

Characterization of baroreflex gain in the domestic pigeon (*Columba livia*)

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Abstract

Birds have a remarkable capacity to regulate circulation yet little is known about the avian baroreflex. Although both linear regression and curve-fitting techniques are frequently used to assess baroreflex function in mammals, only the former technique has been used in birds. We characterized baroreflex gain in domestic pigeons (*Columba livia*) and compared gain values derived from applying linear regression to ramp changes in mean arterial pressure (MAP) to values derived from fitting a four-parameter sigmoidal function to steady-state alterations in MAP. We found that, unlike mammals, pigeons do not display circadian patterns in MAP, HR or gain derived from bolus injections of vasoactive drugs. The pressor, but not depressor response, was attenuated by administration of the NMDA-antagonist ketamine, suggesting that central processing of the baroreflex may be similar in birds and mammals despite anatomical differences in arterial baroreceptive zones. Because graded infusions of vasoactive drugs could not consistently produce a plateau in the HR response, fitting data to a sigmoidal curve was difficult. Thus, we propose that variations of the Oxford method and linear regression analysis are superior method to assess baroreflex gain in pigeons than curve fitting.

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1. Introduction

Birds evolved many unique physiological adaptations to meet the demanding physical performance required of them. Activities associated with metabolically costly behaviors such as flying require a high level of performance from the cardiovascular system. Adequate tissue perfusion depends on the maintenance of arterial pressure within fairly narrow limits. For example, during flight, the heart rate (HR) of pigeons can increase fourfold, and the cardiac output can increase sevenfold, yet the mean arterial blood pressure (MAP) is maintained close to pre-flight rest levels (Butler et al., 1977). In diving birds, the HR can decrease by as much as 90% and is accompanied by a decrease in cardiac output (CO), yet the MAP remains within normal physiological limits (Lillo and Jones, 1982; Smith and Jones, 1990). Thus, birds appear to have

evolved considerable cardiovascular control to support their activity, especially birds that are strong fliers and divers.

Similar to mammals, birds have a well-developed baroreflex (Taha et al., 1983; Bagshaw, 1985; Bagshaw and Cox, 1986; Taha, 1987). Baroreceptors are specialized nerve endings embedded in arterial walls that discharge impulses centrally when mechanically stimulated. The efferent limb modulates HR and arterial tone in order to maintain arterial pressure within a specific range. In mammals, the walls of the right and left carotid sinuses, bilaterally innervated by branches of the glossopharyngeal nerves, and the walls of the aortic arch, innervated by branches of the vagal nerves, have by far the greatest density of baroreceptors. Almost all baroreflex control is thought to originate from these two regions, and the majority of this control is linked to the carotid sinus. Birds do not have a carotid sinus similar in structure and placement to that in mammals (Muratori, 1934; Nonidez, 1935). Although histological findings suggest ultrastructural evidence for baroreceptors in the carotid arteries (Abdel-Magied et al., 1982), it is believed that avian baroreflex control of the cardiovascular system stems almost exclusively from the ascending aorta.

Baroreceptor gain is defined as the change in HR for a given change in blood pressure. In birds and mammals, gain has been

Abbreviations: HR, heart rate; MAP, mean arterial pressure; PE, phenylephrine; NP, sodium nitroprusside; NMDA, *N*-methyl-D-aspartate.

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assessed for decades using a technique referred to as the Oxford method (Smyth et al., 1969; Sagawa, 1978; Smith and Jones, 1990, 1992; Carretta et al., 1996; Fazio et al., 2001; Lipman et al., 2003). The Oxford method uses changes in MAP caused by bolus injection of a vasoactive drug and the resulting change in arterial pressure is plotted against concomitant changes in HR; the slope of this relationship is defined as the baroreflex “gain” or “sensitivity”. Using variations of this method, gain has been evaluated in adult ducks (Millard, 1980; Smith and Jones, 1990, 1992) and embryonic chickens (Altimiras and Crossley, 2000). Frequently, mammalian baroreflex gain is determined using steady-state changes in HR and MAP induced via continuous infusion of a vasoactive drug that are fit to a four-parameter sigmoidal function originally described by Kent et al. (1972) (Reid and Chou, 1990; Wong et al., 1993; Segar et al., 2002; Gardner et al., 2004; Thomas and Woods, 2004). This technique is preferred by some researchers over the Oxford method because it accounts for the physiological limits of HR at the limits of physiological arterial pressure ranges. This technique has not been applied to birds.

Another important consideration in assessing baroreflex gain is the dependence of gain on circadian patterns. Many studies show that in mammals, baroreflex gain negatively correlates to circadian periods of activity (Smyth et al., 1969; Hossmann et al., 1980; Su et al., 1987b; Takakuwa et al., 2001; Sei et al., 2002; Klawe et al., 2004). Although no study has examined circadian patterns of circulation control in birds, arterial pressure is significantly lower in free-ranging turkeys at night although HR remained similar during both night and day (Krista et al., 1981). This suggests that baroreflex in birds, like mammals, may have a circadian component.

