s breathing cycle. No other distinct peaks were observed in the spectral profiles of either SBP, DBP or HR.

3.2. Effects of atropine and propranolol

The effects of atropine are illustrated in Fig. 2. Atropine induced a marked increase in HR (104.7±6.1 vs. 174.6±7.9 bpm, $P<0.001$). A moderate BP rise was associated with the tachycardia although the change reached significance only for DBP (111.9±3.2 vs. 135.7±8.7 mmHg, $P<0.01$).

The respiratory peak (RP) of HR was dramatically reduced by atropine (7.5±1.2 vs. 2.1±0.4 bpm, $P<0.001$). The RP of SBP was also decreased by atropine but to a proportionally lesser extent (4.5±0.8 vs. 3.0±0.4 mmHg, $P<0.05$). A slight decrease of the RP of DBP was also observed (4.0±0.6 vs. 2.7±0.3 mmHg, $P<0.01$).

The effects of propranolol are shown in Fig. 3. Propranolol decreased HR (108.2±5.1 vs. 89.3±5.1 bpm, $P<0.001$). No changes in the average SBP or DBP were detected.

The RP of HR was diminished by the beta-adrenoceptor blocking drug (6.8±1.1 vs. 3.5±0.7 bpm, $P<0.01$), and the overall variability of HR reflected by the standard deviation (S.D.) of the HR distribution was concomitantly reduced (14±1.5 vs. 9.0±0.9 bpm, $P<0.01$). Propranolol induced a decrease in the RP of DBP (3.7±0.6 vs. 2.5±0.4 mmHg, $P<0.01$) and SBP (3.9±0.6 vs. 2.5±0.4 mmHg, $P<0.05$). The S.D. of DBP was also significantly reduced by propranolol (7.1±0.6 vs. 6.1±0.7 mmHg, $P<0.05$).

Table 2
Baseline values of the baroreceptor–heart rate reflex curves (top) and baseline values of the cross-spectral analysis between systolic blood pressure and heart rate (bottom) obtained in the 12 pigs (mean±S.E.M.)

Upper plateau (bpm)	Lower plateau (bpm)	Maximum gain (bpm/mm Hg)	BP50 (mm Hg)	Resting SBP (mm Hg)	Resting HR (bpm)
147.8±10.8	62.6±3.3	1.9±0.2	143.9±4.9	142.6±4.6	102.2±5.4

Coherence	Gain (bpm/mm Hg)	Phase (radians)
0.70±0.06	1.4±0.10	1.3±0.22

3.3. Baroreflex indices

Average indices of the baroreceptor-HR reflex of untreated pigs are shown in Table 2. The three average barocurves are illustrated in Fig. 4. Propranolol induced a decrease in the upper plateau (147.8±10.8 vs. 118.8±5.1 bpm, $P<0.05$).

Atropine shifted the barocurve to upper values. Atropine markedly increased the resting HR (102.2±5.4 vs. 186.4±7.8, $P<0.001$). The differences reached significance for the upper plateau (147.8±10.8 vs. 208.3±9.9 bpm, $P<0.01$) and the lower plateau as well (62.6±3.3 vs. 141.5±7.9 bpm, $P<0.001$).

Cross-spectral analysis between SBP and HR of untreated pigs showed a peak of high average coherence (0.70±0.06) detected at the respiratory frequency (mean: 0.184 Hz±0.019 Hz) with an average gain of 1.4±0.10 bpm/mmHg and a phase of 1.2±0.2 radians (HR preceding SBP by 1.06 s). Atropine reduced the coherence to 0.2±0.05 ($P<0.001$), the gain to 0.5±0.1 mmHg ($P<0.001$) and the phase to −0.4±0.38 radians ($P<0.001$) (SBP preceding HR by 0.3 s), while atenolol did not modify these cross-spectral indexes. In addition, a significant linear relationship was observed between the gain of the baroreflex obtained from the pharmacology and the gain obtained from the cross spectral analysis ($r=0.70$, $P<0.05$).

