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EVALUATION OF SPONTANEOUS BAROREFLEX SENSITIVITY IN CONSCIOUS DOGS

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SUMMARY

1. We evaluated a method of measuring cardiac baroreflex sensitivity (BRS) derived from spontaneous changes in systolic pressure (SP). SP was measured directly from an arterial catheter, and pulse interval (PI) was measured from the ECG signal in seven conscious, resting dogs.

2. Beat-to-beat changes in PI (dPI) were positively correlated with beat-to-beat changes in SP (dSP) in all dogs tested, suggesting spontaneous baroreflex function. The slope of the regression of dPI on dSP was used as an index of spontaneous BRS.

3. The spontaneous BRS was abolished by hexamethonium, atropine and bilateral carotid sinus denervation. Low dose atropine sulphate produced a paradoxical increase in spontaneous BRS, which has been observed in other studies. The spontaneous BRS was positively correlated with the average pulse interval in resting dogs.

4. Random modulation of heart rate after vagotomy failed to reproduce the strong positive correlation between dSP and dPI; this demonstrated that the correlation was not the result of mechanical coupling between heart rate and arterial blood pressure.

5. The BRS was measured pharmacologically in six dogs using a bolus injection of a vasoconstrictor. The pharmacological BRS was positively correlated with the spontaneous BRS measured after the bolus injection.

6. Finally, the spontaneous BRS was negatively correlated with the average arterial pressure in resting dogs. We conclude that the spontaneous BRS is a useful quantitative indicator of baroreflex function in conscious resting dogs.

INTRODUCTION

A reliable method of evaluating baroreflex sensitivity (BRS) is needed both for investigating the neural mechanisms which control heart rate, and for diagnosing cardiovascular and autonomic pathology. Currently used methods of measuring BRS can be classified as either perturbational or non-perturbational. The perturbational methods depend on a variety of mechanical or pharmacological manipulations which

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either directly or indirectly alter arterial pressure (AP). These methods include the neck suction collar method (Ernsting & Parry, 1957; Eckberg, Cavanaugh, Mark & Abboud, 1975), the tilt table method (Stefadouros, El Shahawy, Stefadouros & Witham, 1975), the lower body negative pressure (LBNP) method (Abboud, Eckberg, Johannsen & Mark, 1979), and the vasoactive drug method (Smyth, Sleight & Pickering, 1969). Perturbations in AP stimulate the baroreceptors in the carotid sinus and aortic arch regions of the arterial circulation, and elicit, via reflex changes in cardiac vagal efferent tone, a readily measurable change in pulse interval (PI).

Each of the perturbational methods is limited by either theoretical or practical considerations (Smyth *et al.* 1969; Doba & Reis, 1974; Ludbrook, Mancia, Ferrari & Zanchetti, 1977; Bergel, Peveler, Robinson & Sleight, 1979; Eckberg, 1980). Furthermore, by inducing changes in arterial pressure, the perturbational methods pose a potential hazard to certain patients.

The need for non-perturbational measures of BRS has prompted investigators to develop methods of analysing the spontaneous fluctuations in AP and PI normally observed in conscious animals and humans. These methods are based on the assumption that spontaneous fluctuations in AP also stimulate the baroreceptors and give rise to the observed fluctuations in PI by way of the baroreflex mechanism. One non-perturbational method is based on the identification of sequences of three or more heart beats in which the systolic pressure (SP) and PI both progressively change in the same direction (Bertinieri, DiRienzo, Cavallazzi, Ferrari, Pedotti & Mancia, 1985; Bertinieri, DiRienzo, Cavallazzi, Ferrari, Pedotti & Mancia, 1988; Parati, DiRienzo, Bertinieri, Pomidossi, Casadei, Groppelli, Pedotti, Zanchetti & Mancia, 1988). Another method is based on spectral analysis of spontaneous SP and PI variability (Robbe, Mulder, Rüddel, Langewitz, Veldman & Mulder, 1987).

In this study we describe and characterize a non-perturbational method that evaluates BRS in conscious dogs from an analysis of spontaneous beat-to-beat differences in systolic pressure (dSP) and pulse interval (dPI). A positive correlation is observed between the beat-to-beat differences, and the BRS is determined from the slope of the regression line. The correlation between dSP and dPI has been previously observed in isolated cases, and it has been assumed that this correlation is the result of a baroreflex mechanism (Karemaker & Borst, 1980; DeBoer, Karemaker & Strackee, 1985). In our experiments we test the validity of this assumption by interrupting the afferent and efferent pathways of the baroreflex. We also present a critical evaluation of the usefulness of the regression slope as an indicator of the spontaneous BRS. In our evaluation, we consider alternative explanations for the observed correlation between dSP and dPI, including mechanical coupling between heart rate and blood pressure, and variability of blood pressure and heart rate associated with respiration.

METHODS

Animal preparations

Experiments were performed on seven healthy female mongrel dogs weighing 16–24 kg. All dogs were in good general health, and were free of heartworms and intestinal parasites. The dogs were trained daily, over a two week period, to stand quietly for 30–40 min at a time. The dogs were then instrumented, using aseptic surgery, with chronic indwelling arterial and venous Silastic catheters (0·04 in i.d., 0·08 in o.d., 72 cm long, Dow-Corning Corp., Midland, MI, USA). Each dog was anaesthetized with sodium pentobarbitone (30 mg kg^{-1}, I.V.), intubated, and mechanically

ventilated. A midline abdominal incision was made to expose the blood vessels of the hindlimbs. The arterial catheter was inserted into the right internal iliac artery and advanced 12 cm into the abdominal aorta. The venous catheter was inserted into the right deep circumflex iliac vein and advanced 12 cm into the inferior vena cava. The abdominal incision was closed and the catheters were tunnelled subcutaneously to exit through the skin on the dorsum between the scapulae. The catheters were protected by wide elastic bandages and cloth body jackets worn by the dogs at all times.

