The effect of clonidine on cardiac baroreflex delay in humans and rats

Short title: Clonidine and baroreflex delay.

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Abstract

The delay \( \tau \) between rising systolic blood pressure (SBP) and baroreflex bradycardia has been found to increase when vagal tone is low. The alpha-2 agonist, clonidine increases cardiac vagal tone, and this study tested how it affects \( \tau \). In 8 conscious supine human volunteers clonidine (6 \( \mu \)g/kg p.o.) reduced \( \tau \), assessed both by cross correlation (xBRS) and sequence methods (both \( p< 0.05 \)). Experiments on urethane-anaesthetized rats reproduced the phenomenon and investigated the underlying mechanism. Heart rate (HR) responses to increasing SBP with an arterial balloon catheter showed reduced \( \tau \) (\( p<0.05 \)) after clonidine (100 \( \mu \)g/kg i.v.). The central latency of the reflex was unaltered, however, as shown by the unchanged timing with which antidromically identified cardiac vagal motoneurons (CVM) responded to the arterial pulse. Testing the latency of the heart rate response to brief electrical stimuli to the right vagus showed that this was also unchanged by clonidine. Nevertheless, vagal stimuli delivered at a fixed time in the cardiac cycle (triggered from the ECG R-wave) slowed HR with a 1 beat delay in the baseline state but a 0 beat delay after clonidine (\( n=5, p<0.05 \)). This was because clonidine lengthened the diastolic period, allowing the vagal volleys to arrive at the heart just in time to postpone the next beat. Calculations indicate that naturally generated CVM volleys in both humans and rats arrive around this critical time. Clonidine thus reduces \( \tau \) not by changing central or efferent latencies, but simply by slowing the heart.

Key words: baroreflex, sequences, delay, lag, latency, cardiac vagal motoneurons, alpha-2 agonist, clonidine.
Introduction

The gain of the arterial baroreceptor-heart rate reflex (cardiac baroreflex) has been calculated from the relation between spontaneous (10; 13) or drug-induced (47) increases in systolic blood pressure (SBP) and the accompanying decrease in heart rate (HR). In humans, the best correlations were initially obtained by relating cardiac intervals to the preceding SBP (delay $\tau=1$ beat) (47), although later studies (14; 38) found a delay of 0 beat was appropriate in subjects with lower initial heart rates. In unanesthetized rats, delays of between two and fourteen beats gave the best correlation for spontaneous baroreflex measurements (35): the best fit for tachycardic responses was 2-3 beats longer than that for bradycardic responses (35). The “sequence” technique (sBRS) (13) traditionally uses a constant delay $\tau=1$ beat to measure spontaneous baroreflex slope in humans, although the method may be applied using delays of any whole number of beats. This approach has been expanded to give a near-continuous assessment of the slope of the cardiac baroreflex (time-domain cross-correlation baroreflex sensitivity : xBRS) (56). The algorithm of xBRS (56) adjusts $\tau$ in seconds to obtain the highest correlation between BP and HR.

In general, the delay $\tau$ becomes longer when vagal tone is low and sympathetic tone is high. It is positively related to HR and age, but negatively related to baroreflex slope (56). It increases in normal subjects when they are standing compared with when they are lying down (1s standing vs. 0s supine) (17; 56). Patients with congestive heart failure (2) or syncope (17) show longer delays than healthy volunteers.

The alpha-2 agonist, clonidine, increases vagal tone (25; 26; 28; 30; 41; 52) and lowers sympathetic tone (33; 48). Given these actions, we predicted that clonidine would decrease $\tau$. Using data previously collected from healthy human volunteers (37), sBRS (13) and xBRS (56) algorithms were used to test this prediction. To generalize the findings in a second species and to pursue the mechanisms responsible, data on HR and the activity of cardiac vagal motoneurons (CVM) (32; 40; 53) were taken from a published study on anesthetized rats (53), re-analyzed and supplemented with further experiments. These allowed the central and efferent components of the reflex pathway to be studied independently of each other, clarifying the site and mode of action of clonidine on cardiac baroreflex delay.
MATERIALS AND METHODS