The purpose of this study is to define baroreflex gain in the adult pigeon (*Columba livia*). We chose this species because they are bred to be strong fliers, as opposed to domestic fowl, and have been shown to have a remarkable physiological HR range during exercise. Baroreflex gain was determined by bolus injections of vasoactive substances (Oxford method) and with continuous infusion of the same drugs. In order to identify any circadian component to baroreflex function, mean arterial pressure, heart rate and baroreflex gain were determined at five time points per day. Gain values using the continuous infusion method were also compared in anesthetized and conscious animals to determine the effects of anesthesia on baroreflex function. Lastly, we determined whether baroreflex gain was more accurately described using the Oxford method or a curve-fitting method. Characterizing baroreflex control of cardiovascular function in strong fliers, such as pigeons, can provide insight into adaptive cardiovascular mechanisms evolved by animals that experience extreme ranges in cardiovascular performance on a daily basis.

2. Materials and methods

Domestic pigeons (*C. livia*) of approximately 7 to 12 months of age (216 g–345 g) were purchased from a local breeder between 4 and 15 days prior to experimentation. Birds were housed indoors in a temperature-controlled room (20 °C) within

the CSUEB animal facility in individual cages, provided food and water ad libitum, and exposed to a 12:12 h light/dark cycle (light phase 0800 to 2000 h). To minimize the effects of housing social birds individually, the cages were positioned so that the birds had visual contact with each other. Surgical procedures and initial recovery occurred in a separate room and birds were returned to the same location within the animal facility for extended recovery and physiological measurements. All protocols and procedures were approved by the CSUEB Institutional Animal Care and Use Committee.

2.1. Surgical procedures

Each bird was anesthetized with an intramuscular injection of ketamine HCl (Ketaset, Wyeth, Madison, NJ, USA) (35 mg/kg) and xylazine (Rumpun, Bayer Corporation, West Haven, CT, USA) (6 mg/kg). After a surgical plane was reached, the ventral aspect of the right humerus was plucked and prepared. An incision was made along the length of the humerus, and the superficial ulnar artery and the basilic vein were isolated. The artery was cannulated with 2% TDMAC (Polysciences, Inc., Warrington, PA, USA)-coated polyethylene tubing (PE-50) that was stretched as necessary to fit the vessel and was filled with heparinized saline (100–150 U/ml). The cannula was advanced until the tip was within the brachiocephalic trunk. The vein was similarly cannulated. Each cannula was sutured into place with 4-0 silk and the incision was closed. The incision area was covered with Tegaderm™ (3M, St. Paul, MN, USA) and the wing was secured in a folded position using stretch cotton gauze and a veterinary stretch bandage. Excess tubing was secured to the wing wrap with tape. All birds were given oral antibiotics (Clavamox Drops, SmithKline Beecham, Westchester, PA, USA) at a dose of 35 mg/kg BID and the cannulas were flushed with heparinized saline at least once daily.

2.2. Experimental protocols

2.2.1. Protocol 1: influence of circadian rhythm on heart rate, blood pressure and baroreflex gain

2.2.1.1. Protocol 1a. A total of 7 pigeons were used for this experiment. Approximately 36 h after surgery, the arterial cannula was led out of the cage and attached to a calibrated fluid-filled pressure transducer mounted on a ring stand at the rear of the cage. The top of the cage was then covered with paper towels in order to allow light penetration but obscure visualization. The signal was continuously monitored with MacLab 4e (AD instruments, Mulford, MA, USA). Except for daily maintenance, the birds had no physical or visual contact with researchers for the next 24 h and pressure measurements were accomplished by prompting the software to record. 10-min BP recordings were taken at 0400, 0900, 1400, 1900, and 2300 h. HR measurements were taken from the interval between systolic points.

2.2.1.2. Protocol 1b. Following Protocol 1a, baroreflex gain was determined during the subsequent 24 h on each animal. For

this protocol, in addition to the arterial cannula the venous cannula was also led out of the cage. During this period, each bird was given an intravenous injection of a vasoactive drug at 0400, 0900, 1400, 1900, and 2300 h. Phenylephrine (PE) (L-phenylephrine HCl, Sigma Chemical, St. Louis, MO, USA), an α_1 adrenergic agonist, was given at a bolus dose of 75 $\mu\text{g}/\text{kg}$ followed by 0.18 ml of lactated Ringers solution (LRS). Sodium nitroprusside (NP) (Sigma Chemical, St. Louis, MO), a nitric oxide donor, was injected at a dose of 100 $\mu\text{g}/\text{kg}$ followed by 0.18 ml LRS. These drugs are frequently used to elicit baroreflex responses and doses were determined in preliminary experiments and elicited reliable but submaximal responses. It is worth noting that birds have a higher metabolic rate and clear pharmacological substances faster than mammals. Thus, they usually require substantially higher concentrations of pharmacological and therapeutic substances in order to achieve a similar effect (see Ritchie et al., 1994). Injection volumes were large enough to clear the cannula of drug but too small to impact MAP or HR. Drugs were administered in random order and approximately 15 min apart from each other.