3.4. Diabetic animal model

Body weight was significantly reduced after 6 months of diabetes (41.4±1.7 vs. 31.8±1.4 kg, $P<0.001$). STZ induced a marked and sustained rise in glycemia in the STZ group compared to the controls as shown in Fig. 5.

3.5. Effects of the duration of diabetes on cardiovascular variables

The effects of the duration of diabetes on BP and HR parameters are summarized in Fig. 6. Diabetes decreased BP at 3 months (for SBP 142.9±4.2 vs. 125±4.5 mmHg, $P<0.001$; for DBP 103.4±2.9 vs. 91.4±4.4 mmHg, $P<0.05$) and 6 months (for SBP 137.9±6.3 vs. 120.5±3.0 mmHg, $P<0.001$; for DBP 99.7±6.7 vs. 86.7±2.8 mmHg, $P<0.05$) as well.

Diabetes induced a significant increase in HR 6 months after the diabetes was initiated (66.6±2.7 vs. 77.4±3.8 bpm, $P<0.01$).

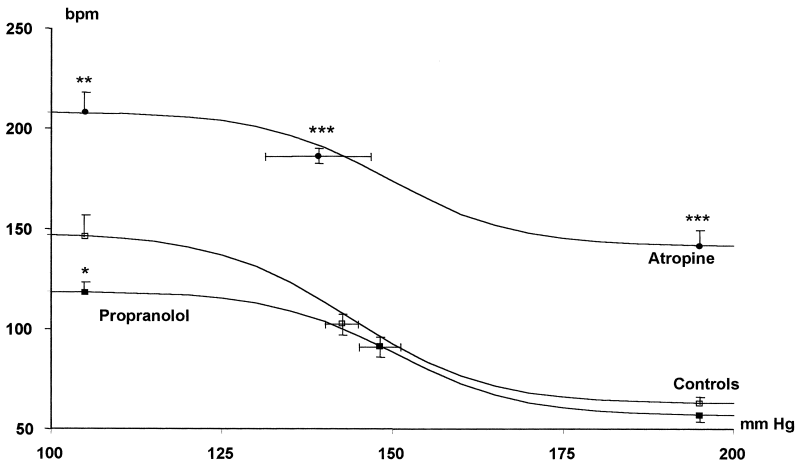

Fig. 4. Average sigmoid baroreceptor–heart rate reflex curves obtained in control conditions, after atropine and after propranolol ($n=12$). The middle points refer to resting blood pressure and heart rate levels. *$P<0.05$, **$P<0.01$, ***$P<0.001$ for the comparison between the atropine or propranolol levels (resting or plateaus) to the control values.

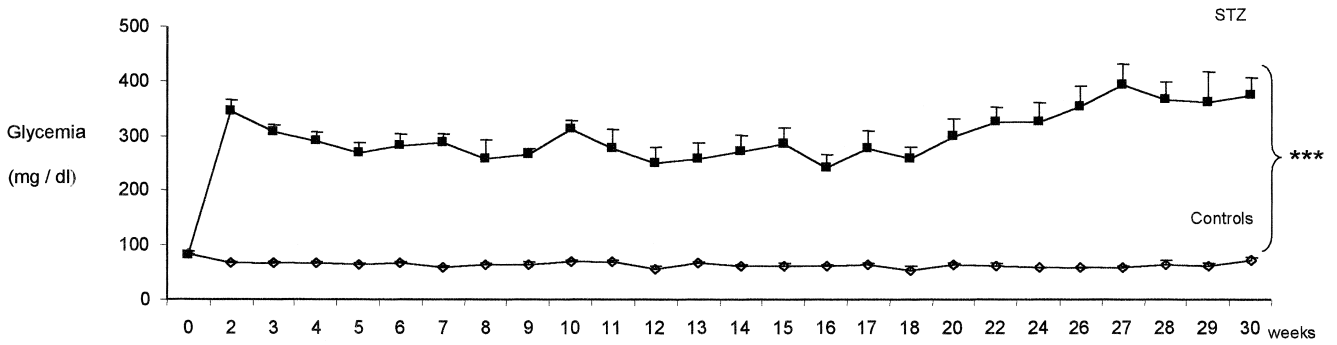

Fig. 5. Plasma glucose levels obtained during the 30 weeks of observation in the seven streptozotocin (STZ) treated pigs and the five control animals. ***$P<0.001$.

