Central Adrenomedullin Augments the Baroreceptor Reflex in Conscious Rabbits

Kiyoshi Matsumura, Isao Abe, Takuya Tsuchihashi, Masatoshi Fujishima

Abstract—We examined the roles of central adrenomedullin, proadrenomedullin N-terminal 20 peptide (PAMP), and calcitonin gene–related peptide (CGRP) on the baroreceptor reflex in conscious rabbits. Intracerebroventricular injection of adrenomedullin (0.2 and 1 nmol/80 μL) elicited dose-related increases in arterial pressure and renal sympathetic nerve activity. On the other hand, a suppressor dose of intracerebroventricular infusion of adrenomedullin (1 nmol/300 μL per hour) caused significant increases in baroreflex sensitivities assessed by renal sympathetic nerve activity and heart rate compared with vehicle infusion (G_{min}; −14.9±1.7 versus −8.0±0.7%/mm Hg, P<0.01, and −8.1±0.8 versus −5.1±0.5 bpm/mm Hg, P<0.01, respectively). Intracerebroventricular infusion of CGRP (1 nmol/300 μL per hour), which is structurally homologous to adrenomedullin, also enhanced the baroreflex controls of renal sympathetic nerve activity and heart rate. However, the intracerebroventricular infusion of PAMP (30 nmol/300 μL per hour) failed to alter the baseline levels of arterial pressure and baroreflex sensitivities. These results suggest that central adrenomedullin and CGRP, but not PAMP, participate in cardiovascular regulation to augment the baroreflex controls of renal sympathetic nerve activity and heart rate in conscious rabbits. (Hypertension. 1999;33:992–997.)

Key Words: adrenomedullin | baroreflex | calcitonin gene–related peptide | central nervous system | renal sympathetic nerve activity

Adrenomedullin is a vasorelaxant peptide isolated from the acid extract of human pheochromocytoma.1 Immunoactive adrenomedullin has been detected in various tissues, such as the adrenal medulla, heart, lung, kidney, and brain.2,3 Various lines of evidence suggest that adrenomedullin participates in cardiovascular regulation not only by its direct effect on vascular smooth muscle but also through its action in the central nervous system.4,5 Adrenomedullin shows structural homology with calcitonin gene–related peptide (CGRP), and its vasodilatory effect is inhibited by CGRP antagonist CGRP(8-37) in the isolated rat mesenteric artery.6 CGRP(8-37) also suppresses the central pressor action of adrenomedullin.7 These findings suggest that adrenomedullin and CGRP may share the same receptors peripherally and centrally. On the other hand, the DNA sequence encoding the precursor of adrenomedullin, proadrenomedullin, has been identified in human as well as rat tissue.7,8 The first paired basic amino acids of this precursor (Lys43-Arg44) are a representative site for proteolytic cleavage, which yields proadrenomedullin N-terminal 20 peptide (PAMP).7,8 PAMP reduces norepinephrine overflow from peripheral sympathetic nerve endings of the arteries,9 which in turn decreases arterial pressure. Intracerebroventricular (ICV) injection of CGRP, adrenomedullin, and PAMP caused increases in arterial pressure in rats,4,5,10,11 suggesting that these peptides participate in cardiovascular and sympathetic regulation of the central nervous system. Moreover, Okamoto et al12 showed that intravenous injection of CGRP modulates baroreceptor reflex in anesthetized rabbits, although the exact site where CGRP acts centrally or peripherally has not been determined. We hypothesized that central adrenomedullin, PAMP, and CGRP participate in cardiovascular regulation by modulating the baroreceptor reflex. However, baroreceptor reflex is influenced to a great extent by the anesthesia used in the experiments.13 Thus, the present study was designed to investigate the role of central adrenomedullin, PAMP, and CGRP in the baroreflex gains of renal sympathetic nerve activity (RSNA) and heart rate (HR) in conscious rabbits.

