Effect of systemic B-type natriuretic peptide on cardiac vagal motoneuron activity

E. Toader,1 R. M. McAllen,2 A. Cividjian,1 R. L. Woods,2 and L. Quintin1

1Department of Physiology, Centre National de la Recherche Scientifique Unité Mixte de Recherche, University of Lyon, Lyon, France; and 2Howard Florey Institute, University of Melbourne, Victoria 3010, Australia

Submitted 3 May 2007; accepted in final form 22 September 2007

Toader E, McAllen RM, Cividjian A, Woods RL, Quintin L. Effect of systemic B-type natriuretic peptide on cardiac vagal motoneuron activity. Am J Physiol Heart Circ Physiol 293: H3465–H3470, 2007. First published September 28, 2007; doi:10.1152/ajpheart.00528.2007.—Intravenous B-type natriuretic peptide (BNP) enhances the bradycardia of reflexes from the heart, including the von Bezold-Jarisch reflex, but its site of action is unknown. The peptide is unlikely to penetrate the blood-brain barrier but could act on afferent or efferent reflex pathways. To investigate the latter, two types of experiment were performed on urethane-anesthetized (1.4 g/kg iv) rats. First, the activity was recorded extracellularly from single cardiac vagal motoneurons (CVMs) in the nucleus ambiguus. CVMs were identified by antidromic activation from the cardiac vagal branch and by their barosensitivity. Phenyl biguanide (PBG), injected via the right atrium in bolus doses of 1–5 μg to evoke the von Bezold-Jarisch reflex, caused a dose-related increase in CVM activity and bradycardia. BNP infusion (25 pmol·kg⁻¹·min⁻¹ iv) significantly enhanced both the CVM response to PBG (n = 5 rats) and the reflex bradycardia, but the log-linear relation between those two responses over a range of PBG doses was unchanged by BNP. The reflex bradycardia was not enhanced in five matched time-control rats receiving only vehicle infusions. In five other rats the cervical vagi were cut and the peripheral right vagus was stimulated supramaximally at frequencies of 1–20 Hz. The bradycardic responses to these stimuli were unchanged before, during, and after BNP infusion. We conclude that systemic BNP in a moderate dose enhances the von Bezold-Jarisch reflex activation of CVM, in parallel with the enhanced reflex bradycardia. That enhancement is due entirely to an action before the vagal efferent arm of the reflex pathway.

cardiopulmonary; von Bezold-Jarisch; phenyl biguanide; bradycardia; heart; chemoreflex; parasympathetic; vagus; single unit activity

INCREASED SYMPATHETIC DRIVE and reduced vagal activity are of interest for their therapeutic potential in cardiovascular disease (12, 14, 29). Many studies have shown the benefits of reducing sympathetic activity in cardiovascular disease (10), but actions on the vagal arm of the autonomic nervous system have been under-exploited. It is likely that enhancing vagal drive to the heart will be beneficial because efferent vagal stimulation can improve the outcome after myocardial infarction in dogs (29). Mechanisms that may enhance vagal tone are therefore of interest for their therapeutic potential in cardiovascular disease.

The cardiac natriuretic peptides—atrial natriuretic peptide (ANP), B- and C-type natriuretic peptides (BNP and CNP)—have actions that may be interpreted as cardioprotective (31). Indeed, BNP (Nesiritide) has proved clinically useful in the treatment of congestive heart failure (5, 16). One of these presumed cardioprotective actions is to enhance bradycardic reflexes such as the von Bezold-Jarisch (cardiopulmonary) chemoreflex and the “ramp” (cardiac) high-pressure baroreflex. This action has been shown in several species and may be produced by all three natriuretic peptides, although BNP is the most potent (23, 27). This action is mediated by particulate guanylate cyclase (pGC) receptors (25), but their location is as yet uncertain. On the one hand, experiments on rats and sheep have found that BNP, in a dose that enhanced the von Bezold-Jarisch and “ramp” (cardiac) baroreflex, did not alter the bradycardic response to arterial baroreceptor loading [as assessed by the steady-state baroreceptor reflex (26, 27)]. These findings have been interpreted as showing modulation specifically of reflexes from the heart, with cardiac afferents being the most likely site of hormone action, echoing early studies on ANP (1, 28). On the other hand, Paterson and colleagues (8) demonstrated that higher concentrations of BNP (and CNP) enhanced vagal efferent neurotransmission to the guinea pig heart in vitro by an action mediated via cGMP (8). Whereas the latter workers found ANP ineffective, Atchison and Ackermann (2) had previously demonstrated that more moderate doses of ANP in vivo enhanced the bradycardic effect of vagal stimulation in rats. Corresponding data on BNP are lacking.