The overall variability (S.D.) of SBP, DBP and HR was reduced at the early stage (3 months) and latter stage (6 months) of diabetes (for SBP: 7.0±0.73 vs. 5.9±0.75 mmHg, $P<0.05$ at 3 months and 7.7±0.7 vs. 5.3±0.4 mmHg, $P<0.01$ at 6 months; for DBP: 7.9±0.9 vs. 6.1±0.7, $P<0.05$ at 3 months and 7.4±0.6 vs. 5.3±0.5 mmHg, $P<0.05$ at 6 months; for HR: 10.0±0.9 vs. 7.1±0.5 bpm, $P<0.01$ at 3 months and 9.8±0.9 vs. 7.0±0.8 bpm, $P<0.01$ at 6 months). The frequency component analysis demonstrated the RP of HR was lowered at 3 months (5.6±0.7 vs. 3.2±0.4 bpm, $P<0.01$). This alteration was still there at 6 months although controls exhibited smaller HR RP (4.9±0.7 vs. 3.7±0.5 bpm, $P<0.05$). Finally the gain of the cross-spectral analysis was reduced at the early stage (3 months) of diabetes (1.6±0.4 vs. 1.1±0.2 bpm/mmHg, $P<0.05$).

4. Discussion

4.1. Cardiovascular variables of the normal minipig

The present results indicate the resting HR of the sexually mature pig approximates 110 bpm at 5 months of age with a decline with age to 70 bpm at 8 months. BP levels were less affected by age as reflected by a stable SBP close to 150 mmHg at any age. The DBP remained above 100 mmHg in adult pigs. It is noteworthy that the respiratory rate of the pigs was low (0.19 Hz at 5 months, 0.13 Hz at 8 months and 0.13 Hz at 11 months) compared to the HR level, resulting in a HR to breathing rhythm ratio greater than 4 (10.8 at 5 months, 10.8 at 8 months and 10.8 at 11 months as well), which is usually observed in mammals. A recent study of respiration in the miniature swine also indicated a slow respiratory rhythm of 0.14 Hz [23]. The BP and HR variability profile recorded during a conscious, quiet state revealed another peculiarity of this species, that is the dominant role of respiration in generating variations in BP and HR. The recordings did not allow the discrimination of a variability component of lower rate than respiration. Respiration is usually referred to as the high frequency component of variability, as opposed to the other component which is often named low-frequency component or Mayer waves [24–26]. Similar observations of a small low-frequency component have been done in the rabbit: Mayer waves are basically undetectable unless a stimulus such as hemorrhage or hypoxia is applied [27,28]. However, as a consequence of the bradypnea, one cannot exclude a slow non respiratory cardiovascular rhythm (low-frequency component) could be trained and superimposed within the respiration frequency [29]. Kuwahara et al. [23] recently performed power spectral analysis of the BP and HR signals in miniature pigs and suggested the low-frequency component was located at a range lower than respiration of ≈0.03 Hz corresponding to 33-s period oscillations. This implies stationary recordings longer than those obtained in our conditions for a descriptive study.