2.2.2. Protocol 2: measurement of baroreflex gain in ketamine-anesthetized and conscious birds

A total of 11 pigeons were used for these experiments. Surgery was performed as described in Protocol 1 and the birds were prevented from becoming conscious with supplemental doses (approximately half of initial dose administered as needed) of ketamine. Birds were rolled slightly on their right side prior to experimentation. The ribcage must expand in a ventral direction during respiration and returning the bird to a prone position can compromise respiration. Since birds of short stature, such as pigeons, maintain a relatively horizontal body position and do not experience significant orthostatic stress, we did not anticipate an effect of body position on baroreflex function. Bolus injections of PE (75 $\mu\text{g}/\text{kg}$) and NP (100 $\mu\text{g}/\text{kg}$) were given in random order approximately 10 to 15 min apart from each other (four to five injections total) and changes in BP and HR were recorded. Following injections of PE and NP, the birds were allowed to recover from the anesthetic. Approximately 24 h after recovery, PE and NP were administered as bolus injections to determine baroreflex gain during the awake state.

2.2.3. Protocol 3: measurement of baroreflex gain using HR values collected during steady state changes in MAP

The aim of this experiment was to determine how gain values derived from steady state changes in MAP compared to gain values derived from plotting instantaneous HR against changing MAP values. A total of 5 birds completed this protocol. Surgery was performed as described and the birds were allowed to recover for approximately 40 h. The experimental protocol, including infusion intervals and dose–response ranges, was based on commonly used mammalian protocols (Meyrelles et al., 1996; Cardoso et al., 2005). An infusion pump was used to deliver 5 graded doses of PE (5–35 $\mu\text{g}/\text{kg}/\text{min}$) and NP (30–100 $\mu\text{g}/\text{kg}/\text{min}$) in LRS at a rate of 0.25 ml/

min. Preliminary studies showed that these ranges elicited minimal and maximal responses (data not shown). Graded doses for each drug were given in ascending order, and injection series between the two drugs were spaced 5 to 12 h apart. The total infused volume did not exceed 1% of body weight for each bird. The order of drug administered was random and, with the exception of two birds, each drug was administered in two series of infusions. A control infusion of LRS was administered prior to drug infusion in each bird and no significant change in arterial pressure was observed.

2.3. Data analysis

MAP and HR were determined post-hoc with MacLab software. Baroreflex analyses were performed with Microsoft Excel, SPSS (Jandel Scientific, IL, USA) and SigmaStat (Jandel). Instantaneous HR was determined by multiplying the inverse of the systolic period by 60 and instantaneous MAP was determined using the formula [(pulse pressure/3)+diastolic pressure]. All values are reported as means \pm S.E.M. and statistical significance was determined at a level of 5% alpha error between groups for a single measure ($p < 0.05$).

Repeated measure analysis of variance (ANOVA) was used to determine the dependence of HR and MAP on time of day. To determine baroreflex gain using the Oxford method, the increase or decrease from resting MAP to maximal change (referred to as the ramp) after drug administration was identified in each bird (Fig. 1). Mean arterial pressure was determined for each beat and plotted against instantaneous HR and analyzed by linear regression (HR as the dependent variable) using the least squares method. The slope of the regression was used as an index of baroreflex gain. Comparison of means was done via paired *t*-tests. Paired *t*-tests were also used to compare baroreceptor gain values for birds in which gain was determined during anesthesia and full consciousness.

For baroreceptor gain determined by continuous infusion of graded doses of PE or NP, steady state arterial pressure was reached within 3–5 min of infusion (Fig. 2). Approximately 15

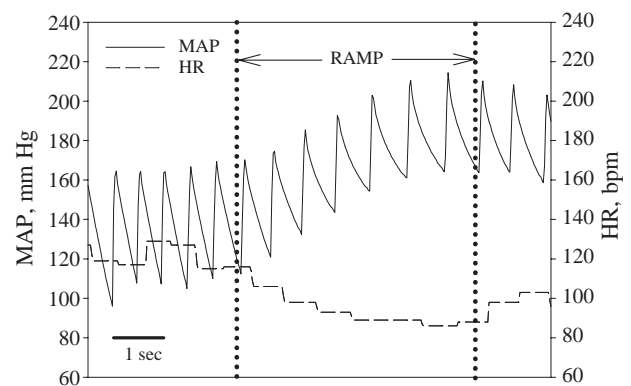


Fig. 1. Determining baroreflex gain using a variation of the Oxford method. After injection of a vasoactive drug (phenylephrine (PE) is shown), arterial pressure and heart rate (HR) change in opposite directions. During the period of arterial pressure change, the mean arterial pressure (MAP) for each heartbeat was calculated and plotted against the instantaneous HR. The slope of this line is considered the index of baroreflex gain.