Methods

Preparation of Animals

The experiments were conducted on male Japanese White rabbits weighing 2.5 to 3.0 kg. The experimental protocol was approved by the Committee on the Ethics of Animal Experimentation of the Faculty of Medicine, Kyushu University. Rabbits were anesthetized with pentobarbital sodium (30 mg/kg IV). Three days before experimentation, bipolar electrodes were implanted on the left renal sympathetic nerve, and a stainless steel cannula was placed in the right lateral cerebral ventricle. RSNA was recorded as...
described previously. Briefly, under aseptic conditions, the left kidney was exposed retroperitoneally, and a branch of the renal nerve was separated from the renal plexus and the surrounding connective tissues with the use of a dissecting microscope. RSNA was recorded by a pair of electrodes made from Teflon-insulated 7-stranded steel wire (Medwire). The area of the nerve and wire interface was embedded in silicone cement (Elastosil RT 604A and B cement, Wacker Chemicals).

A 23-gauge stainless steel cannula was implanted into the right lateral cerebral ventricle, 4 mm lateral to the bregma and 6 mm below the cerebral surface. The position of the cannula in the lateral cerebral ventricle was confirmed by the staining of all 4 ventricles after injection of 0.1 mL dye at the end of the experiments. The cannula was fixed to the skull with 3 jeweler's screws and dental cement. A 27-gauge obturator was used to seal the cannula. After surgery, disodium sulfamethicillin (200 mg IV) was given to the rabbits to prevent postoperative infections.

At least 3 days after the surgical procedures, the following experiments were performed on conscious rabbits placed in the holding box. On the day of the experiment, polyethylene catheters (PE-50) were inserted into the central ear artery and marginal ear vein under 1% lidocaine local anesthesia. The arterial catheter was connected to a pressure transducer (model P50, Gould Inc) to measure arterial pressure. HR was monitored with a cardiotachometer (model 1332, NEC San-ei).

RSNA was amplified (model DPA-100E, Dia Medical System) and filtered (100 to 3000 Hz), and the waveforms were integrated after a full-wave rectification using an integrator amplifier (model 1322, NEC San-ei) with the sample-hole function reset to baseline by an internal timer set at 5 seconds. The residual integrated RSNA that existed after administration of hexamethonium bromide (30 mg/kg IV) was taken as the noise level associated with nerve recording. This value was subtracted from absolute values of integrated RSNA before further data analysis was performed.

All drugs for ICV infusion were dissolved in artificial cerebrospinal fluid (aCSF) (in mmol/L: NaCl 133.3, KCl 3.4, CaCl2 1.3, MgCl2 0.6, NaH2PO4 0.6, NaHCO3 32.0, glucose 3.4).

Effect of ICV Infusion of Adrenomedullin on Cardiovascular and Sympathetic Responses

To determine the effect of adrenomedullin on cardiovascular and sympathetic responses, 0.2 and 1 nmol of human adrenomedullin (Peptide Institute) were injected intracerebroventricularly (n=6 for each). These doses of adrenomedullin were dissolved in 80 μL aCSF. The administration of each dose of adrenomedullin was separated by a period of 60 minutes. Arterial pressure, HR, and RSNA were monitored continuously.