The autonomic blockers markedly affected average cardiovascular parameters, baroreflex curves and the variability of BP and HR. The marked increase in HR following atropine administration demonstrated the dominance of the vagal tone at rest. This increase in HR could well be responsible for the BP elevation observed after muscarinic blockade. Atropine pretreated pigs exhibited a baroreflex curve shifted to higher HR levels, with a reduced HR range due to a decreased bradycardic response to phenylephrine. The most interesting observation was the dramatic reduction in the amplitude of the respiratory peak of HR variability following atropine, which demonstrates that respiratory sinus arrhythmia depends mainly upon vagal modulation. Kuwahara et al. [23] also observed a marked reduction of respiratory sinus arrhythmia of the miniature swine with atropine. However, an effective dosing of propranolol, which induced a bradycardia in relation with the beta-adrenoceptor cardiac blockade together with a moderate BP reduction, also revealed a significant contribution of the cardiac sympathetic modulation in the respiratory oscillation of HR. This acute effect of the beta blocker could reflect the implication of the cardiac sympathetic activity in the genesis of respiratory sinus arrhythmia in pigs, possibly by decreasing the non-respiratory component. Baroreceptor responses to arterial

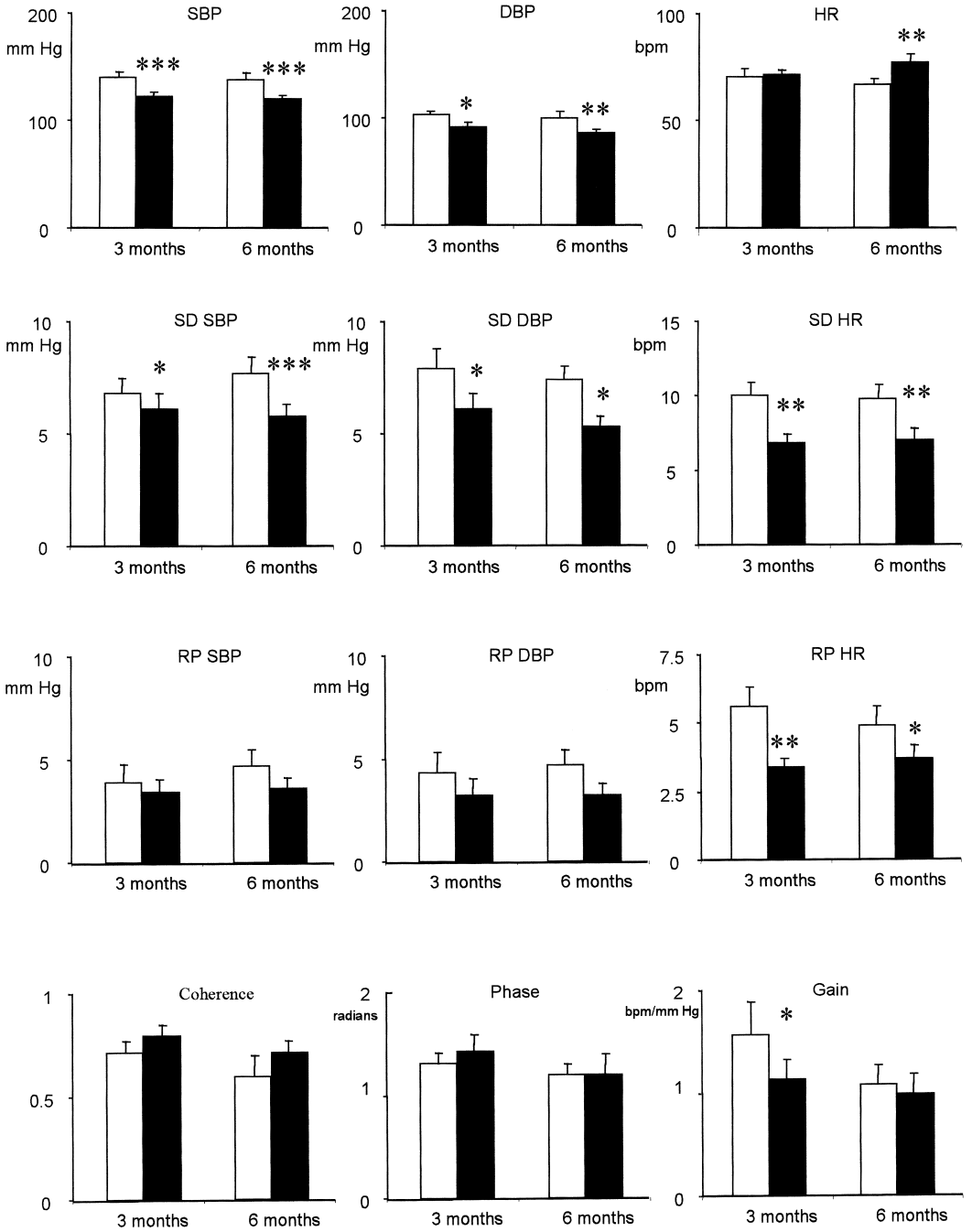

Fig. 6. Effects of diabetes duration (3 or 6 months, $n=7$) on the cardiovascular variables. Diabetic pigs are compared to five controls at the same age. *$P<0.05$, **$P<0.01$, ***$P<0.001$ for the comparison with the control value.

pressure variations produced by respiratory movements could well determine changes in cardiac sympathetic activity. The slow breathing rate in this species could favour the contribution of the sluggish cardiac sympathetic activity in the genesis of the respiratory peak of HR. Thus, this peak could be influenced by the dual interaction of the vagal and the sympathetic cardiac nerves. The cardiac sympathetic contribution to the baroreflex-mediated HR changes was illustrated by the effect of propranolol, which lowered the upper plateau of the barocurve obtained during steady state conditions [19]. It is noticeable that both atropine and propranolol reduced the respiratory peak of BP. This could indicate that the fluctuations in BP in the respiratory range, which partly result from oscillations in left ventricular ejection due to the mechanical effect of respiration, could also reflect oscillations in HR. Although there are species differences in BP and HR variability profiles, it is noticeable the contribution of respiratory sinus arrhythmia to respiratory BP fluctuations has already been found in dogs and in humans [30,31]. Compared to

