The following is the abstract of the article discussed in the subsequent letter:

Moffitt, Julia A., Angela J. Grippo, and Alan Kim Johnson. Baroreceptor reflex control of heart rate in rats studied by induced and autogenic changes in arterial pressure. Am J Physiol Heart Circ Physiol 288:H2422–H2430, 2005. First published January 6, 2005; doi:10.1152/ajpheart.0057.2004.—The function of the arterial baroreflex has traditionally been assessed by measurement of reflex changes in heart rate (HR) or sympathetic nerve activity resulting from experimenter-induced manipulation of arterial blood pressure (the Oxford method, also termed the pharmacological method). However, logistical and flexibility limitations of this technique have promoted the development of new methods for assessing baroreflex function such as the evaluation of changes in spontaneous arterial pressure and HR. Although this new spontaneous method has been validated in dogs and humans, it has not been rigorously tested in rats. In the present study, the method of correlating spontaneous changes in systolic blood pressure and HR was evaluated in resting, normotensive Sprague-Dawley rats. This technique was found to be neither reliable nor valid under the conditions employed in the present protocol. We also tested a variation of the spontaneous method that evaluates particular sequences of data during which arterial pressure and pulse interval are changing in the same direction for at least three consecutive heart beats (the sequence method). The sequence method did not provide extra reliability or validity over the spontaneous method. We conclude that due to the restricted range of variability obtained by measuring spontaneous blood pressure fluctuations, the spontaneous and sequence techniques do not provide data that are comparable to the traditional method of assessing HR changes triggered by arterial blood pressure increases and decreases induced by vasoactive drugs. However, it is possible that surgical stress obscured the relationship between blood pressure and HR, and therefore additional studies are needed to determine whether the spontaneous and sequence methods can be applied to rats during different behavioral states.

Baroreceptor Reflex Sensitivity Estimated by the Sequence Technique Is Reliable in Rats

To the Editor: In a recently published article, Moffitt et al. (5) asked if measurements of spontaneous beat-to-beat blood pressure fluctuations and their corresponding heart rate changes enable calculation of a reliable index of baroreceptor-heart rate reflex sensitivity in rats. The authors concluded that the “spontaneous method” was found to be neither reliable nor valid when compared with the Oxford method (7) based on short-term infusions of phenylephrine and sodium nitroprusside as the standard technique. Moffitt et al. (5) also assessed the baroreceptor-heart rate reflex sensitivity by using a “sequence method” that selected for analysis the sequences of blood pressure fluctuations and corresponding changes in pulse interval only when both parameters changed in the same direction. They concluded that the “sequence method did not provide extra reliability or validity over the spontaneous method.” Although the negative conclusion regarding the spontaneous method is conceivable, the one related to the sequence method was unexpected and surprising because many cardiovascular investigators have used it reliably and effectively to determine baroreceptor-heart rate reflex sensitivity in rodents (4, 6, 8–10).

The purpose of this letter is to clarify that the negative conclusion by Moffitt et al. (5) regarding the sequence method is due to the computational algorithm applied in their study. In addition, we demonstrate how applying the algorithm originally outlined by Bertinieri et al. (1) to the original data files from the study of Moffitt et al. (5) confirms the validity of the sequence method as a measure of baroreceptor-heart rate reflex sensitivity.

The sequence method, as first described by Bertinieri et al. (1), identifies sequences of four or more heart beats, where blood pressure and pulse interval change in the same direction. Moffitt et al. (5) pooled all pairs of systolic blood pressure and corresponding pulse intervals from all sequences and calculated one single linear regression line. In contrast, the sequence technique as first described by Bertinieri et al. (1), and as used by us and others (4, 6, 8–10), requires the calculation of a linear regression for each individual sequence. The average of the slopes of all individual regression lines is then used as an index of baroreceptor-heart rate reflex sensitivity.