HUMAN VOLUNTEERS

Data from a previous study (37) were re-analyzed. According to the Declaration of Helsinki and following approval from the ethics committee of the Hospices Civils de Lyon and signed informed consent, eight healthy male physicians with normal BP and without any disease, aged 25–46 years, were studied, as reported earlier (37). They were instructed to avoid tobacco, alcohol and caffeine for 12 h and strenuous exercise for 24 h before recordings. Continuous monitoring of the electrocardiogram (ECG) was performed usually through lead V5 (PhysioControl VSM1, Redmond, WA), and of BP with a noninvasive Finapres 2300 (Ohmeda, Madison, WI) (22) leveled to the heart. Recordings were conducted in the supine position in a quiet and darkened room used only for this study, between 08 AM-4 PM. The volunteers were fully familiar with the environment and the investigators; some volunteers napped during part of the recordings. Subjects were instructed not to speak during recordings. Recordings were made under baseline conditions for at least 20 min, followed by oral ingestion of 6 µg /kg clonidine (Catapressan®, Boehringer-Ingelheim, France) and 120 min later by a further recording session of 20 min.

The spontaneous baroreflex sensitivity was assessed with the “sequence” (sBRS) (13) and cross-correlation (xBRS) (56) methods. For the sequence method, the software selected all sequences lasting 3 beats or more in which there were concordant increases or decreases in SBP and RR interval (13). Linear regression was applied to each sequence detected during the study period. The slope of this regression represents the cardiac baroreflex sensitivity (ms/mmHg). For each sequence the software also determined a delay between SBP and RR interval of either 0 or 1 beat, on the basis of the best correlation (r²>0.7; RR interval threshold=2 ms; SBP threshold=1 mm Hg; sampling rate : 1 kHz;). The ratio of [number of sequences of delay 0/(number of sequences of delay 0+number of sequences of delay 1)] was calculated. Sequences were categorized by the number of beats in the sequence (4). Because sequences of 3 or more beats were far fewer after clonidine (see Results and Discussion), the analysis was extended to include sequences of 2 beats.

For the cross correlation method (xBRS), a 10 s window slides over the SBP and RR interval data set. The signals are interpolated and resampled at 1 s intervals. Within the window, RR interval is cross-
correlated with the BP signal delayed by 0, 1, 2, 3, 4 or 5 s. The delay with the highest cross-correlation is taken as the best delay, \( \tau \). With this best delay \( \tau \), the regression slope divided by the coefficient of correlation is recorded as one determination of \( x_{BRS} \) when the correlation coefficient is positive and significant at \( p<0.05 \). For the results both \( x_{BRS} \)-values and delay \( \tau \) have been averaged over the period of the calculations. The \( x_{BRS} \) values were obtained with proprietary software (BMEYE, Amsterdam, Netherlands). For this study, only the sequences of delay 0, 1 and 2s were taken into consideration. This was in order to select for the rapid parasympathetic actions on the heart (9; 20; 21) and obtain data uncontaminated by sympathetic actions, whose influence may be seen only in sequences of 4 or more beats (4).

### RATS

#### Preparation

**CVM recording experiments**

Experiments were approved by the Rhône-Alpes Committee for the care of Animals and were performed on Sprague-Dawley male rats, as reported earlier (53) (Harlan, Gannat, France, 325-400g) according to NIH guidelines (3). Data from CVM recordings and HR obtained in the course of a previous study (53) were reanalyzed. The methods were described in the earlier publication (53), but will be outlined here. Surgical preparation was performed under isoflurane anesthesia, which was switched to urethane (1.4 g/kg i.v., administered over 15-30 min) before recording commenced. Rats were paralyzed with metocurine iodide (0.2mg i.v., Metubine\textsuperscript{®}, Lilly, Indianapolis, IN) and mechanically ventilated (f~72/min, 40 % \( O_2 \), end-tidal \( CO_2 \sim 30-35 \) mmHg). A percutaneous transluminal coronary angioplasty (PTCA) catheter with a balloon at its tip (2-3mm, Boston Scientific, Galway, Ireland) was introduced through the right femoral artery up to the thoracic aorta to monitor BP and to generate blood pressure rises when inflated (8). An infusion of saline (<3ml/h) through the PTCA catheter prevented hypovolemia (39) and clotting. When pressure rises were not included in the protocol, a polyethylene catheter was used instead of the PTCA catheter. A 3 lead electrocardiogram (ECG) was set up.