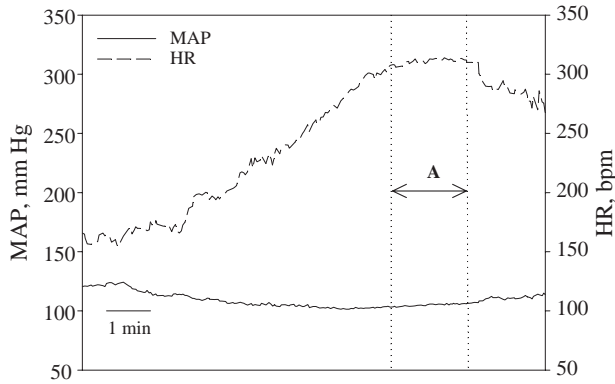


Fig. 2. Determining baroreflex gain from drug infusions. After infusion of a vasoactive drug (sodium nitroprusside (NP) is shown), mean arterial pressure (MAP) and heart rate (HR) change in opposite directions until they reach a plateau value (A). The average HR value at this plateau was determined and plotted against the average MAP. Five graded infusions of vasoactive drugs produced plateau values for MAP and HR within 3 to 5 min. Plateau values of HR were plotted against MAP and gain was determined by fitting the data to a four-parameter sigmoidal function (see text).

heartbeats during the plateau period were averaged to yield one MAP and HR value at steady state for each drug dose. The data were fitted to a four-parameter logistical function (Kent et al., 1972) as:

$$Y = b_4 + b_1 / \{1 + \exp[b_2 * (MAP - b_3)]\} \quad (1)$$

where Y equals predicted HR, b_1 is the range of HR, b_2 is the slope of the curve, b_3 is the mean arterial pressure (MAP) value when HR is at the mid point of its range and b_4 is the minimum HR. The slope of the curve (b_2) is considered as the index of baroreflex gain. In order to obtain gain values that can be compared to those derived from the Oxford method, a linear regression was used to determine the slope of the line for data points representing the steepest part of the fitted curve. In addition, the first derivative of the predicted slope was used to determine the maximum slope of the curve predicted by the model.

3. Results

3.1. Circadian influence on resting HR and MAP (Protocol 1a)

The mean HR for each time period is shown in Fig. 3 ($n=7$). The overall mean HR was 115.7 ± 3.7 bpm and did not vary with time of day ($p=0.36$). However, a significant difference in HR values was observed between birds ($p<0.01$). The overall mean MAP was 115.1 ± 2.5 mm Hg (Fig. 3) and also was not dependent on the time of day ($p=0.42$). The MAP was also dependent on the individual ($p<0.01$).

3.2. Circadian influence on resting baroreflex gain (Protocol 1b)

Data from PE and NP injections at each hour were combined and a linear regression yielded an overall gain for that

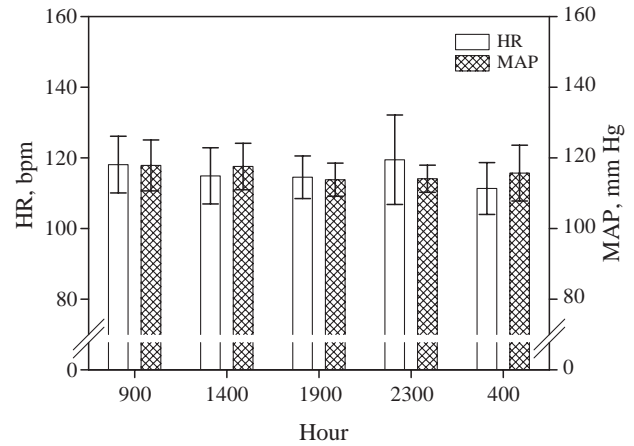


Fig. 3. Circadian effects on heart rate and mean arterial pressure in pigeons. No circadian pattern was observed for either HR ($p=0.36$) or MAP ($p=0.42$). Column heights represent means \pm S.E.M. Time period is given in military time.

hour (Fig. 4). PE-derived gain, NP-derived gain and combined (COM) gain (Fig. 5) were similar at all time periods ($p=0.28$, $p=0.32$, $p=0.10$, respectively). Gain was then grouped into “day” (0900 and 1400 h) and “night” (2300 and 0400 h) (data not shown). There was no difference between “day” or “night” PE-derived slopes ($p=0.21$), NP slopes ($p=0.58$) or for the overall gain ($p=0.46$) (data not shown). Gain for each time period was then averaged into one value per bird. Mean gain from PE injections was -1.7 ± 0.17 bpm/mm Hg and was significantly lower ($p<0.005$) than the mean gain from all NP injections (-3.0 ± 0.22 bpm/mm Hg). When all PE and NP data were combined for each bird, the overall mean gain was -2.8 ± 0.21 bpm/mm Hg. Regardless of the drug used to obtain a slope, the gain was dependent on the bird for PE injections ($p=0.01$), NP injections ($p=0.03$) and the

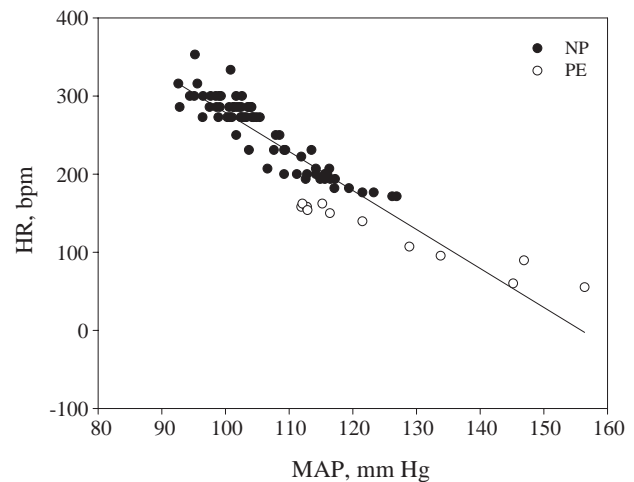


Fig. 4. Representative linear regression analysis for data from PE and NP injections. Injection of vasoactive PE (○) or NP (●) elicited reflex alterations in HR. Regression analysis was performed on responses for each drug separately and combined (shown). Note that the increased HR associated with NP-derived hypotension increases the number of data points contributing to the regression analysis.