Each dog was administered morphine sulphate (0·5 mg kg^{-1}) every 6 h for the first day after surgery to alleviate pain, and one million units of penicillin G every day during the first 4 days after surgery as a prophylactic measure. The catheters were flushed daily with saline solution and filled with a solution containing 1000 U ml^{-1} of heparin and 100000 U ml^{-1} of penicillin G. The skin incisions were washed daily and treated with a topical antibacterial ointment (Neosporin). The dogs were ambulatory within 12–24 h of surgery. At least 10 days were allowed for recovery after surgery before any experiments were conducted.

Instrumentation

Pulsatile arterial pressure (AP) was measured using a light-weight strain gauge pressure transducer (Statham, model P23Db), worn at heart level in a fitted canvas jacket. The electrocardiogram (ECG) was monitored using surface electrodes placed across the chest. Both signals were amplified and plotted on a Sensormedics R611 polygraph chart recorder, using universal coupling amplifiers with a bandwidth of 0–200 Hz, and displayed on a two-channel oscilloscope. The amplified signals were digitally recorded on videotape using a Pulse Code Modulator (PCM; Medical Systems Corporation, model PCM-8) coupled with a standard videocassette recorder (Panasonic, model AG-1720). The tapes were later played back and decoded through the PCM, recreating the original ECG and pulsatile AP signals. The signals were redigitized using a personal computer (IBM PC-AT) equipped with a twelve bit analog-to-digital convertor (Data Translation, model DT-2801). The analog-to-digital convertor was capable of resolving voltage changes of 4·9 mV, and arterial pressure changes of 0·06 mmHg.

Data collection

Data acquisition software was developed in our laboratory. Raw data were collected on a beat-by-beat basis using a program written in Microsoft QuickBASIC (compiled BASIC). Both the ECG and AP signals were sampled at a rate of 290 samples s^{-1} or one sample every 3·4 ms. The beginning of each heart beat was defined by the R-wave of the ECG. The rising edge of the R-wave was detected when the first derivative of the ECG signal exceeded a user-defined threshold. For each heart beat, the computer calculated PI as the time interval between consecutive R-waves. The mean arterial pressure was computed for each heart beat as the average of all arterial pressure samples (collected at a rate of 290 s) within the heart beat. Systolic pressure was computed for each heart beat as the maximum arterial pressure sample within the heart beat.

Calculation of beat-to-beat differences

The data files created by the data collection program were stored as text files and imported into a spreadsheet for statistical analysis. Beat-to-beat changes in systolic pressure and pulse interval were computed using the first difference equations:

$$dSP_n = SP_n - SP_{n-1},$$

$$dPI_n = PI_n - PI_{n-1}.$$

The beat-to-beat differences were stored together with the values of SP and PI in the spreadsheet data files in preparation for the next phase of data processing.

Elimination of artifactual heart beats

Two types of artifacts were occasionally encountered during data collection. Noise spikes in the ECG recording sometimes resulted in the erroneous detection of false R-waves, which appeared in the data files as abnormally short pulse intervals and low systolic pressures. Occasional drop-outs in the ECG signal sometimes resulted in the loss of R-waves. These missed beats appeared in the data files as abnormally long pulse intervals. Both types of artifactual heart beats were excluded from the analysis by discarding all heart beats which met the following criteria: (1) PI was less than 300 ms, or (2) dPI was greater than 1000 ms, or (3) dPI was less than −1000 ms, or (4) dSP was greater than 30 mmHg, or (5) dSP was less than −30 mmHg. The minimum pulse interval criterion

(PI < 300 ms) was not applied to data collected during ganglionic blockade, atropine sulphate infusion, and carotid sinus denervation studies since the pulse interval was already shortened due to the experimental interventions. Using the exclusion criteria previously described, fewer than 2% of the total heart beats were ever discarded from any experiment.

Calculation of baroreflex sensitivity

After removing the artifactual heart beats, linear least squares regression analysis was used to regress dPI on dSP. The slope of the regression line was used as an index of the overall spontaneous BRS during each experiment. The variability in the baroreflex sensitivity within a single trial was assessed using the standard error of the regression coefficient (Zar, 1984).

Experimental conditions

Efforts were made to control the environmental conditions during data collection. Experiments were conducted at approximately the same time each morning. The dogs were fasted for twelve hours before each experiment, but they were given free access to water. In order to minimize distractions, only one person was allowed in the room during data collection. Data were only collected after the dogs had become acclimated to the laboratory setting and the monitoring instruments. All experiments were conducted while the dogs were quietly standing. The dogs were given at least three full days of rest between changes in drug protocols in order to minimize any residual drug effects and possible interactions between drugs.

Control studies

Experiments were performed under control conditions in seven dogs, with a total of twenty-nine trials conducted. Repeat trials were performed on separate days. In each trial, 2000 heart beats were collected over approximately 30 min. Isotonic saline solution was infused (1 ml min^{-1}, I.V.) during data collection as a vehicle control for the drug infusions used in the other experiments. The spontaneous BRS and the mean values of AP, SP, and PI were calculated based on the 2000 heart beats collected from each trial. Results from repeat trials were averaged for each dog.