The thoracic vagus was exposed through a 2nd right intercostal space thoracotomy. The craniovagal cardiac branch was identified by its location and its ability to cause bradycardia when stimulated (20-
50Hz, 2-7 V, 0.05ms) (40). A pair of electrodes made from teflon-coated 125µm silver wire, bared at the tips, was placed under the branch. The arrangement was insulated from the underlying tissues by a piece of thin plastic sheet and embedded in silicon gel (Wacker, Munich, Germany). The viability of the branch was re-checked at intervals throughout CVM unit recording by stimulating through the implanted electrodes, and the experiment was discontinued if this failed to cause a consistent fall in HR. The rat was fixed in a stereotaxic frame, with the head ventroflexed to bring the surface of the medulla horizontal. The tail was pulled to keep the spine under moderate tension to help to stabilize the medulla for recording. The medulla was exposed via the atlanto-occipital membrane.

*Single unit recording*: Carbon fiber electrodes (29) were inserted vertically through the dorsal surface of the medulla 1.5–2.2 mm to the right of the calamus scriptorius to a depth of 1.5-2 mm, corresponding to the external formation of the nucleus ambiguus. Unit activity was recorded differentially between the carbon fiber electrode and a silver wire on the medullary surface. The signal was amplified (x10,000), band-pass filtered (300-3000Hz) and displayed on an oscilloscope. The unit signal was digitized at 18.5 kHz (Instrutech, New York, NY) and recorded on magnetic tape (JVC, Friedberg, Germany) along with BP, ECG, CO₂ stimulus, vocal messages and event markers. Signals were also recorded with a computer-based system (Micro 1401 interface and Spike2 software; CED, Cambridge, U.K), for which the unit signal was digitized at 15 KHz. On-line spike discrimination was performed with a time/amplitude window discriminator (FHC, Brunswick, ME). CVM were sought by their fixed-latency response to stimulation of the cardiac branch (0.5–5 mA, 0.05 ms, ~1 Hz (40)). Antidromic spikes were averaged using Spike2 software, and their amplitude in successive tracks was used to help localize CVM recordings to the optimum site. Once a unit recording of sufficient amplitude and stability had been isolated (Figure 3A), it was subjected to time-controlled collision testing (Figure 3B) (31).

**Vagal stimulation experiments**

New experiments were performed for this part of the study. Ten rats were prepared surgically as described above, but no muscle relaxant was used in these experiments. The right cervical vagus was exposed through a ventral neck incision and cut. The caudal end of the right vagus was placed over a pair of silver wire hook electrodes under a pool of silicon gel (Wacker). During the experiment the effects of vagal stimulation on HR were observed on a paper recorder (Gould Recorder 2600S,
R-R interval was calculated on-line with custom-made RECAN® software (Alpha-2 Ltd, Lyon, France). The minimum voltage for which a brief 20 Hz vagus nerve stimulus resulted in an observable bradycardia (at least 4 bpm) was taken as threshold (range 1-3V) and thereafter stimuli of 5 times threshold were used (range 5-15V, pulse duration 0.05ms).

**Protocols**

Clonidine 100 µg/kg i.v. (50 µg/ml in saline, Sigma, St Louis, MO) was infused over ~10 min. This dose has been shown to increase CVM activity and alter its firing pattern (53). For comparison, a dose of 200 µg/kg has been reported to achieve maximal sympathetic inhibition in the presence of intact baroreceptors (19).

Two or three BP rises of ≤ 30-50 mmHg (15), were generated by balloon inflation before and after clonidine. Intervals of at least 2 minutes were allowed between inflations, which were only repeated after full recovery of BP and HR to baseline values. CVM and HR responses were measured over 1 s periods (length of sampling bin).