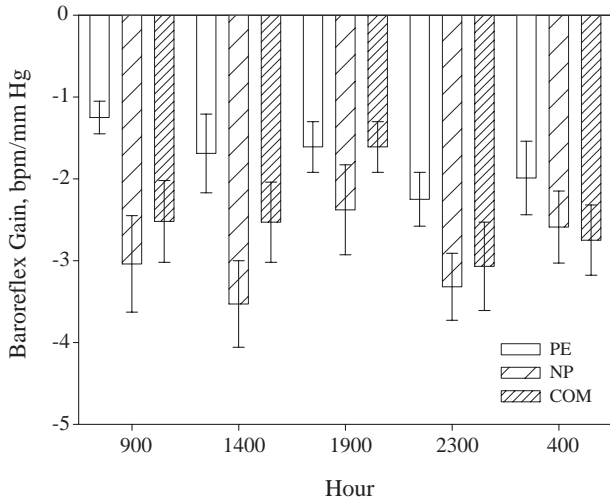


Fig. 5. PE-induced, NP-induced and combined baroreflex gain at five time periods. Repeated measures ANOVA showed no dependence of PE-induced ($p=0.28$), NP-induced ($p=0.32$) or PE and NP combined (COM) data ($p=0.1$) gain on time period. However, paired t -test comparing PE and NP gain at each time period and each pigeon revealed that the gain depended upon the drug ($p<0.005$). Column heights represent means \pm S.E.M.

overall gain per time period ($p=0.02$). There was no statistical interaction between the bird and drug ($p=0.56$).

3.3. Influence of ketamine on baroreflex gain (Protocol 2)

Baroreflex gain was determined in birds maintained under ketamine-induced anesthesia and was compared by drug ($n=7$) and state of consciousness ($n=4$) (Fig. 6). Linear regression on the HR–BP response was determined separately for each drug.

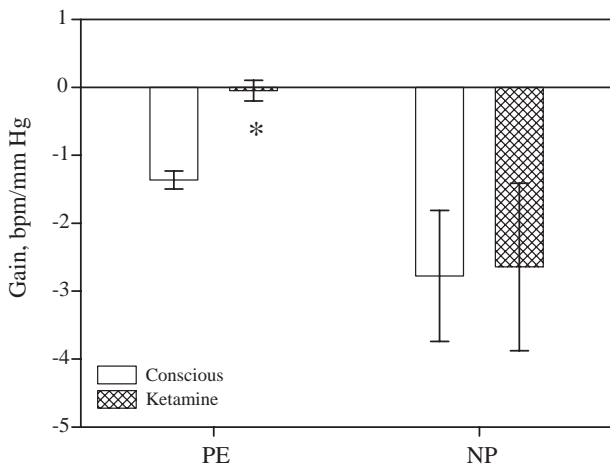


Fig. 6. Comparison of baroreflex gain during ketamine anesthesia and consciousness. Baroreflex gain derived from PE injection was not different from 0, indicating suppression of the baroreflex response during vasoconstriction. After recovery, PE-induced vasoconstriction yielded a gain parameter that was different from gain observed under anesthesia for each bird ($p=0.01$) and as a group ($p<0.01$). When grouped, PE-derived gain was similar to gain obtained during circadian experiments. Gain derived from NP injection was similar during the anesthetized and recovered state ($p=0.21$) and was similar to NP-derived gain observed in circadian experiments ($p=0.59$).

Where more than one drug was given to a bird, the results were combined to yield one value per drug category per bird. The mean slope derived from PE injections in anesthetized birds was -0.05 ± 0.15 bpm/mm Hg and was lower than the mean slope obtained in Protocol 2 ($p<0.01$). For each bird, gain was less negative during anesthesia (-0.07 ± 0.53 bpm/mm Hg) than during a conscious state (-1.37 ± 0.27 bpm/mm Hg) ($p=0.01$). The mean gain for all birds receiving NP injections under anesthesia was -2.64 ± 1.2 bpm/mm Hg and was similar to values obtained in Protocol 1b ($p=0.20$). No differences in NP-derived gain values under anesthesia and subsequent consciousness were observed ($p=0.59$).

3.4. Determination of baroreflex gain using a four-parameter sigmoidal function (Protocol 3)

Resting measurements were taken either before any drug infusion and at least 12 h post infusion ($n=5$ completed protocols). During infusion, approximately 15 consecutive HR and BP pulses were averaged for each bird at approximately 3 min and 5 min to determine if they were influenced by infusion time. No significant difference was noted and regression results did not differ. Data for each parameter at approximately 5 min are presented for each bird (Table 1).