humans, the relatively slow breathing rate of the pig could allow a sympathetic modulation of HR in this species. In addition, the significant relationship obtained between the gain obtained with the pharmacological determination of the baroreflex and the gain of the cross-spectral analysis observed in this study, extends to this species the concept that cross-spectral analysis may provide an index of spontaneous baroreflex sensitivity [20,21]. This non invasive approach was used as an alternative to pharmacological determination of baroreflex sensitivity in diabetic pigs.

4.2. Effects of diabetes

Spontaneous fluctuations of BP and HR could be used to detect autonomic dysfunction in diabetes mellitus in man [9,10]. The time course of the CAN alterations remains to be defined. Telemetric recordings allowed the detection of early signs of cardiovascular dysfunction in diabetic pigs. The cardiovascular profile at 3 months after the onset of diabetes differed from the more altered profile observed after 6 months, suggesting the underlying cardiovascular neuropathy was more severe as the duration of diabetes increased. At 3 months, there was a decrease in BP, no change in resting HR and reduced BP and HR variability. The marked alteration in HR variability corresponded to a reduced respiratory sinus arrhythmia. Early alterations in vagal modulation of HR could contribute to this result. However, the decrease in BP associated with the decrease in HR variability could also be a sign of a sympathetic deficit. It is conceivable that a sympathetic deficit affects both the vascular and the cardiac limbs and therefore the HR variability alteration could be due to a combination of a vagal deficit and a cardiac sympathetic deficit. A balanced deficit could explain why the resting HR was unchanged at 3 months. At a later stage of diabetes the decreases in BP levels and HR variability were still present and the differences versus the control pigs of the same age were highly significant. In addition, the resting HR was high in diabetic pigs. A marked vagal deficit could be responsible for this tachycardia. Higher HR have long been recognized in diabetic patients in association with CAN, with the highest HR found in patients with parasympathetic damage [1].