Ganglionic blockade studies

Experiments were conducted during ganglionic blockade in seven dogs, with a total of ten trials performed. Repeat trials were conducted on separate days. A loading dose of hexamethonium (5 mg kg^{-1}, I.V.) was administered over a ten minute period prior to the start of data collection. During data collection a maintenance infusion of hexamethonium (5 mg kg^{-1} h^{-1}, I.V.) was administered. Two thousand heart beats were collected under resting conditions, and the spontaneous BRS and mean AP, SP, and PI were calculated for each trial. Results from repeat trials were averaged for each dog.

Graded atropine infusion studies

In order to determine the effects of low doses and high doses of atropine sulphate on the dSP–dPI relationship, experiments were conducted during graded drug infusions in three dogs. The experiments consisted of a 10 min baseline without atropine sulphate, followed by four levels of progressively increasing atropine sulphate infusion, each lasting 10 min. After the baseline measurement, the infusion rate was started at 6·25 μg kg^{-1} min^{-1}, and was doubled every 10 min up to a final infusion rate of 50 μg kg^{-1} min^{-1}. The spontaneous BRS and the mean values of AP, SP, and PI were assessed during the last 2 min of each 10 min infusion period, thus ensuring that the experimental conditions (i.e. the drug levels) did not change significantly during the measurements. In order to compensate for differing baseline conditions between dogs, the values of AP, SP, PI, BRS and the correlation coefficients were expressed as a percentage of their baseline values.

Carotid sinus denervation studies

Experiments were conducted after bilateral carotid sinus denervation in two dogs. The denervation operations were performed under aseptic conditions. The dogs were anaesthetized with sodium pentobarbitone (30 mg kg^{-1}, I.V.), intubated, and mechanically ventilated. A mid-line neck incision was made and both carotid sinuses were exposed, ligated and excised. The exposed regions of the internal, external and common carotid arteries were painted with a 7% phenol solution. The external and common carotid arteries remained patent. Although the internal carotid arteries were ligated, cerebral blood flow was maintained by the vertebral arteries. After recovering from anaesthesia, the dogs did not show any outward signs of cerebral ischaemia, as evidenced by their

gait, exercise tolerance, and general alertness. Data were collected 3 days after the denervation surgery. During the experiments, the dogs stood quietly for approximately 30 min while the data were recorded. The spontaneous BRS and the mean values of AP, SP and PI were determined for each dog based on 2000 heart beats.

Random modulation of heart rate

An additional experiment was conducted in one anaesthetized, vagotomized dog, in which the heart rate was randomly modulated using vagal stimulation. The dog was anaesthetized with sodium pentobarbitone (30 mg kg^{-1}, I.V.), intubated, and mechanically ventilated (12–13 breaths min^{-1}, 20 ml kg^{-1} tidal volume, equal inspiratory and expiratory times). A mid-line neck incision was made and the right cervical vagus nerve was isolated, stripped of its fascial sheath, tied and cut. In order to block sympathetically mediated changes in heart rate, propranolol was administered using a loading dose of 2 mg kg^{-1}, I.V., followed by a constant infusion of 2 mg kg^{-1} h^{-1}, I.V. A bipolar electrode was positioned around the distal end of the cut vagus nerve. The nerve was immersed in mineral oil throughout the experiment in order to preserve the nerve and to insulate the surrounding tissue from the stimulator current. The bipolar electrode was driven by a voltage-controlled nerve stimulator built in our laboratory. The stimulator output was adjusted for a 1 ms square pulse with an amplitude of 8 V. The stimulator firing frequency was randomly modulated between 0 and 32 Hz by a control voltage generated with a personal computer (IBM PC-AT) equipped with a digital-to-analog convertor (Data Translation, DT-2801). The rate and variability of the stimulator firing pattern was adjusted to produce the same mean and variance in heart rate as that observed in the conscious, resting dog. Since the dog was tachycardic during anaesthesia, adequate heart rate modulation was possible using cardio-inhibitory vagal stimulation alone.

Comparison of spontaneous BRS with pharmacological BRS

In a separate set of experiments, the BRS was measured using both the spontaneous beat-to-beat difference method and the standard pharmacological method. A total of ten trials were performed in six conscious, resting dogs. Repeat trials were performed on separate days. A single bolus injection of either phenylephrine (5 μg kg^{-1}, I.V.) or methoxamine (100 μg kg^{-1}, I.V.) was used in each trial. The pharmacological BRS was calculated as the slope of the regression line of PI regressed on SP during the rising phase of the arterial blood pressure response (Smyth *et al.* 1969). The spontaneous BRS was measured using 200 heart beats immediately following the rising phase of the arterial pressure response to the drug injection.

Statistical analysis

One-way analysis of variance was used to compare the mean values of BRS, AP, SP, and PI in the control, ganglionic blockade, muscarinic blockade and carotid sinus denervation experiments. *Post hoc* multiple comparisons with the control values were computed using the two-tailed Dunnett test. Mean values of blood pressure and pulse interval during the random heart rate modulation were compared with those in the conscious state using Student's two-tailed t test. The variabilities of the blood pressure and pulse interval values were compared using the variance ratio f test. One-way analysis of variance was used to compare the values of BRS, AP, SP, and PI at each level of the graded atropine infusion experiments. *Post hoc* multiple comparisons with baseline measurements were computed using the two-tailed Dunnett test. Multiple comparisons between pharmacologically measured BRS and spontaneously measured BRS were computed using Student's two-tailed paired t test with a Bonferroni adjustment. Statistical significance for all tests was set at the 95% confidence level.