In contrast to our observations after bolus injection, sustained infusion of either drug resulted in a substantial increased in HR (Fig. 7). High doses of NP increased HR by 221% (268 bpm) over the mean resting HR (121 bpm) and averaged NP infusions increased HR by 173%. Reducing MAP could not produce a plateau in the HR response in all birds (Fig. 8) and complicated curve fitting. PE infusion increased HR to a peak value of 176 (146% over resting HR). Doses of infused PE elicited syncope in some birds, at which case the infusion was halted and the experiment ended. Thus, infusion caused a significant upward shift of the HR range for a given MAP value, although the absolute range was similar for infusion and bolus drug injection (approximately 100 bpm). MAP responses to maximum bolus and infusion doses were similar (Fig. 9). The average value for the model parameter b_3 (MAP at the midpoint of the HR range) was 103 mm Hg, which is 15% below the average resting MAP of 121 mm Hg, indicating that the curve shifted

Table 1
Mean and individual sigmoidal function parameters

Bird	b_1	b_2	b_3	b_4
1	149	0.6	83	134
2	23	0.3	98	159
3	102	3.6	124	143
4	150	0.4	125	159
5	78	9.7	83	189
Mean	100 ± 23.8	2.9 ± 1.8	103 ± 9.4	157 ± 9.4

Data are presented for each bird at after approximately 5 min of vasoactive drug infusion. The parameter b_1 is the range of HR, b_2 is the slope of the curve, b_3 is the MAP value when HR is at the mid point of its range and b_4 is the minimum HR. The slope of the curve (b_2) is considered as the index of baroreflex gain. Means are shown as \pm S.E.M.

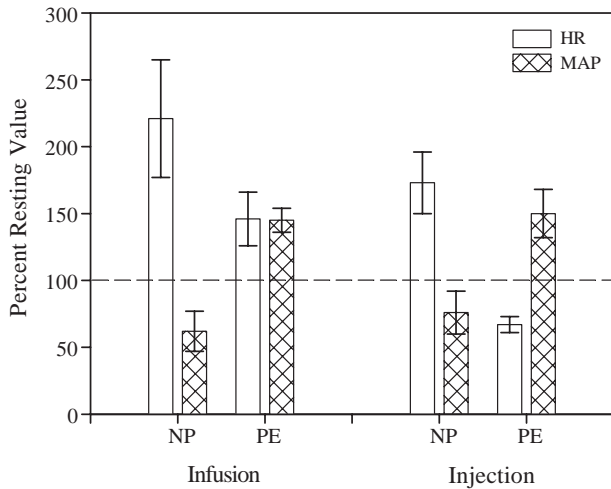


Fig. 7. Comparison of HR and MAP ranges following drug infusion and bolus injection. HR and MAP responses to maximum PE and NP doses for injection and infusion trials were averaged and compared to pre-treatment resting values. HR response range was smaller in infused birds but shifted dramatically upward. MAP ranges were similar between treatments but shifted slightly downward in infused birds.

not only upward but slightly to the left without a change in the overall curve shape.

NP-derived gain during infusion (-2.7 ± 0.8 bpm/mm Hg) was similar to NP-derived gain calculated from bolus injections in Protocol 1 (-3.0 ± 0.3 bpm/mm Hg) ($p=0.76$) (Fig. 7). In contrast, the mean PE derived gain was much higher in the infusion experiments (0.2 ± 0.3 bpm/mm Hg) than for bolus injections (-1.7 ± 0.3 bpm/mm Hg) ($p<0.01$). The mean of the combined slope from the infusion experiments was -1.8 ± 0.4 bpm/mm Hg and was similar to the combined slope mean from Protocol 1b (-2.5 ± 0.3 bpm/mm Hg ($p=0.15$)), suggesting that, despite the shifts in HR and MAP,

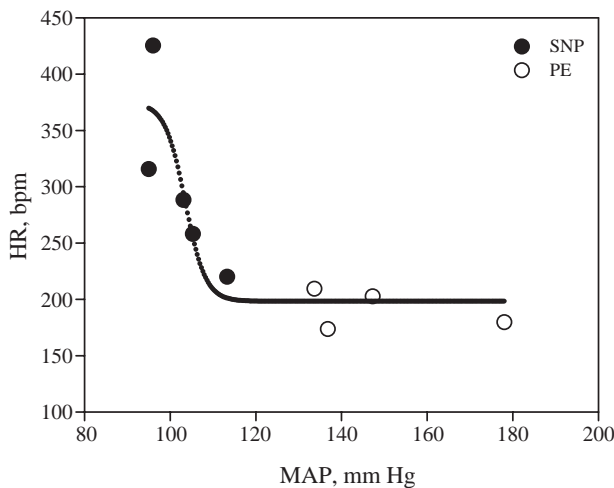


Fig. 8. Representative non-linear regression analysis after infusions. Data derived from PE (○) and NP (●) infusions were combined and fit to a four-parameter sigmoidal function. A plateau in HR change with increasing MAP change is important to the fit of the curve. An upper HR plateau was not reliably observed in pigeons (as shown) and made curve fitting difficult. Note that although the MAP range is similar to that observed after bolus injections of the same drugs, the HR range is significantly higher.