Effect of ICV Infusion of Adrenomedullin on Baroreflex Sensitivity

Three days after the surgical procedure, the effects of adrenomedullin on baroreflex control of RSNA and HR were determined (n=6). Either aCSF or adrenomedullin was infused with a compact syringe pump (model 100, Muromachi Kikai) at flow rate of 300 μL/h. Fifteen minutes after the beginning of the ICV infusion of either aCSF or adrenomedullin (1 nmol/h), the sensitivities of the baroreflex control of RSNA and HR were determined as follows: Progressive infusion of sodium nitroprusside (5 to 80 μg/kg per minute diluted in 0.9% NaCl) was performed at flow rates of 0.029 to 0.467 mL/min with a compact infusion pump (STC-523, Terumo) for 2 minutes to induce a 25- to 30-mm Hg decrease in mean arterial pressure (MAP). Phenylephrine (2 to 32 μg/kg per minute diluted in 0.9% NaCl) was infused at flow rates of 0.029 to 0.933 mL/min for 3 minutes to induce a 30-mm Hg increase in MAP. Half of the rabbits were infused first with sodium nitroprusside and then phenylephrine; the remaining rabbits received an infusion of phenylephrine before sodium nitroprusside. At least 30 minutes elapsed between the infusion of each vasoactive agent to allow MAP, HR, and RSNA to return to baseline values. The control values of MAP, HR, and RSNA were taken as their 3-minute averages before each infusion. The values of the mean RSNA before each infusion were defined as 100%.

Effect of ICV Infusion of PAMP on Baroreflex Sensitivity

Three days after the surgical procedure, the effect of 30 nmol/h of human PAMP (Peptide Institute) on baroreflex control of RSNA and HR was determined (n=6). Fifteen minutes after the beginning of the ICV infusion of either aCSF (300 μL/h) or PAMP (30 nmol/300 μL per hour), the sensitivities of the baroreflex control of RSNA and HR were determined as in the adrenomedullin experiment.

Effect of ICV Infusion of CGRP on Baroreflex Sensitivity

Three days after the surgical procedure, the effect of human CGRP (Peptide Institute) on baroreflex control of RSNA and HR was determined (n=6). Fifteen minutes after the beginning of the ICV infusion of either aCSF (300 μL/h) or CGRP (1 nmol/300 μL per hour), the sensitivities of the baroreflex control of RSNA and HR were determined as in the adrenomedullin experiment.

Data for the MAP-RSNA or MAP-HR relations during increases and decreases in MAP were collected at 5-mm Hg intervals and fitted to a sigmoid logistic function curve. The equation used for the data analysis was based on the following mathematical model

\[ \text{RSNA or HR} = P_1/[1 + \exp(P_2(MAP - P_3))] + P_4 \]

where \( P_1 \) is the range between the upper and lower plateau, \( P_2 \) is a range-independent measure of slope or normalized gain, \( P_3 \) is the blood pressure at the midpoint of the logistic function curve, and \( P_4 \) is the lower plateau. Data were fit to the logistic function curve with the use of a nonlinear regression program in the Statistical Analysis System (NLIN procedure, SAS Institute).

In the present study, the maximum slope \( (\text{G}_{\text{max}} = -P_1 \times P_4/4) \) calculated from the parameters of the logistic function curve was considered to be the sensitivity of the baroreceptor reflex. The slope of the logistic curve at any given MAP was calculated with the computer from the first derivative of the equation described above.

Statistics

All values are expressed as mean±SE. To determine the effects of ICV adrenomedullin on cardiovascular and sympathetic responses, 1-way ANOVA with repeated measurements was performed, followed by Duncan’s multiple range test to determine which means are different from the responses to aCSF. A paired \( t \) test was used to determine the effects of ICV adrenomedullin, PAMP, and CGRP on baroreflex control. A value of \( P<0.05 \) was considered significant.

Results

Effect of ICV Infusion of Adrenomedullin on Cardiovascular and Sympathetic Responses

Baseline values for MAP and HR before the ICV injection of adrenomedullin were 85.3±2.8 mm Hg and 217.5±15.5 bpm, respectively. ICV injection of adrenomedullin elicited dose-related increases in MAP, HR, and RSNA (Figure 1).

Effect of ICV Infusion of Adrenomedullin on Baroreflex Sensitivity

Figure 2 illustrates representative traces of arterial pressure, HR, and RSNA in the assessment of baroreceptor reflex function during ICV infusion of adrenomedullin in a conscious rabbit. ICV infusion of adrenomedullin (1 nmol/h) did
not cause any changes in MAP, HR, or RSNA, but it significantly enhanced the baroreflex control of RSNA (G max; 214.9 ± 61.7 versus 28.0 ± 60.7%/mm Hg, P<0.01) and HR (G max; 28.1 ± 60.8 versus 25.1 ± 60.5 bpm/mm Hg, P<0.01) (Tables 1 and 2, Figure 3). The changing range of RSNA in the sigmoid curve (P1) was significantly increased by ICV infusion of adrenomedullin.