Vagal stimuli were triggered from the R-wave of the ECG. In a first experimental series (5 rats) a train of 1-5 stimuli (2.5-5ms between stimuli) was set at a range of delays from the R-wave, increasing and later decreasing back to zero in steps of approximately 10ms. In the second series (5 rats) a brief stimulus train (5ms between stimuli) was triggered to begin at the R-wave, and its duration (5-50ms) was adjusted to evoke a consistent 1-5ms increase in the R-R interval. In both series, stimulus trains were repeated once every 10 s and responses were averaged over at least 10 repetitions.

At the end of experiments animals were killed with an overdose of chloral hydrate (200 mg i.v.).

**Data analysis**

Balloon inflation tests on HR responses: SBP and R-R interval signals were filtered at 0.8Hz and 0.3Hz respectively using a 201-coefficients low-pass finite impulse response filter. Only the first 5s of the balloon-induced pressure rise were used to calculate the slope of the SBP-RR relationship, to focus on the parasympathetic component of the reflex (9). Here, the delay was calculated by
measuring the number of heart beats between the mid-heights of the SBP and R-R interval rises. Then, linear regression was applied to calculate the SBP-RR interval slope.

*CVM responses to the arterial pulse:* To quantify the delay between the arterial pulse and the resultant CVM discharge (driven by cyclic baroreceptor afferent traffic) an average was constructed of the arterial pressure waveform triggered by every CVM spike (Figure 3). The time between the peak of the averaged BP waveform and the triggering CVM spike time was taken as the mean delay. This was measured for each CVM before and after clonidine treatment.

**Statistical analysis**

The best delay and the ratios of number of delays were not normally distributed. Therefore non-parametric Friedman analysis (STATISTICA 5.1, Statsoft, Tulsa, OK), followed by Wilcoxon paired test was used. Data are expressed as mean±SD in text and ±SEM in Figures. p<0.05 was chosen as significant.
Results:

Human volunteers

As reported previously (37), clonidine (6 µg/kg p.o) significantly increased RR interval (from 1043±25 to 1319±58 ms) and decreased SBP (from 118±4.1 to 99.4±3.1 mmHg) but it did not change respiratory rate (12.6±0.74 before and 12.4±0.73 breaths/min after clonidine). Clonidine also significantly increased the slope of the cardiac baroreflex, measured both by phenylephrine challenge (+ 60%) and by the sequence method (+19%) (37). In addition to these reported changes, clonidine typically reduced SBP lability while increasing HR variability (Figure 1). Clonidine also reduced the number of SBP-RR interval sequences of 3 beats or more, virtually eliminating long sequences (Table 1). The number of 2-beat sequences increased, however (Table 1).

The present re-analysis of that data set using the sBRS method (13) showed that clonidine 6 µg/kg p.o. increased the proportion of sequences of delay 0 beat vs. 1 beat in every subject (Figure 2A; grouped data : Figure 2B: p< 0.05). Because after clonidine there were relatively few sequences of 3 or more beats (as required by the classical sequence method (4; 13)), the analysis was extended to include 2 beat sequences. The proportional shift from 1 beat to 0 beat delays also applied, without exception, to 2-beat sequences (Table 1). Using the xBRS method (56), the mean delay was found to decrease from 0.43±0.26 s to 0.10±0.11 s (p<0.05; Figure 2C).

Rats

In anesthetized rats, all CVM were identified by antidromic activation (Figure 3 A,B), following established methods and criteria (8; 32; 40). As reported, clonidine (100 µg/kg) decreased arterial pressure and HR while increasing CVM activity (Figure 3C) and the slope of its response to SBP (53). The following results are novel.

Arterial pulse-related CVM activity

The systolic upswing of the arterial pulse wave triggers a burst of activity in the arterial baroreceptors, which in turn activates CVM, giving cardiac rhythmicity to their discharge (8; 32; 40). CVM activity was maximal around the systolic peak in arterial pressure, as shown by the neuron illustrated in Figure 4 A. To quantify that time relation while taking account of every CVM spike, CVM spikes were used as
triggers to construct an average of the arterial pulse waveform (Figure 4B). The time between the trigger spike and the peak of averaged pulse wave was then measured for each of the 8 CVM (Figure 4C). Clonidine (100 µg/kg) caused no significant change in that time relation (Figure 4 C, D).