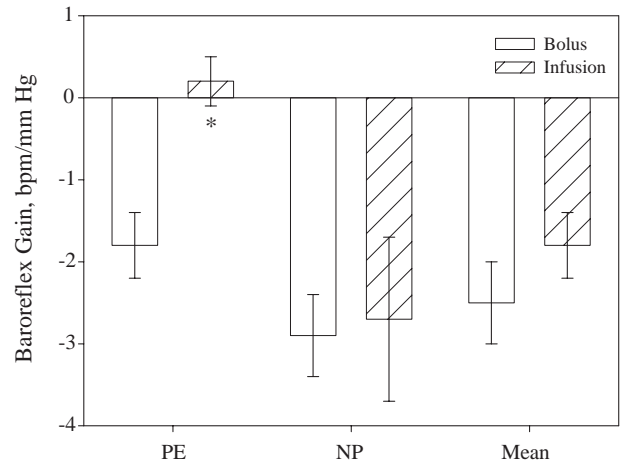


Fig. 9. Comparison of baroreflex gain values for the bolus injection and the infusion method. Gain calculated from NP infusion (-2.7 bpm/mm Hg) was similar to gain calculated from bolus injections (-3.0 bpm/mm Hg). Mean, or combined, gain derived from infusions and injections also did not differ (-1.8 and -2.5 bpm/mm Hg, respectively). However, the mean PE gain for infusion experiments yielded a positive value (0.2 bpm/mm Hg) as opposed to the more characteristic negative value (-1.7 bpm/mm Hg) obtained from PE injections.

the two methods yield similar results when assessed by linear regression.

4. Discussion

The average MAP reported here is very similar to values reported by Woodbury and Hamilton (1937), but lower than pressures reported by Ringer et al. (1955) (mean for males = 151 mm Hg and females = 147 mm Hg) and Butler et al. (1977) (142 ± 6 mm Hg). The mean HR (116 ± 4 bpm) was almost identical to the values reported by Butler et al. (1977) (115 ± 2 bpm), but lower than those described by either Ringer et al. (1955) or Grubb (1982). In the present study, data collection occurred without visual contact between the experimenters and the birds and while the bird remained in its cage. Because the values observed in this study are as least as low as other reports in the literature, we assume our values adequately represent resting HR and MAP in domestic pigeons.

Neither HR nor MAP depended on the time of day recorded. Using radio telemetry, Krista et al. (1981) found domestic turkeys to have a lower MAP during night, although HR did not change with time of day. Telemetry systems allow for the collection of data continuously or at frequent intervals without disturbance to the animal, and are ideal for the measurement of circadian cardiovascular patterns. In the present study, a physical presence was required to record data. These birds were not habituated to handling and, despite measures taken to minimize human contact, they may have entered a heightened state of awareness during measurements. It is possible that, due to human presence, a true circadian pattern associated with HR or MAP may have been masked. It is also possible that peaks in activity, which would influence cardiovascular parameters, were missed due to the infrequent recording intervals. However, using a very similar protocol, de Jong et al. (2001) observed a circadian component to circulating levels of corticosterone

and noradrenaline but not adrenaline in chickens, suggesting that our protocol may be sufficient to ascertain hemodynamic patterns influenced by neurohumoral control.

It is also possible that the post-surgical recovery time was not sufficient to allow a true recovery to baseline physiological values and impacted the results presented in this study. We chose not to immobilize the birds after surgery or use collars that preclude birds from preening or disturbing surgical sites after surgery. Thus, with time after surgery, there is a potential for damage to wing bandage and cannula as well as an observed tendency for cannula patency to decline and an increased risk of infection associated with an externalized cannula (despite administration of a broad-spectrum antibiotic). These factors weighed heavily in our experimental design. Our observations of comparatively low resting HR and MAP in Protocol 1a suggest that a relatively short recovery period may be adequate, albeit not ideal, for this type of study.

Similarly, there was no observed circadian effect on baroreflex gain in pigeons. In humans, baroreflex gain increases during night (resting) hours and decreases during day (active) hours (Smyth et al., 1969; Hossmann et al., 1980; Kawano et al., 1995; Takakuwa et al., 2001; Klawe et al., 2004). Studies on rats demonstrated a higher baroreflex gain during day (resting) hours than during evening (active) hours (Su et al., 1987b; Sei et al., 2002) suggesting that circadian changes in baroreflex gain may be more closely related to circadian patterns of activity rather than a light/dark cycle. An absence of a circadian effect on baroreflex gain in pigeons may be characteristic of avians as a group, pigeons as a species, or the experimental design, although this cannot be confirmed without further studies.