RESULTS

Spontaneous baroreflex sensitivity

A typical example of the spontaneous beat-to-beat relationship between dSP and dPI is shown in Fig. 1. The slope of the least squares regression line, indicative of the spontaneous BRS, was 27·8 ms $mmHg^{-1}$ ($r = 0{\cdot}7842$). Under control conditions, the spontaneous BRS was variable between dogs. Among the seven dogs tested, the mean $\pm$ S.E.M. of BRS was 32·2 $\pm$ 7·4 (range 14·4–62·4) ms $mmHg^{-1}$. The average range

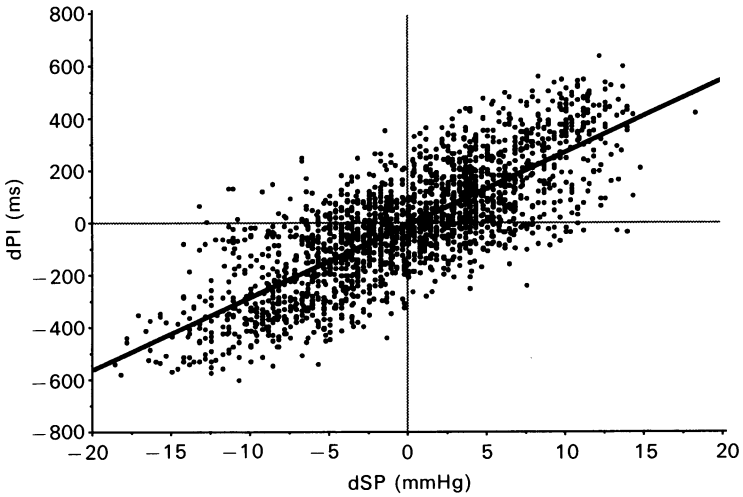

Fig. 1. A typical example of the positive correlation between beat-to-beat changes in systolic pressure (dSP) and the simultaneous beat-to-beat changes in pulse interval (dPI). The regression is based on 2000 heart beats measured in a conscious, quietly resting dog. The slope of the regression line (27·8 ms $mmHg^{-1}$, correlation coefficient = 0·7842, $P < 0·001$) indicates the spontaneous baroreflex sensitivity (BRS).

TABLE 1. Spontaneous BRS and effects of baroreflex ablation

	BRS (ms $mmHg^{-1}$)	Correlation coefficient	AP (mmHg)	SP (mmHg)	PI (ms)
Intact baroreflex ($n = 7$)	32·20 ± 7·40	0·584 ± 0·054	100·8 ± 2·4	143·6 ± 5·4	744·9 ± 56·4
Ganglionic blockade ($n = 7$)	0·03 ± 0·05*	0·119 ± 0·038*	99·6 ± 4·0	123·7 ± 4·0*	405·0 ± 31·5*
Muscarinic blockade ($n = 3$)	−0·09 ± 0·03*	0·084 ± 0·027*	114·4 ± 7·4	135·8 ± 7·9	321·6 ± 6·2*
Carotid sinus denervation ($n = 2$)	1·39 ± 1·11*	0·136 ± 0·006*	112·2 ± 0·4	154·8 ± 2·7	481·9 ± 59·6*

Values indicate mean ± S.E.M.; BRS = spontaneous baroreflex sensitivity; Correlation coefficient = Pearson correlation coefficient; AP = arterial pressure; SP = systolic pressure; PI = pulse interval; * = $P < 0·05$ *vs.* intact baroreflex.

of BRS within each dog, indicating day-to-day variability, was 23·5 ms $mmHg^{-1}$. The average S.E.M. of BRS for each dog was 5·5 ms $mmHg^{-1}$. The average S.E.M. of BRS within each 30 min experiment was 0·89 ms $mmHg^{-1}$.

Effects of blockade

The effects of ganglionic blockade, muscarinic blockade, and carotid sinus denervation on the average spontaneous BRS, correlation coefficient, AP, SP, and PI are summarized in Table 1. During ganglionic blockade, the spontaneous BRS was reduced 99·9% from control, and the correlation coefficient between dSP and dPI was reduced 80%. The average AP was unchanged, while SP was reduced 14%, and PI was reduced 46%.

The effect of muscarinic blockade was assessed following the maximal infusion rate in the graded atropine infusion experiments. The dogs received a cumulative atropine dosage of 937·5 $\mu g\ kg^{-1}$. The spontaneous BRS was abolished, and the correlation coefficient between dSP and dPI was reduced 86%, with a change in the

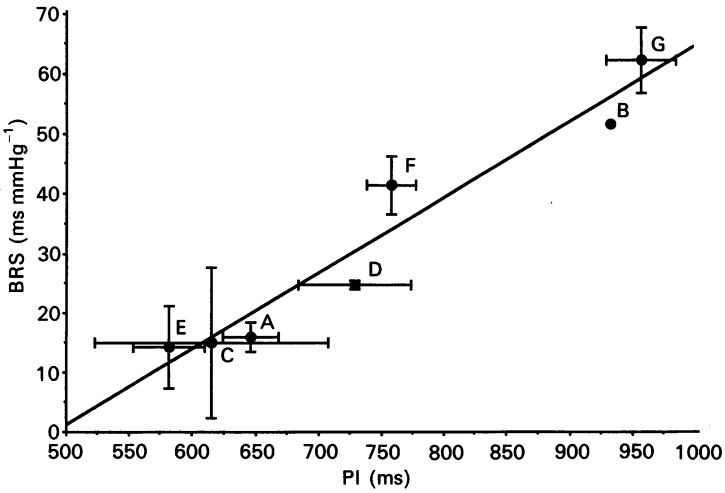

Fig. 2. Spontaneous baroreflex sensitivity (BRS) was positively correlated with the average pulse interval (PI). The data points represent the average values in each of seven conscious, quietly resting dogs. Error bars indicate the S.E.M. Labels (A–G) correspond to the same dogs in each of the remaining figures. Regression line: BRS = 0·127 × PI − 62·6, correlation coefficient = 0·970, $P < 0·001$, $n = 7$ dogs.

sign of the correlation. Both AP and SP were not significantly different from control, while PI was reduced 57%.