The present study was undertaken first to test directly whether BNP, in systemic doses that enhance cardiac reflexes, has any effect on the activity of cardiac vagal motoneurons (CVMs). This approach effectively opens the vagal cardiac reflex loop, allowing us for the first time to distinguish actions before and after its efferent arm. Second, we sought to test whether BNP has any demonstrable action on the parasympathetic efferent arm of the von Bezold-Jarisch reflex pathway between the CVMs and the heart.

MATERIALS AND METHODS

Preparation. Experiments were performed on 32 Sprague-Dawley male rats (350–400 g, Harlan, Gannat, France) and were approved by the Rhône-Alpes Committee for the Care of Animals. Rats were anesthetized with 5% isoflurane in oxygen and given a tracheotomy, after which they were mechanically ventilated with 2% isoflurane in oxygen during surgery. End-tidal CO₂ was adjusted to 25–28 mmHg during surgery. Rectal temperature was maintained at approximately 37.5°C with a servo-controlled heating blanket (Harvard, Edenbridge, Kentucky). The bladder was cannulated and allowed to drain spontaneously. The right femoral vein and artery were catheterized for the injection of drugs and the monitoring of arterial blood pressure, respectively. Normal saline was infused at ~3 ml/h through the arterial catheter to keep it patent and to avoid hypovolemia (19). A third catheter was inserted into the right atrium to evoke the von Bezold-Jarisch reflex.

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
advanced through the right jugular vein until its tip rested close to the right atrium. Subcutaneous silver wire electrodes were placed across the thorax to record the ECG.

**CVM recording and time-control experiments.** The right thoracic vagus was exposed through a thoracotomy at the second intercostal space. The craniovagal cardiac branch was identified anatomically and then tested for its ability to slow the heart (0.05 ms pulses of 2–7 V, delivered at 20 Hz via a paired silver wire electrode) (20). A small polyethylene sheet was inserted beneath the branch to insulate it from the underlying tissue. A pair of electrodes made from Teflon-coated 0.005-in silver wire (A-M Systems, Carlsborg, WA), bare at the tips, was placed under the cardiac branch and was fixed in contact with the nerve by embedding the area in silicon gel (Wacker, Munich, Germany). The leads were also sutured to the thorax to avoid pulling on the nerve. The thoracotomy was then closed, and the viability of the nerve was rechecked by stimulating through the implanted electrodes. If this failed to give a bradycardia, the experiment was discontinued.

The rat was fixed prone in a stereotaxic frame, with the head ventroflexed. The tail was clamped and kept under mild tension. The dorsal portion of the medulla (now approximately horizontal) was exposed via a dorsal approach, and part of the occipital bone was removed with rongeurs. The atlanto-occipital membrane and dura were incised and reflected.

When surgery was complete, anesthesia was switched from isoflurane to urethane (1.4 g/kg iv, administered over ~30 min). End-tidal pCO2 was adjusted to 30–35 mmHg. When a stable plane of anesthesia had been established, sufficient to abolish withdrawal reflexes, the animal was paralyzed with metocurine iodide (0.2 mg iv, Metubine, Lilly, Indianapolis, IN). Paralysis was allowed to wear off between doses so that the adequacy of anesthesia could be checked before repeating the paralysis. If necessary, additional urethane (10–20% of original dose) was given to deepen anesthesia. At the end of the experiments, rats were euthanized with an overdose of chloral hydrate (0.2 g iv).