Su et al. (1987a) used PE and nitroglycerine (a NO donor) to elicit a baroreflex response in juvenile rats and reported a dependence of gain on the drug administered. In the present study, gain derived from PE injections differed significantly from those derived by NP injections. PE injections generally invoked peak response within 5 s while NP injections took as long as 10 or 20 s to reach full effect. Although both drugs have their main effect on the peripheral resistance, each drug has a different mechanism of action. PE is an α_1 agonist, causing the formation of inositol triphosphate and diacylglyceride, and leads to vasoconstriction. NP is a nitric oxide donor, stimulating guanylate cyclase and resulting in vasodilation (Murad, 1986). Differences in receptor binding properties and signal amplification efficiency may lead to differential responses of baroreflex pathway. Also, each drug may impact components involved in the reflex pathway other than the peripheral vasculature, such as the heart or central pathways.

Ketamine is a dissociative anesthetic used in humans and other animal species. Studies have shown that ketamine suppresses *N*-methyl-D-aspartate (NMDA)-initiated neural excitation within the nucleus tractus solitarius (Yamamura et al., 1990; Ogawa et al., 1993) and the spinal cord (Anis et al., 1983; Martin and Lodge, 1985; Mills et al., 1988) by acting as a noncompetitive NMDA receptor antagonist. Administration of NMDA into central structures associated with the baroreflex causes a decrease in arterial pressure (Anis et al., 1983;

Gordon, 1987; Ogawa et al., 1993; Gordon and Sved, 2002) via a decrease in sympathetic cardiovascular tone and an increase in cardiac vagal tone. Ogawa et al. (1993) showed that ketamine blocked the baroreflex response initiated by arterial traction. Inoue and Arndt (1982) described a reduction in efferent vagal cardiac discharge (and a resultant increase in HR) after ketamine administration in cats. These studies suggest that not only does ketamine effectively negate baroreceptor input, but may facilitate sympathetic activity by partially removing a tonic inhibitory effect from the caudal ventrolateral medulla or other areas of the brain. The role of NMDA receptors in regulating the baroreflex in birds is not known. However, we observed an attenuation of the pressor response without affect of the depressor response when the pigeons were anesthetized with ketamine. This observation is consistent with a reduction in vagal tone associated with blockade of NMDA receptors.

In mammals, infusions of PE and NP incur graded steady state changes in MAP and the data can be fit to a sigmoidal curve to reflect the physiological limits of HR at very high and low arterial pressures. Fitting data obtained in this study to a sigmoidal function proved difficult for many reasons. First, HR did not reliably plateau at higher pressures in these birds. Second, infusion of both drugs shifted the HR range upward to values 1.5 to 2.2 times higher than resting values without obvious distress to the bird. In contrast, excessive hypertension (PE ≥ 40 $\mu\text{g}/\text{kg}/\text{min}$) and hypotension (NP ≥ 100 $\mu\text{g}/\text{kg}$) caused balance loss or syncope, suggesting that extreme changes in arterial pressure are not well tolerated. Since birds, especially strong fliers such as pigeons, have the capacity to increase HR fourfold while maintaining MAP (Butler et al., 1977), it may not be possible to assess the upper and lower limits of their HR range using pharmacological techniques. Thus, our results may reflect unique characteristics of cardiovascular regulation in domestic pigeons.

Conflicting reports exist on the effects of stress on the baroreflex response. Experiments show that the efferent limb of the baroreflex is inhibited during stress (Nosaka, 1996), allowing for the simultaneous rise in MAP and HR. Other experiments using conscious, unrestrained animals challenged with stressful stimuli suggest that the baroreflex arc functions normally during stress but may reset to accommodate the stress-related pressor response (Shammas et al., 1988; Hatton et al., 1997). Thus, a hypertensive animal with a reset baroreflex will have the same HR as a normotensive animal, although the hypertensive animal has a higher resting MAP. Resetting can begin to occur quickly. Moreira et al. (1989) investigated acute resetting in rats and found that after only 2 min of induced hypertension, the systolic threshold for baroreceptor response increased 33% of the total rise in diastolic pressure. Similarly, resetting after only 5–10 min of sustained MAP changes has been documented in dogs (Coleridge et al., 1981; Heesch et al., 1984) and rabbits (Dorward et al., 1982). It is therefore possible that a pressor response in pigeons quickly resets the baroreflex and may be an adaptive response to flight. This could explain the fact that a HR plateau was difficult to obtain at extreme arterial pressures.

In conclusion, we did not observe a circadian pattern in HR, MAP or baroreflex gain in domestic pigeons. However, birds have obvious activity patterns based on solar cues, and further studies using non-invasive techniques will be required to rule out a circadian dependence of cardiovascular function. We propose that the Oxford method is the superior method to assess baroreflex gain in pigeons due to the remarkable ability of birds to produce large changes in HR in response to induced changes in arterial pressure. Adaptations to flight and other physiologically strenuous activity allow for an enormous range in HR and preclude the use of models based on defining the physiological limits of HR. Despite the differences in the functional ranges of HR between birds, experimental mammalian models and humans, values observed for baroreflex gain are remarkably similar. Further, evidence presented here suggests that central processing of the baroreflex arc may have similar elements in birds and mammals. A detailed investigation of the avian baroreflex response can provide important insight into the evolution of cardiovascular control in vertebrates.

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