Following bilateral carotid sinus denervation, the spontaneous BRS was reduced 96% from control. The correlation coefficient between dSP and dPI was reduced 77%. Both AP and SP were not significantly different from control, while PI was reduced 35%.

Relationship between heart beat interval and spontaneous BRS

Figure 2 shows the scattergram of the spontaneous BRS plotted against PI. Each point in the graph represents the average of all trials, for each conscious dog, while resting under control conditions (i.e. autonomic reflexes and carotid sinuses intact). A strong positive correlation exists between BRS and PI ($r = 0·970$, $P < 0·001$, $n = 7$), described by the regression line, BRS = 0·127 × PI − 62·6. When the values from the twenty-nine individual trials were used to calculate a regression line between BRS and PI (not shown), a positive relationship having essentially the same slope and intercept was found (BRS = 0·134 × PI − 66·2, $r = 0·899$, $P < 0·001$, $n = 29$).

Random modulation of heart rate

Figure 3 shows the beat-by-beat regression of dPI on dSP during control conditions in one conscious resting dog, and during random heart rate modulation after vagotomy in the same anaesthetized dog. The correlations were significant in

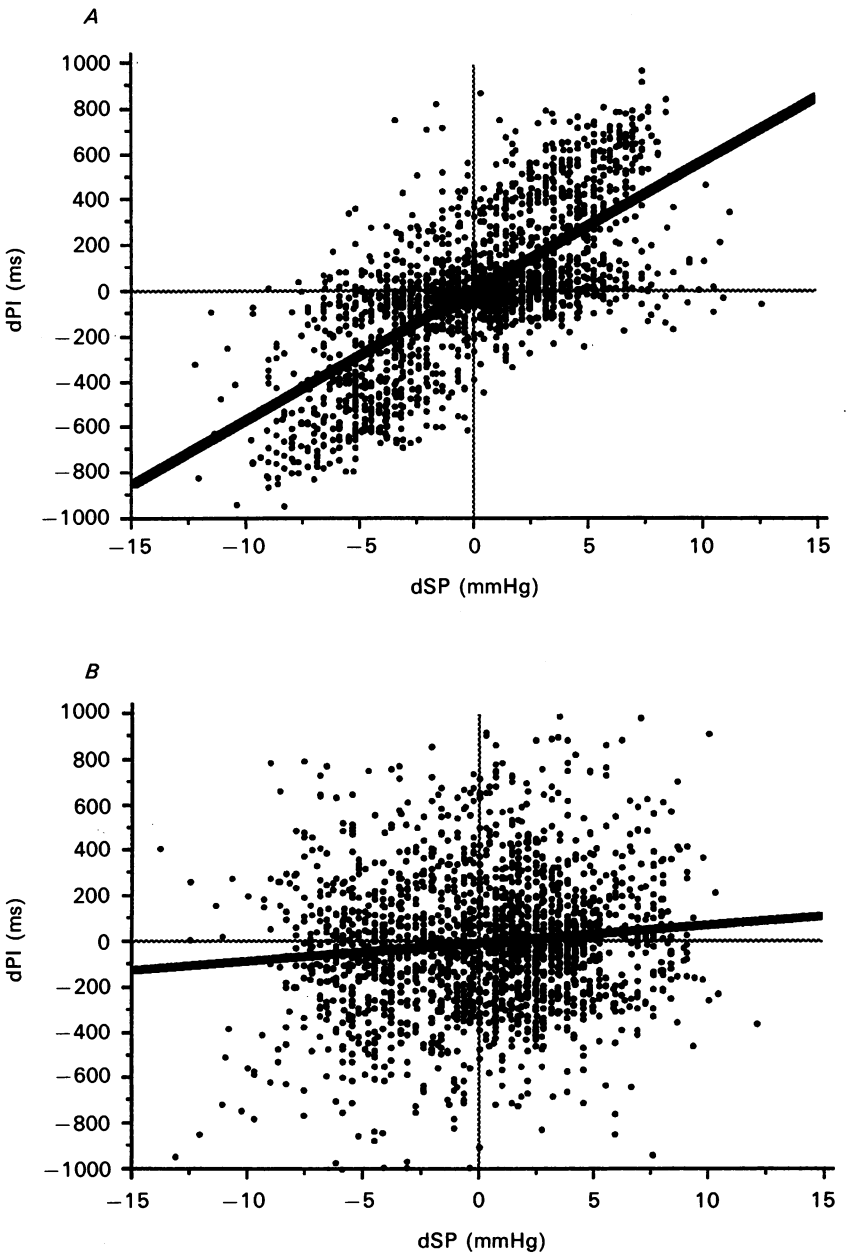

Fig. 3. *A*, spontaneous baroreflex sensitivity was measured in one conscious, quietly resting dog using a regression analysis of dPI *vs.* dSP. The slope of the regression line indicates a spontaneous BRS of 56·9 ms $mmHg^{-1}$; correlation coefficient = 0·707, $P < 0·01$, $n = 2000$ heart beats. *B*, spontaneous BRS was measured in the same anaesthetized and vagotomized dog during heart rate modulation using random vagal stimulation. Vagal stimulation parameters were adjusted to approximate the same mean and S.D. in pulse interval as was observed in the conscious animal. Regression line: slope = 7·85, correlation coefficient = 0·109, $P < 0·01$, $n = 2000$ heart beats.