Carbon fiber electrodes were made as described by Kuras and Gutmaniene (13), with the fiber protruding 10–15 μm beyond the end of the glass micropipette. Electrical contact was made via 2 M NaCl in the pipette shaft. Unit activity was recorded differentially with respect to a reference silver wire on the medullary surface, digitized, using a Grass P16 preamplifier (Grass, Quincy, MA). The signal was amplified (×10,000) and filtered (100–3,000 Hz) before being displayed on an oscilloscope and stored, along with blood pressure, ECG, and stimulus and event markers on magnetic tape (JVC, Friedberg, Germany) and computer (CED Micro 1401 interface and Spike2 analysis program; CED, Cambridge, UK).

Electrodes were inserted vertically through the dorsal surface of the medulla, aiming for a region 1.5–2.0 mm to the right of the calamus scriptorius, and at a depth corresponding to the external formation of the nucleus ambiguus (9). CVMs were sought by their antidromic response to stimulation of the craniovagal cardiac branch, using a search stimulus of 0.5–1 mA, 0.05 ms width, delivered at ~1 Hz. A signal-averaging program in Spike2 was used to help locate CVMs. Once a unit recording of sufficient amplitude and stability had been isolated and showed spontaneous activity, it was subjected to time-controlled collision testing (15, 20). Spike discrimination was performed online with a time-amplitude window discriminator (FHC, Brunswick, ME) and off-line from the analog signal (digitized at 15–20 kHz), using the Spike2 program. The accuracy of discrimination was carefully checked off-line and edited to eliminate mistakes.

**Vagal stimulation experiments.** The right and left cervical vagi were exposed through a large ventral incision in the neck and were 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). The maximal stimulus voltage to activate cardiac vagal motor axons was measured by delivering 20 Hz stimuli of 0.05 ms width. Test stimuli were thereafter delivered at 1.5 times the maximal voltage. The effects of vagal stimulation were observed on a chart recorder, and the heart rate was measured from a digital meter, triggered by the R-wave of the ECG and smoothed with a 1-s time constant (Gould Recorder 2600S, Cleveland, OH). The ECG waveform was displayed on the computer screen at the time and also checked off-line from the recorded signal. Supramaximal stimulus trains were delivered in a set sequence: 1, 2, 5, 10, 20, 40, 20, 10, 5, 2, and 1 Hz. Each stimulus train (except at 40 Hz) was continued until a plateau heart rate response was achieved. The next stimulus was given once stable control conditions were reestablished. In each case the difference in heart rate between the plateau and the prestimulus control was measured. Junctional rhythms often appeared during 40 Hz of stimulation, and these data were discarded.

**Protocols.** In CVM recording experiments, phenyl biguanide (PBG, Lilly) was dissolved freshly in saline at a concentration of 50 μg/ml. After filling the jugular catheter, bolus doses of 1, 2 (both in 5 rats), and either 4 (2 rats) or 5 μg (1 rat) of PBG were administered, using a Hamilton syringe. A minimum of 10 min of recovery time was allowed between PBG doses to avoid tachyphylaxis. Two to three repeat injections were made of each PBG dose before and during BNP infusion. The CVM response to each PBG injection was defined as the extra spikes occurring within the 30 s following the stimulus. The fall in heart rate was measured with respect to the prestimulus control value.

After the control series of tests in both types of experiment, BNP (rat BNP-45, Bachem, Bubendorf, Switzerland) was infused at a rate of 25 pmol·kg⁻¹·min⁻¹ via the femoral vein. After 10–15 min were allowed to reach a steady state, test PBG stimuli were repeated, using the same PBG doses as before (2 to 3 repeats of each dose) in each rat. In the case of vagal stimulation experiments, the same sequence of supramaximal stimulus trains was repeated before, during, and 15–30 min after the BNP infusion was stopped.

Time-control experiments were performed on five rats, which were each individually matched to a successful CVM recording experiment. The surgical preparation was the same, except that no microelectrodes were inserted. Each control rat received PBG doses on the same time schedule as in the counterpart CVM recording experiment but received vehicle infusion in place of BNP. The falls in heart rate with PBG were measured as detailed above.