**HR responses to aortic balloon inflation**

The optimum delay for the reflex bradycardia in response to the increase in SBP due to arterial balloon inflation was shortened by clonidine (baseline: 5.8±4.9 beats; clonidine: 1.9±2.1 beats; n=8, p<0.05). The mean delay $\tau$ was reduced also for the increase in CVM activity (from 0.9±0.9 beat to 0.2±0.4 beat; n=8 cells; p<0.05).

**HR responses to vagal efferent stimulation**

To analyze whether clonidine changed the delay between the vagus and the heart rate response, the right cervical vagus was stimulated electrically in two series of experiments. In the first series, the stimulus was triggered to occur at selected delays after the ECG R-wave. In Figure 5 the lengthening of the R-R interval is plotted as the ordinate, while the abscissa is time between the vagal stimulus and the following delayed (or undelayed) R-waves. As may be seen from the grouped baseline data (Figure 5 A), there was a consistent latency of ~ 0.13 s from the vagal stimulus before any R-wave was delayed, and the maximum effect occurred between 0.2 and 0.3 s after the stimulus. Figure 5B shows data from one rat before and after 100 µg/kg clonidine, showing the lack of effect on the latency and time course of the vagal action on HR. The mean latency of the response in 5 rats was not significantly changed by clonidine (130±5.6 ms before vs. 128±8.3 ms after).

In a second series of 5 rats, the vagal stimulus was synchronized to the ECG R-wave. As may be seen in Figure 6, this caused a maximum increase in R-R interval with a 1 beat delay in the baseline state (Figure 6 left), but in every rat the maximum increase happened one beat earlier (0 beat delay) after 100 µg/kg clonidine (Figure 6 right). The reduction in $\tau$ was significant for the grouped data (Figure 6 F). Note also that in every rat the baseline R-R interval was substantially shorter in the baseline state (Figure 6 A-E, left) than it was after clonidine (Figure 6 A-E, right).
Discussion

These data show for the first time that clonidine reduces the cardiac baroreflex delay $\tau$, as assessed by sBRS (13) and by xBRS (56) in humans. This result was predicted on the basis that $\tau$ has been found to vary with sympathovagal balance, which in turn is altered by clonidine. That finding has also been confirmed in a second species: when $\tau$ was measured by similar methods in anesthetized rats, it was also reduced by clonidine. Further experiments on rats then elucidated the mechanisms involved.

Clonidine and the cardiac baroreflex

It is established that clonidine increases the gain of the cardiac baroreflex in humans (37; 46; 51) and other species (28), (53). This is attributable at least partly to increased sensitivity of the arterial stretch receptors themselves (1; 28; 30), to an action in the nucleus of tractus solitarius (44; 49) and probably also to increased excitability of CVM, whose firing pattern after clonidine changes to include many short bursts (doublet and triplet spikes) (53). While clonidine’s action on sympathetic premotor neurons is attributed to alpha-2 adrenoreceptors rather than imidazole receptors (18), it is not certain whether this also applies to its vagal actions. Those receptors have not yet been shown to be expressed by CVM, although alpha-2 binding sites are present in the region of the ventrolateral medulla that includes the nucleus ambiguus (42; 50; 54).

Cardiac baroreflex delay: central pathways

While the effects of clonidine on baroreflex gain are well established, its actions on reflex delay are not. The term “delay” ($\tau$) is used here to indicate the apparent postponement of a response with respect to the stimulus. The term ‘latency’ is used to refer to the dead period where the system cannot respond, however strong the stimulus and however high the gain. The central processing time of the cardiac baroreflex may belong to this latter category. Spike-triggered averaging of the arterial pulse wave provides an accurate timing estimate because it is based on a tightly grouped stimulus and multiple data points over many cardiac cycles. It therefore gives a robust measure that is relatively independent of the low mean firing rate of CVM. The finding that clonidine had no significant effect on the timing of CVM activity within the cardiac cycle, despite the increase in reflex gain (53), indicates that this measurement reflects the unchanging central latency of the cardiac baroreflex.
Baroreceptor afferents, being rate-sensitive, start to fire at the beginning of the systolic upswing in arterial pressure (43). CVM activity is maximal around the systolic peak in arterial pressure, approximately 50 ms later (Figure 3). The present observations therefore indicate that the central latency of the vagal cardiac baroreflex in rats is fixed and \( \leq \) 50 ms. It was evidently unchanged by clonidine.