Diabetes also affected the coupling between BP and HR at 3 months of diabetes, suggesting an early alteration of baroreflex sensitivity. The gain of the cross-spectral analysis remained at a low level at 6 months in the diabetes group. Controls also exhibited a reduced gain at the same age of 11 months. The values of gain followed the same pattern as the RP of HR. This could reflect the mixed vagal and sympathetic influences upon the RP of HR, which are also the determinants of the baroreflex sensitivity. It was already shown that diabetic neuropathy impairs the baroreceptor-cardiac reflex sensitivity in man [9].

5. Conclusion

The present protocol aimed at developing a model of diabetes in pigs resulted in animals exhibiting indices of autonomic failure at an early stage of diabetes, together with an impairment of the baroreflex sensitivity. The study of cardiac autonomic regulation demonstrated a dual vagal and sympathetic control of respiratory sinus arrhythmia, which appeared to be the main determinant of short-term variability in this species. The spectral and cross-spectral analysis of BP and HR could be used to develop therapeutic strategies of cardiovascular autonomic neuropathy.

References

[1] Ziegler D. Diabetic cardiovascular autonomic neuropathy: prognosis, diagnosis and treatment. Diabetes Metab Rev 1994;10:339–383.
[2] Kitney RI, Byrne S, Edmonds ME, Watkins PJ, Roberts VC. Heart rate variability in the assessment of autonomic diabetic neuropathy. Automedica 1982;4:155–167.
[3] Ewing DJ, Martyn CN, Young RJ, Clarke BF. The value of cardiovascular autonomic function tests: 10 years experience in diabetes. Diabetes Care 1985;8:491–498.
[4] Genovely H, Pfeifer MA. RR-variation: the autonomic test of choice in diabetes. Diabetes Metab Rev 1988;4:255–271.
[5] Weise F, Heydenreich F, Gehrig W, Runge U. Heart rate variability in diabetic patients during orthostatic load — a spectral analytic approach. Klin Wochenschr 1990;68:26–32.
[6] Bellavere F, Balzani I, De Masi G et al. Power spectral analysis of heart-rate variations improves assessment of diabetic cardiac autonomic neuropathy. Diabetes 1992;41:633–640.
[7] Ziegler D, Laux G, Dannehl K et al. Assessment of cardiovascular autonomic function: age-related normal ranges and reproducibility of spectral analysis, vector analysis, and standard tests of heart rate variation and blood pressure responses. Diabetic Med 1992;9:166–175.
[8] Oka H, Mochio S, Sato K et al. Spectral analyses of R–R interval and systolic blood pressure in diabetic autonomic neuropathy. J Auton Nerv Syst 1995;52:203–211.
[9] Weston PJ, Panerai RB, McCullough A et al. Assessment of baroreceptor-cardiac reflex sensitivity using time domain analysis in patients with IDDM and the relation to left ventricular mass index. Diabetologia 1996;39:1385–1391.
[10] Frattola A, Parati G, Gamba P et al. Time and frequency domain estimates of spontaneous baroreflex sensitivity provide early detection of autonomic dysfunction in diabetes mellitus. Diabetologia 1997;40:1470–1475.
[11] Ducher M, Thivolet C, Cerutti C et al. Noninvasive exploration of cardiac autonomic neuropathy — Four reliable methods for diabetes? Diabetes Care 1999;22:388–393.
[12] Chaouloff F, Blanc J, Baudrie V, Laude D, Elghozi JL. Cardiovascular and adrenaline-releasing effects of the 5-HT1A receptor agonist 8-hydroxy-2-(di-n-propylamino)tetralin in streptozotocin diabetic rats. Life Sci 1991;48:2543–2552.
[13] Maeda CY, Fernandes TG, Timm HB, Irigoyen MC. Autonomic dysfunction in short-term experimental diabetes. Hypertension 1995;26:1100–1104.
[14] Roth DA, White CD, Hamilton CD, Hall JL, Stanley WC. Adrenergic desensitization in left ventricle from streptozotocin diabetic swine. J Mol Cell Cardiol 1995;27:2315–2325.
[15] Fazan Jr. R, Dias da Silva VJ, Ballejo G, Salgado HC. Power spectra of arterial pressure and heart rate in streptozotocin-induced diabetes in rats. J Hypertens 1999;17:489–495.