both experiments, although the slope of the regression line was considerably lower during random heart rate modulation. In the conscious state, the mean ± S.D. of AP was 92·9 ± 4·5 mmHg, the mean ± S.D. of SP was 121·9 ± 4·7 mmHg, and the mean ± S.D. of PI was 870·6 ± 230·9 ms. During the random heart rate modulation the

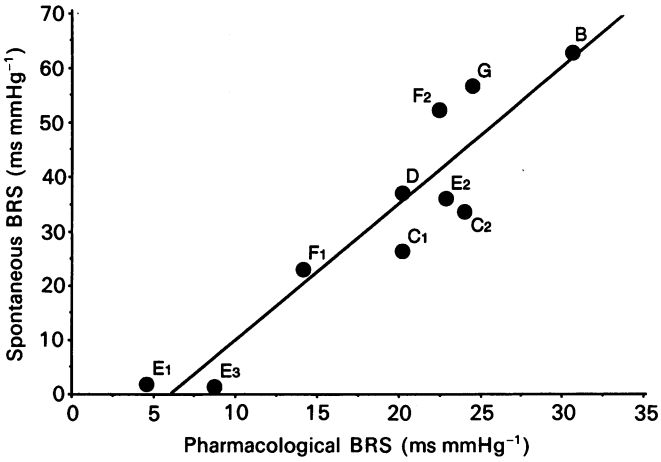

Fig. 4. Pharmacologically measured BRS was positively correlated with the spontaneously measured BRS. The pharmacological BRS was measured during the arterial pressure rise following a bolus injection of either phenylephrine or methoxamine. The spontaneous BRS was measured using 200 heart beats following the arterial pressure rise after each drug injection. Data points represent the results of ten trials in six conscious, quietly resting dogs, labelled A–G. Numbers next to each letter indicate repeat trial numbers for the same dogs. Repeat trials were conducted on separate days. Correlation coefficient = 0·930, $P < 0·001$.

mean of AP was 104·1 mmHg ($P < 0·05$ *vs.* conscious state) and the S.D. was 6·0 mmHg ($P < 0·05$ *vs.* conscious state). The mean of SP was 132·1 mmHg ($P < 0·05$), and the S.D. was 4·9 mmHg. The mean of PI was 1033·1 mmHg ($P < 0·05$ *vs.* conscious state), and the S.D. was 230·3 ms.

Comparison of spontaneous BRS with pharmacological BRS

Figure 4 shows the scattergram of the pharmacologically measured BRS plotted against the spontaneous BRS measured immediately after the drug injection. The figure summarizes the results from ten trials conducted in six dogs. The pharmacological BRS was significantly correlated with the spontaneous BRS ($r = 0·930$, $P < 0·001$, $n = 10$). The average value of the pharmacological BRS was 19·2 ± 2·5 ms mmHg^{-1}, and the average value of the spontaneous BRS was 33·1 ± 6·7 ms mmHg^{-1}. The pharmacological BRS was significantly less than the spontaneous BRS ($P < 0·01$). The average SP measured during the 200 heart beats before the drug injection was 144 mmHg, while that measured during the 200 heart beats after the drug injection was 174 mmHg. The average PI was 619 ms before the drug injection, and 834 ms after the drug injection.

Graded atropine infusion

The results of the graded atropine infusion are shown in Fig. 5. The values are expressed as a percentage of the baseline values. The average baseline of the spontaneous BRS was $27{\cdot}9 \pm 12{\cdot}3$ ms mmHg^{-1}, and the average baseline corre-

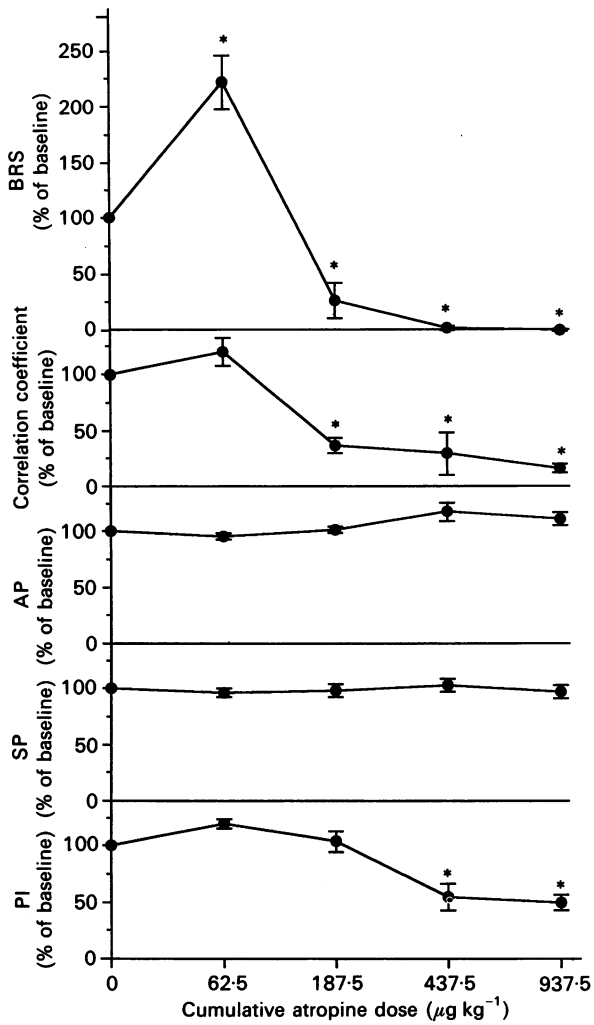

Fig. 5. Spontaneous baroreflex sensitivity was measured during a graded infusion of atropine sulphate in three dogs. Spontaneous BRS, Pearson correlation coefficient of the dPI *vs.* dSP regression, mean arterial pressure (AP), mean systolic pressure (SP), and mean pulse interval (PI) are plotted against cumulative atropine dose. Baseline measurements were taken prior to the start of atropine infusion (time = 0). The infusion rate was started at 6·25 μg kg^{-1} min^{-1}, and doubled every 10 min thereafter (12·5, 25 and 50 μg kg^{-1} min^{-1}). Data points represent measurements made during the last 2 min of each 10 min infusion. All values are expressed as a percentage of the baseline measurements. Error bars indicate the standard error of the mean. Asterisks indicate significant difference from baseline ($P < 0{\cdot}05$, two-tailed Dunnett test).