**Cardiac responses and peripheral delay**

Could clonidine delay the vagal action on the heart? (Sympathetic actions may be ignored because they are too slow to mediate reflex changes with a 0-2 beat delay (9; 21; 34; 55)). It is well established that there is a fixed latency imposed by vagal neuroeffector transmission at the cardiac pacemaker (6; 21; 23). This is due to the time taken between the occupation by acetylcholine of postjunctional \( M_2 \) muscarinic receptors and the changes in pacemaker cell currents mediated by G protein-coupled signalling pathways (12). The experiments illustrated in Figure 5 measured that latency in rats under the present experimental conditions. The minimum time between a stimulus delivered at the cervical vagus and the next R-wave that could be delayed was approximately 130 ms. To estimate the true neuroeffector latency one needs to subtract the efferent conduction time (\(~5\) ms) and to measure the delay with respect to the P-wave rather than the R-wave (a further \(~25\) ms to subtract), giving a true neuroeffector latency value of \(~100\) ms.. As has been well described previously (6; 11), a vagal efferent volley can only lengthen a particular heartbeat if it arrives early enough in the cardiac cycle to do so – i.e. by at least the neuroeffector latency time in advance of the next pacemaker action potential. If the vagal signal arrives at the heart too late in the cardiac cycle, the effect is postponed until the following beat (6; 11).

**Arrival time determines which beat responds best to vagal efferent volleys**

The arrival time at the heart of vagal efferent volleys is sufficient to explain why, even though the vagal neuroeffector latency was unchanged, stimuli synchronized to the R-wave slowed an earlier heartbeat after clonidine treatment than they did in the baseline state (Figure 6). It is a simple consequence of the longer cardiac cycle after clonidine, which in turn is attributable to reduced sympathetic activity (33; 48), increased vagal efferent activity (25; 26; 28; 30; 41; 52; 53) and direct inhibition by clonidine of the cardiac hyperpolarization-activated current (24). Under baseline conditions, vagal stimuli synchronized to the R-wave would have arrived at the heart just too late to extend the current beat: the latency
(measured from cervical vagus to R-wave) in this case was 130 ms, while the next R-wave was due after ~ 140 ms, when the vagal effect on heart rate would have been minimal (Figure 5). Under clonidine, however, the following R-wave was not due for ~ 170 ms, allowing a sufficient time window for the efferent volley to arrive and extend the current beat. By the arrival of the following beat (R-wave due ~ 340 ms after the stimulus in clonidine-treated animals), the action on the pacemaker would have been in decline (Figure 5), and the beat duration less extended.

**Cardiac responses to naturally-timed CVM volleys**

CVM activity is driven by arterial baroreceptors responding to the arterial pulse wave (32). In rats, it is grouped around the time of systole (Figure 3A). The effect of these naturally timed volleys on the heart should thus be very similar to that of electrical stimuli synchronized to the R-wave. Their action would be too late to extend the current heartbeat under baseline conditions, but early enough to do so in the lengthened cardiac cycles after clonidine treatment. This would explain why the reflex heart rate response to SBP is exerted with a 0 beat delay after clonidine but a 1 beat delay in the baseline state. A diagram outlining these issues is shown in Figure 7.

In humans the timescales are different and our knowledge is incomplete, but the same principles may apply. Electrical stimuli delivered to baroreceptor afferent fibres in the carotid sinus nerves can significantly delay the current heartbeat only if they are delivered at least 550 ms before the next anticipated P-wave (5). Stimuli delivered at progressively earlier times in the cardiac cycle result in progressively more powerful slowing (5; 45). Natural baroreceptor afferent volleys arise with the systolic upswing in arterial pressure, which occurs at carotid (38) and brachial (16) arteries ~ 300 ms after the P-wave. From these values, resting pulse intervals of less than ~ 850 ms (HR>70.5 beats/min) would be expected to prevent pulse-related baroreceptor afferent traffic from arriving in time to extend the current diastole. Experimentally, the transition between 0 beat and 1 beat delay has been reported to occur at resting heart periods of 700-800 ms (14; 38). The discrepancy here is not great, and might in part be due to aortic baroreceptors being activated slightly earlier than the time used for calculation. Longer cardiac intervals would progressively open the way for naturally occurring baroreceptor volleys to prolong the current heartbeat (Figure 7).