**Analysis.** Repeat responses to the same dose of PBG in the same condition (e.g., before BNP) were averaged. To examine the effect of BNP, each animal’s mean bradycardic and CVM responses to PBG (all doses combined) before BNP were compared with its responses to the same PBG doses during BNP infusion. The significance of the BNP effect on all animals was assessed by paired t-test. In time-control experiments, the effect of vehicle infusion on the bradycardic response to PBG injections was assessed by paired t-test in the same way. Baseline blood pressure, heart rate, and CVM spike rate were also measured in each rat during control conditions and 15 min after the onset of BNP infusion: the within-animal effect of BNP was compared for the group, using paired t-tests. The same procedure was used to assess the effect of vehicle infusion on blood pressure and heart rate. To compare vagal stimulus-response relations, the bradycardia (in beats/min) was plotted against the log of the stimulus frequency or the log of the CVM response. Least-squares regression Analysis.
with the full protocol. Their properties matched those previously described (17, 20). Their conduction velocities were in the B-fiber range (10.0 ± 1.2 m/s). Their spontaneous activity (mean rate, 0.14 ± 0.10 spikes/s) was strongly modulated by the arterial pulse, as shown by cardiac cycle-triggered histograms (Fig. 1B). They were also activated by rises and inhibited by falls in arterial blood pressure, as reported previously (20) (not shown here). This combination of antidromic activation from the cardiac vagus and barosensitivity was considered sufficient to identify these neurons as CVMs (17, 20).

When PBG was injected close to the right atrium, CVM activity abruptly increased in parallel with the classic von Bezold-Jarisch reflex bradycardia and hypotension. A representative recording is illustrated in Fig. 2A. CVM activity peaked within 1 s of its onset and then declined to a plateau level, which usually persisted for 30 s or more (Fig. 2A). The CVM response and the bradycardia were dose related (Fig. 3, A and B).

When BNP was infused intravenously at 25 pmol·kg⁻¹·h⁻¹, there was a modest fall in resting arterial pressure (from 119.0 ± 4.7 to 105.8 ± 4.5 mmHg) but no change in mean heart rate or CVM activity (381 ± 14.4 to 382.8 ± 16.2 beats/min and 0.14 ± 0.10 spikes/s unchanged, respectively). In response to PBG injections in the presence of BNP, there was a modest enhancement in both the mean CVM response and the mean bradycardia (Fig. 3, A and B; P < 0.05 in each case).

It was found empirically that the change in heart rate in each response to PBG was closely related to the logarithm of the CVM response (expressed as the extra spikes within the first 30 s). Figure 4A illustrates for one CVM how that relation was unchanged in the presence of BNP: the enhanced CVM responses and the enhanced bradycardias shifted upward along the same line of relation. For none of the five CVMs tested did the slope or the intercept of the regression line of data obtained before BNP differ significantly from that of data during BNP infusion. Figure 4B illustrates the corresponding regression lines for each CVM, constructed from all responses to PBG before and during BNP infusion: \( r^2 \) values were between 0.83 and 0.98. It thus appears that there was a strong, fixed relationship between CVM activity and bradycardia, which was unaffected by BNP.

In five time-control rats, resting blood pressure was 126.2 ± 10.0 before and 122 ± 10.1 mmHg during vehicle infusion.
The corresponding values for resting heart rate were 391.6 ± 6.1 and 394.6 ± 7.9 beats/min. The mean reflex bradycardia in response to PBG (all doses) was 64.5 ± 17 before and 60.2 ± 16 beats/min during vehicle infusion (−7%). None of these changes was significant ($P < 0.05, n = 5$).

To test whether BNP had any direct action on the reflex pathway distal to the CVM, the cervical vagi were cut in five other rats, and the cardiac end of the right vagus was directly stimulated. The bradycardic response to a range of increasing stimulation frequencies up to 20 Hz showed an approximately linear-log relation (Fig. 5). The responses obtained before, during, and after infusion of BNP (at the same rate as in CVM recording experiments) were superimposable (Fig. 5), and their regression lines were not significantly different before and during BNP ($P = 0.90$ for slope, $P = 0.28$ for intercept).