lation coefficient was 0·4850±0·1210. The average baseline AP and SP were 103·1±1·2 mmHg and 140·3±0·6 mmHg, respectively, and the average baseline PI was 674·7±83·9 ms. The wide range of spontaneous BRS and PI values is typical of the variability observed between dogs. The spontaneous BRS showed a 121% increase from baseline at the end of the first 10 min of atropine infusion (62·5 μg kg^{-1} cumulative dosage). At the end of the next 10 min infusion (187·5 μg kg^{-1} cumulative dosage), the spontaneous BRS was reduced to 26% of baseline. At the end of the third atropine infusion (437·5 μg kg^{-1} cumulative dosage) the spontaneous BRS was abolished, and it remained so through the fourth atropine infusion period (937·5 μg kg^{-1} cumulative dosage). The correlation coefficient showed a 21% increase from baseline at the end of the first 10 min atropine infusion, but the increase did not reach statistical significance. During the following three atropine infusions, the correlation coefficient was reduced, reaching a final value of 17% of baseline. The pulse interval also showed a 20% increase from baseline at the end of the first atropine infusion, but the increase was not significant. PI remained unchanged through the second atropine infusion, but was reduced to approximately 50% of baseline at the end of the third and fourth infusion periods. Both AP and SP were unchanged from baseline during the entire graded atropine infusion.

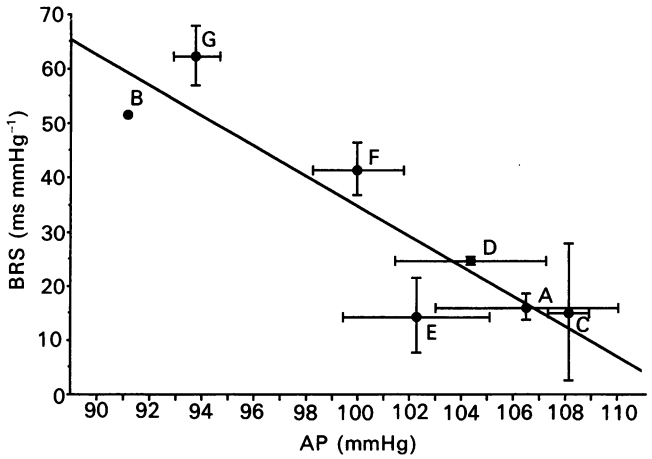

Fig. 6. Spontaneous baroreflex sensitivity (BRS) was negatively correlated with mean arterial pressure (AP). The data points represent the average values in each of the seven conscious, quietly resting dogs. Error bars indicate the S.E.M. Regression line: BRS = $-2·784 \times AP + 313·1$, correlation coefficient = 0·905, $P < 0·001$, $n = 7$ dogs.

Relationship between mean arterial pressure and spontaneous BRS

Figure 6 shows the regression between the spontaneous BRS and the mean AP for each of the seven dogs tested under control resting conditions. A significant negative correlation was observed between BRS and AP. When the regression was calculated using the twenty-nine individual control trials, the correlation was no longer

statistically significant ($P = 0{\cdot}057$), but the disruption to the correlation was due to a single outlier corresponding to the very first trial conducted. Exclusion of the first trial from the regression resulted in a significant correlation (BRS = $-1{\cdot}505 \times \mathrm{AP} + 185{\cdot}1$, $r = 0{\cdot}520$, $P < 0{\cdot}005$, $n = 28$).

DISCUSSION

It was hypothesized that the consistent positive correlation between spontaneous beat-to-beat changes in SP and the corresponding changes in PI demonstrated a continuously active baroreflex responding to small and apparently random changes in blood pressure. In support of this hypothesis, conditions which abolished or attenuated the afferent and efferent pathways of the baroreflex likewise abolished or attenuated the spontaneous BRS. The abolition of the spontaneous BRS by ganglionic blockade demonstrated that the spontaneous BRS required the presence of the autonomic nervous system. The graded attenuation and eventual abolition of the spontaneous BRS by atropine was consistent with the peripheral muscarinic blockade of the vagal parasympathetic efferent pathway at the sino-atrial node. The abolition of the spontaneous BRS following bilateral carotid sinus denervation demonstrated the necessity of an intact carotid sinus for the maintenance of the spontaneously observed relationship between dPI and dSP.

The ganglionic blockade, muscarinic blockade, and carotid sinus denervation experiments were limited in two respects. The first limitation was that the experiments were confounded by elevated heart rates. One could not conclude that the correlation between dSP and dPI is of baroreflex origin, because the elevated heart rates observed during these experiments were associated with low spontaneous BRS values, as shown in Fig. 2. Thus, an additional ablation experiment was necessary in which heart rate was comparable to that of a conscious resting dog with intact baroreflexes.