**Limitations**
Most of the results considered here are taken from *post hoc* reanalyses of previous studies. Not all conditions were controlled: blood pressure was lower after clonidine treatment and presumably there was reduced sympathetic nerve traffic to the heart as well as to the blood vessels. Given the findings that the latency of the central reflex pathway and the neuroeffector latency at the heart were unchanged by clonidine, it seems unlikely that the lowered blood pressure was a significant factor influencing the present results. Indeed, the lower pressure seen by baroreceptor afferents would, if anything, have acted to reduce baroreceptor afferent traffic and lessen its reflex impact. The lower HR after clonidine, however, was pivotal for the mechanisms discussed above. The possibility that the sedative action of clonidine had any effect relevant to the present findings in humans is unlikely. When nitrazepam and clonidine were administered separately to patients in doses that caused similar levels of sedation, nitrazepam evoked no circulatory effect while clonidine did (27).

**Perspectives**

Clonidine is known to reduce the set-point of the sympathetic baroreceptor reflex (48) and to increase the gain of the cardiac baroreflex (46) (37). To these we may now add a third action: shortening the cardiac baroreflex delay. Circulatory adjustments rely on fast-acting mechanisms (7; 11), and speeding up the cardiac baroreflex from acting with a one beat delay to making within-beat compensatory changes may significantly improve circulatory stability. The fact that beat-to-beat adjustments in heart rate and stroke volume are enhanced by an increased gain and a shortened delay may at least partly underlie the change in pattern from high blood pressure lability with limited HR variability before clonidine to stable blood pressure with enhanced HR variability afterwards (Figure 1; see also Figure 3D in (36)). Additionally it may explain the shift from long to short baroreflex sequences after clonidine (Table 1), on the basis that the enhanced reflex is usually able to compensate blood pressure fluctuations within 2 beats instead of 3 or more. It would be worthwhile for future studies to determine the contribution made by changes in $\tau$ to the improvement of blood pressure stability after clonidine, and more generally when cardiac autonomic balance is shifted in the vagal direction.
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Contributions: AC observed the reduction in delay in rats, performed the first and second series of rats experiments and wrote the sBRS software. ET performed the 3d series of rat experiments and re-analyzed all the data. KHW wrote the xBRS software. RMA and JMK provided the key mechanism. RMA designed the rat experiments. LQ designed and performed the volunteers experiments and observed the reduction in delay in volunteers. RMA, JMK, and LQ wrote the discussion.

Conflict of interest: none declared.


Table 1: Numbers of sequences with delays 0 beat and 1 beat, categorized by sequence duration, before and after clonidine.

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C: Chart records showing the firing rate of a CVM averaged over half a respiratory cycle (Hz), detected CVM spikes and blood pressure (BP). The left record was taken in the baseline state and the right record after 100 µg/kg clonidine. The increase in CVM basal firing rate and enhanced responsiveness to arterial pressure after clonidine are documented elsewhere (53).

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A: arterial pulse-triggered histograms of the activity of a representative CVM under control conditions (filled bars, 268 cardiac cycles) and after clonidine (open bars, 264 cardiac cycles), when its activity was increased. The averaged arterial pulse and trigger point (arrow) are shown below. B shows an alternative approach to handling the same data: CVM spike-triggered averages of the arterial pressure
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**Figure 5. Cardiac interval response to brief vagal electrical stimuli in anesthetized rats.** Stimuli were triggered to occur at selected delays after the R-wave of the ECG. For each delay, the preceding and succeeding the R-R intervals were averaged over 10 repetitions. A. Grouped data from 5 rats under baseline conditions. Cycle lengthening (ordinate) is plotted against the time from the stimulus to the delayed (or undelayed) R-wave. To best display the consistent time profile, data from each rat were normalized to a common ordinate value at 200 ms, close to the response peak. B. Data from an individual rat (rat n°4) before and after 100 µg/kg clonidine, plotted in the same way as A, but with the ordinate in ms (not normalized).