**DISCUSSION**

The present study demonstrates that CVMs in the rat, with cell bodies in the nucleus ambiguus and B-fiber axons in the right cardiac branch, are activated by right atrial PBG. This confirms recent findings in the cat (30). We found, moreover, that PBG activated CVM dose dependently and that this was closely paralleled by a dose-related bradycardia. Both the bradycardia and the CVM activation were significantly enhanced in the presence of the cardiac BNP, but the relation between the CVM activation and reflex bradycardia was unchanged. Control experiments established that the reflex enhancement was due to BNP rather than to time or vehicle. Moreover, the bradycardic response to stimulation of the efferent vagus at a range of frequencies was also unaltered by
BNP. Together, these results indicate that BNP acts to enhance the von Bezold-Jarisch reflex bradycardia at a site upstream to the vagal effenter arm of the baroreflex arc.

The action of BNP, as well as ANP and, to a lesser extent, CNP to enhance the von Bezold-Jarisch reflex bradycardia, is well established (22–25): this action is mediated by particulate GC receptors since it is blocked by the pGC-receptor antagonist HS-142–1 (25). The site of this action of BNP and the other natriuretic peptides has been inferred to be on cardiac afferents. The reasons for this are that BNP enhances von Bezold-Jarisch (23) and high pressure, ramp cardiac mechano-receptor reflexes (27) but, at the same doses, is without effect on steady-state (principally arterial) baroreflexes (26). Also in keeping with a cardiac rather than arterial origin of this effect is the observation that the ability of ANP to enhance ramp mechanoreceptor and von Bezold-Jarisch reflexes was still evident in rats after sinoaortic denervation (22). It is therefore likely that BNP enhances the PBG-induced activity in unmyelinated cardiac ventricular afferents (21). This may involve an interaction between 5-hydroxytryptamines (5-HTs) and natriuretic peptide receptors on or near cardiac afferent terminals (6, 25). Chemo- and mechanosensitive cardiac afferents terminate centrally in the caudal part of the nucleus tractus solitarii (4) where they activate neural pathways to CVMs in the nucleus ambiguus (Ref. 11 and this study).

On the efferent side, ANP has been proposed to have a ganglionic site of action (on the sympathetic side) by Floras (7) and a presynaptic vagal effenter action from Atchison and Ackermann (2). Paterson and colleagues (8) also showed that high doses of CNP, and to a lesser extent BNP, enhanced vagal effenter transmission in guinea pig hearts in vitro but saw no such effect with ANP. The reasons for such discrepancies are uncertain but may involve differences between natriuretic peptides, species, and dose. The present study used modest doses of BNP in vivo, which effectively enhanced bradycardic reflex responses, yet there was no evidence for any action of the peptide on the vagal effenter pathway. Indeed, CVM responses and reflex bradycardias remained robustly linked. The possibility that BNP acts at a central nervous site to enhance cardiac reflexes has not yet been eliminated, however. Although the peptide is unlikely to cross the blood-brain barrier, it could conceivably act on neurons of the sensory circumventricular organs: the area postrema, subfornical organ, or the organum vasculosum laminae terminalis (18). This awaits further study.

The baseline effects of BNP in this study were modest. Arterial pressure fell by ~13 mmHg, yet there was no compensatory tachycardia nor was there any change in CVM activity in response to the fall in baseline arterial pressure. In this respect also, the quantitative relationship between CVM activity and heart rate remained robustly linked. Although anesthesia may blunt arterial baroreflex responses, this lack of compensation to the hypotensive effects of BNP is observed also in the conscious state in dogs, sheep, and humans (3, 23, 32).

The present data add to the understanding of the enhancement of cardiac vagal activity induced by BNP, which is evidently by an action upstream to the cardiac vagal effenter pathway. This action may contribute to the therapeutic benefits of BNP (Nesiritide) in congestive heart failure (5, 16).

ACKNOWLEDGMENTS

We thank Dr. J. M. Pequignot for support during this study.

GRANTS

This study was supported by Groupe de Physiologie Appliquée and Alpha-2 Ltd. studentships (to E. Toader), Block grants from the Université Lyon-1/Centre National de la Recherche Scientifique (CNRS) to Unité Mixte de Recherche 5123 (2002–6), a Professeur Invité fellowship from the Université Lyon-1 to R. M. McAllen (2004–5; 2005–6), and Programme International de Coopération Scientifique-CNRS (to L. Quintin).

REFERENCES