[16] Palmisano BW, Clifford PS, Hoffmann RG, Seagard JL, Coon RL, Kampine JP. Depression of baroreflex control of heart rate by halothane in growing piglets. Anesthesiol 1991;75:512–519.
[17] Japundzic N, Grichois ML, Zitoun P, Laude D, Elghozi JL. Spectral analysis of blood pressure and heart rate in conscious rats: effects of autonomic blockers. J Auton Nerv Syst 1990;30:91–100.
[18] Parlow J, Viale JP, Annat G, Hughson R, Quintin L. Spontaneous cardiac baroreflex in humans. Comparison with drug-induced responses. Hypertension 1995;25:1058–1068.
[19] Head GA, McCarty R. Vagal and sympathetic components of the heart rate range and gain of the baroreceptor-heart rate reflex in conscious rats. J Auton Nerv Syst 1987;21:203–213.
[20] Robbe HW, Mulder LJ, Ruddel H, Langewitz WA, Veldman JB, Mulder G. Assessment of baroreceptor reflex sensitivity by means of spectral analysis. Hypertension 1987;10:538–543.
[21] Saul JP, Berger RD, Albrecht P, Stein SP, Chen MH, Cohen RJ. Transfer function analysis of the circulation: unique insights into cardiovascular regulation. Am J Physiol 1991;261:H1231–H1245.
[22] Weise F, Laude D, Girard A, Zitoun P, Siche JP, Elghozi JL. Effects of the cold pressor test on short-term fluctuations of finger arterial blood pressure and heart rate in normal subjects. Clin Auton Res 1993;3:303–310.
[23] Kuwahara M, Suzuki A, Tsutsumi H, Tanigawa M, Tsubone H, Sugano S. Power spectral analysis of heart rate variability for assessment of diurnal variation of autonomic nervous activity in miniature swine. Lab Anim Sci 1999;49:202–208.
[24] Pagani M, Lombardi F, Guzzetti S et al. Power spectral analysis of heart rate and arterial pressure variabilities as a marker of sympatho–vagal interaction in man and conscious dog. Circ Res 1986;59:178–193.
[25] Janssen BJ, Oosting J, Slaaf DW, Persson PB, Struijker-Boudier HA. Hemodynamic basis of oscillations in systemic arterial pressure in conscious rats. Am J Physiol 1995;269:H62–H71.
[26] Bertram D, Barrès C, Cuisinaud G, Julien C. The arterial baroreceptor reflex of the rat exhibits positive feedback properties at the frequency of Mayer waves. J Physiol 1998;513:251–261.
[27] Mayer S. Studien zur physiologie des herzens und der blutgefässe. Sitzungsberichte der Kaiserlichen Akademie der Wissenschaften 1876;74:281–307.
[28] Gaudet EA, Godwin SJ, Lukoshkova E, Head GA. Effect of central endogenous angiotensin II on sympathetic activation induced by hypoxia. Clin Exp Hypertens 1997;19:913–923.
[29] Novak V, Novak P, de Champlain J, Nadeau R. Altered cardiorespiratory transfer in hypertension. Hypertension 1994;23:104–113.
[30] Akselrod S, Gordon D, Madwed JB, Snidman NC, Shannon DC, Cohen RJ. Hemodynamic regulation: investigation by spectral analysis. Am J Physiol 1985;249:H867–H875.
[31] Taylor JA, Eckberg DL. Fundamental relations between short-term RR interval and arterial pressure oscillations in humans. Circulation 1996;93:1527–1532.