**Figure 6. Responses to vagal stimuli synchronized to the ECG R-wave**

Stimuli to the right cervical vagus were synchronized to the R wave of the ECG, as described in the text. A-E: Averaged responses (20 sweeps) in 5 different anesthetized rats at baseline (left) and after clonidine 100µg/kg i.v (right). Stimulus trains (at asterisk) were triggered on the R-wave of the ECG and lasted 5 ms (C) or 10 ms (A, B, D, E). Maximum bradycardia occurred with a 1 beat delay under baseline conditions, but in every case this became 0 beats after clonidine. F summarises the effect of clonidine on the delay τ. B indicates baseline; C 100, clonidine 100 µg/kg. * : p<0.05.

**Figure 7 Schematic diagram to explain the basis of the cardiac baroreflex delay and its modification by clonidine.**

The sequence of events is depicted from the bottom upwards; the horizontal axis represents time, with the appropriate calibrations for rats and humans indicated in the diagram. Baroreceptor afferents (Baro) fire in response to the upswing of the arterial pulse wave (BP). The central latency between baroreceptor input and CVM output is estimated to be ~ 50 ms in rats (this study). The neuroeffector latency plus the central latency plus conduction time has been measured as 550 ms in man, with the central latency estimated to account for ~250 ms of this (5). The neuroeffector latency plus conduction
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Under baseline conditions (left), the pulse-related baroreflex volley would start to act on the sinus node during the pacemaker action potential, postponing the inhibitory action (shown here as hyperpolarization) to the next beat (dotted line). Under clonidine (right), the longer cardiac interval allows the inhibitory action to prolong the current beat (dotted line).
Table 1: Numbers of sequences with delays 0 beat and 1 beat, categorized by sequence duration, before and after clonidine.

| Volunteer | a    | b    | c    | d    | e    | f    | g    | h    | i    | j    | k    | l    | m    | n    | o    | p    | q    | r    | s    | t    | u    | v    | w    | x    | y    | z    |
|-----------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|
| number of beats within a sequence | baseline | clonidine | baseline | clonidine | baseline | clonidine | baseline | clonidine | baseline | clonidine | baseline | clonidine | baseline | clonidine | baseline | clonidine | baseline | clonidine | baseline | clonidine | baseline | clonidine | baseline | clonidine | baseline | clonidine |
| delay0 | 2 | 138 | 127 | 277 | 234 | 161 | 98 | 275 | 89 | 240 | 163 | 341 | 86 | 180 | 150 | 378 | 100 | 243 | 97 | 252 | 51 | 149 | 130 | 297 | 84 | 159 | 126 | 303 | 151 | 188 | 122 | 318 | 47 |
| delay1 | 3 | 98 | 128 | 56 | 29 | 28 | 21 | 4 | 87 | 34 | 13 | 93 | 50 | 78 | 2 | 29 | 10 | 13 | 1 | 99 | 55 | 43 | 2 | 93 | 54 | 106 | 1 | 72 | 25 | 14 | 2 |
| delay2 | 4 | 26 | 16 | 2 | 1 | 1 | 1 | 2 | 11 | 3 | 1 | 1 | 4 | 2 | 1 | 25 | 9 | 18 | 12 | 4 | 2 | 34 | 18 | 9 | 4 | 7 | 11 | 2 | 6 |
| delay3 | 5 | 4 | 2 | 2 | 7 | 2 | 1 | 10 | 2 | 12 | 6 | 8 | 4 | 5 | 4 | 1 | 26 | 10 | 11 | 8 | 12 | 1 | 12 | 10 | 11 | 6 | 4 | 6 | 2 |
| total number of sequences | 247 | 209 | 407 | 293 | 190 | 127 | 297 | 93 | 325 | 197 | 354 | 86 | 284 | 205 | 144 | 95 | 107 | 100 | 276 | 109 | 266 | 52 | 280 | 196 | 340 | 86 | 271 | 192 | 409 | 152 | 264 | 149 | 333 | 49 | 268 | 39 | 172 | 38 | 357 | 61 | 114 | 79 |

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