The second limitation of the previous ablation experiments was the assumption of causality. The hypothesis that the correlation between dSP and dPI represented the activity of the baroreflex assumed that spontaneous changes in SP caused (via stimulation of the baroreceptors) the observed changes in PI. An alternative hypothesis could assume the reverse causal relationship. The spontaneous changes in PI could have caused the observed changes in SP by purely mechanical means. This mechanical coupling hypothesis was partially validated using computer simulations we developed which were based on a windkessel model (i.e., the aorta represented by an elastic chamber and the peripheral vessels by a rigid tube) of the arterial vasculature, fixed values for the peripheral resistance and pulse pressure, and either random or previously recorded values of PI. The simulations revealed significant correlations between dPI and dSP, although the correlations were not as strong as those observed in conscious dogs. The slope of the regression (analogous to the BRS) was positively correlated with the average PI, similar to the relationship shown in Fig. 2. Although the underlying assumptions of the computer simulations may have been inaccurate, the finding that a simplistic vascular model could partially explain the observed correlation between dPI and dSP made further investigation into the vascular coupling hypothesis necessary.

The random heart rate modulation experiment was conducted in order to address

the two limitations previously described. A weak but significant positive correlation between dPI and dSP remained during random vagal stimulation in an anaesthetized, vagotomized dog, although the slope of the regression line was greatly reduced. The results demonstrated that spontaneous BRS could be attenuated while heart rate was comparable to that of a conscious resting dog. The results further showed that although vascular coupling may have accounted for part of the correlation between dPI and dSP, it was inadequate in explaining the large spontaneous BRS measured in conscious resting dogs.

An alternative explanation of the correlation between dSP and dPI is the simultaneous effect of breathing on heart rate and systemic arterial pressure. Respiratory sinus arrhythmia (RSA) has been used as an index of parasympathetic cardiac control (Eckberg, 1983). It is abolished or significantly reduced by atropine or vagotomy (Anrep, Pascual & Rössler, 1936; Levy, DeGeest & Zieske, 1966; McCrady, Vallbona & Hoff, 1966), and it is linearly correlated with vagal parasympathetic tone (Katona & Jih, 1975). Slow spontaneous breathing is also associated with changes in intrathoracic pressure, venous return to the heart, and systemic arterial pressure (Freyschuss & Melcher, 1976). However, this explanation is unlikely since inspiration is associated with an increase in heart rate and arterial pressure, and expiration is associated with a decrease in heart rate and arterial pressure (Freyschuss & Melcher, 1976). Therefore, a negative correlation between dSP and dPI is predicted, while the observed correlation was consistently positive.

In order to provide a basis for comparing the results of the present study with previous work, the beat-to-beat difference method was compared with the standard pharmacological method. The spontaneous and pharmacological measures of BRS were positively correlated, although the magnitude of the spontaneous BRS was nearly twice as large. The pharmacological BRS was based on the absolute values of SP and PI, while the spontaneous BRS was based on the beat-to-beat changes in the variables. The beat-to-beat difference transformation had the effect of high-pass filtering the SP and PI time series; steady-state and slowly varying baroreflex responses were attenuated. Thus, the spontaneous BRS reflected only the rapid dynamic component of the baroreflex response. Furthermore, the pharmacological BRS was measured during a transitory state, the rise in arterial pressure following a bolus injection of a vasoconstrictor. The bradycardia which accompanied the rise in arterial pressure was promptly followed by a partial return of heart rate toward the pre-injection baseline, consistent with rapid baroreflex resetting. According to our data, if BRS was calculated using steady-state values of SP and PI measured before and after the vasoconstrictor injections, then steady-state BRS would have been approximately half of the pharmacological BRS measured during the transient rise in pressure. Thus, the baroreflex sensitivity was largely dependent on the rate of change of arterial pressure, rather than the absolute magnitude of the change. Baroreceptor rate sensitivity has also been demonstrated in isolated carotid sinus preparations (Spickler & Kezdi, 1967). Since the baroreflex response depends upon transient changes in arterial pressure, and the baroreflex acts to rapidly buffer against these pressure changes, the beat-to-beat difference method is appropriate for quantifying the dynamic baroreflex.

Several interesting findings were also noted in the analysis of the control and graded atropine infusion experiments. First, spontaneous BRS was negatively

correlated with the mean AP, in agreement with previous studies in normotensive humans (Smyth *et al.* 1969; Parati *et al.* 1988). The results were also consistent with previous studies which found a decreased BRS in hypertensive humans (Bristow, Honour, Pickering, Sleight & Smyth, 1969; Gribbin, Pickering, Sleight & Peto, 1971).

Second, our study confirmed the strong positive correlation between the spontaneous BRS and PI demonstrated elsewhere (Smyth *et al.* 1969; Bertinieri *et al.* 1988; Parati *et al.* 1988). A possible explanation for this correlation is that both the resting heart rate and the baroreflex modulation of heart rate are largely determined by the level of vagal parasympathetic activity in the conscious dog. Shortening of the pulse interval is usually the result of a decrease in vagal parasympathetic activity. When vagal activity is diminished, the dynamic range over which the baroreflex can modulate the heart rate is also diminished, thus decreasing the measured BRS. This explanation assumes that baroreflex-mediated increases in vagal parasympathetic activity are ultimately limited by other neural pathways which regulate the maximal vagal activity.

Third, in the graded atropine infusion experiments, there was a paradoxical increase in spontaneous BRS after a low dose of atropine. This increase was consistent with other studies which demonstrated increased cardiac vagal efferent activity following low dose atropine, presumably by a central stimulatory mechanism (Katona, Lipson & Dauchot, 1977; Raczkowska, Eckberg & Ebert, 1983; Eckberg, Mohanty & Raczkowska, 1984). A similar parasympathomimetic effect has been described following low-dose scopolamine (Dibner-Dunlap, Eckberg, Magid & Cintrón-Treviño, 1985).

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