In five rats, recordings were obtained on two consecutive days under baseline conditions and during cardiac autonomic blockade by using atropine, propranolol, and the combination of both (for details, see Ref. 5). The sampling rate was 1,000 Hz although the methods inadvertently referred to the sampling rate as 200 Hz. The freely available HemoLab software (http://www.intergate.com/~harald/HemoLab/HemoLab.html) was used to calculate baroreceptor-heart rate reflex sensitivity by using the sequence technique according to Bertinieri et al. (1). Figure 1 shows the correlation between the baroreflex sensitivities on days 1 and 2. The squared correlation coefficient $R^2 = 0.942$ ($R = 0.971$), indicating an excellent correlation between the baroreceptor-heart rate reflex sensitivities on both days. The average reflex sensitivity on day 1 was $0.90 \pm 0.09$ ms/mmHg and on day 2 was $1.29 \pm 0.28$ ms/mmHg (means ± SE, $P = 0.11$). This tendency to higher gains on day 2 may be related to recovery from surgery and anesthesia.

Cardiac autonomic blockade by atropine, propranolol, or the combination of both dramatically reduced the number of sequences from 6.3 ± 1.6 sequences/1,000 heart beats on day 1 and 9.6 ± 3.5 sequences/1,000 heart beats on day 2 to <2 sequences/1,000 heart beats (Fig. 2, right). This finding reflects the diminished response of the sinus node to baroreflex-mediated changes in cardiac autonomic activity under the conditions of muscarinic and/or $\beta_1$-adrenergic receptor blockade.

Because of the low number of sequences during cardiac autonomic blockade, estimates of the sensitivity of the baroreflex based on the sequence technique become less accurate. Nevertheless, the estimate for baroreflex gain during combined cardiac autonomic blockade (Fig. 2, bottom left) was significantly reduced compared with baseline values ($P = 0.01$, paired Student’s $t$-test).

![Graph showing correlation of baroreceptor-heart rate reflex sensitivity determined from blood pressure recordings on 2 consecutive days using the sequence technique ($n = 5$).](http://www.ajpheart.org)
The sequence technique (propranolol) did not alter baroreflex sensitivity as estimated by the number of sequences per 1,000 heart beats (right). Recordings were obtained in 5 rats on 2 consecutive days (baseline values for both days are presented in top row). Cardiac autonomic blockade using atropine (2nd row), propranolol (3rd row), and combined application of atropine plus propranolol (total block, bottom row) were performed in a randomized order on either day 1 or day 2. Therefore, the control values for BRR gain and number of sequences differ for the different experimental conditions. Data are means ± SE (n = 5). P values (paired Student’s t-test) are for comparison with day 1 (top row) or control conditions (rows 2–4).

While muscarinic receptor blockade (atropine) tended to reduce baroreflex gain (P = 0.10), β1-adrenergic receptor blockade (propranolol) did not alter baroreflex sensitivity as estimated by the sequence technique (P = 0.83). The lack of a reduction in baroreflex sensitivity during β1-adrenergic receptor blockade may be seen as an indication that the sequence technique is more sensitive to fast parasympathetic modulation of heart rate compared with the slower sympathetic modulation of sinus node function. It may be possible to overcome this apparent limitation by only selecting longer sequences (e.g., 7 heart beats or more) and longer recording durations or by introducing a time delay between blood pressure and pulse interval during identification of the baroreflex sequences. Another possibility is that the lack of a reduction in baroreflex sensitivity during β1-adrenergic receptor blockade is due to the randomization of the experimental protocol. Four of the five propranolol experiments were done on day 1 (24 h after surgery) when baroreflex sensitivity was still low even under control conditions. Thus β1-adrenergic receptor blockade did not further reduce baroreflex sensitivity.

In conclusion, although the spontaneous method of calculating baroreceptor-heart rate reflex sensitivity (pooling of heart beats) is not reliable in rats, the sequence method (based on the gains of individual sequences and the number of sequences) appears to be highly reliable and valid.

REFERENCES


Harald M. Stauss
Department of Exercise Science
The University of Iowa
Iowa City, Iowa
e-mail: harald-stauss@uiowa.edu

Julia A. Moffitt
Department of Physical Education
Cornell College
Mt. Vernon, Iowa

Mark W. Chapleau
Departments of Internal Medicine and Physiology and Biophysics
and the Cardiovascular Center
The University of Iowa
and The Veterans Affairs Medical Center
Iowa City, Iowa

Francois M. Abboud
Departments of Internal Medicine and Physiology and Biophysics
and the Cardiovascular Center
The University of Iowa
Iowa City, Iowa

Alan Kim Johnson
Departments of Psychology, Pharmacology, and Exercise Science and the Cardiovascular Center
The University of Iowa
Iowa City, Iowa