**Effect of ICV Infusion of PAMP on Baroreflex Sensitivity**

ICV infusion of PAMP (30 nmol/h) did not cause any changes in MAP, HR, or RSNA. It also failed to change baroreflex control of RSNA (G max; −9.0 ± 1.3 versus −8.9 ± 0.7%/mm Hg) and HR (G max; −5.6 ± 0.5 versus −5.4 ± 0.5 bpm/mm Hg) (Tables 1 and 2).

**Effect of ICV Infusion of CGRP on Baroreflex Sensitivity**

ICV infusion of CGRP (1 nmol/h) did not cause any changes in MAP, HR, or RSNA, but it significantly enhanced the baroreflex control of RSNA (G max; −18.4 ± 2.1 versus −9.2 ± 0.9%/mm Hg, P<0.01) and HR (G max; −11.0 ± 0.9 versus −6.3 ± 0.4 bpm/mm Hg, P<0.01) (Tables 1 and 2).

**Discussion**

The principal findings of the present study are that (1) ICV injection of adrenomedullin caused dose-related increases in arterial pressure and RSNA and (2) the central administration of adrenomedullin as well as CGRP augmented the baroreflex controls of RSNA and HR in conscious rabbits. Both of these findings suggest that adrenomedullin participates in cardiovascular and sympathetic regulation not only by its direct effect on vascular smooth muscle but also by its action in the central nervous system. The pressor response accompanied by increased sympathetic activity induced by ICV injection of adrenomedullin was consistent with the previous findings of Saita et al. They reported that ICV adrenomedullin elicited the initial transient decrease and the following sustained increase in RSNA in conscious rats. In the present study, however, this initial transient decrease in RSNA was not observed. This different response in RSNA might be attributable to the differences of species (rat versus rabbits) or the types of adrenomedullin (rat adrenomedullin versus human adrenomedullin) used in the experiments.

To eliminate the peripheral actions of adrenomedullin, PAMP, and CGRP, small doses of these peptides were continuously infused intracerebroventricularly with a syringe pump. A subpressor dose of an ICV infusion of adrenomedullin or CGRP augmented the baroreflex controls of RSNA and HR. To our knowledge, this is the first study to demonstrate the central effects of adrenomedullin or CGRP on baroreflex control of sympathetic nerve activity in conscious animals. Okamoto et al demonstrated that intravenous CGRP decreased arterial pressure accompanied by a greater response of RSNA compared with that induced by intravenous injection of sodium nitroprusside in anesthetized rabbits, and they suggested that intravenous CGRP augments baroreflex control of RSNA. However, they could not conclude whether CGRP acts peripherally or centrally. Conversely, Kim et al reported that intracisternal injection of CGRP suppressed the tachycardiac responses induced by intravenous injection of sodium nitroprusside in conscious rats. In their study, however, the intracisternal injection of a high dose of CGRP (5

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**Figure 1.** Central effects of 2 doses (0.2 and 1 nmol) of adrenomedullin and aCSF (80 μL) on changes in MAP, HR, and integrated RSNA in 6 rabbits. Values are mean±SE. *P<0.05, **P<0.01 compared with the respective responses to aCSF by Duncan's multiple range test.

**Figure 2.** Representative tracings show changes in arterial pressure, HR, and RSNA induced by intravenous infusions of phenylephrine (A) or sodium nitroprusside (B) during ICV infusion of adrenomedullin (1 nmol/h) in a conscious rabbit.
nmol) decreased arterial pressure, accompanied by a significant increase in HR.17 Thus, the following intravenous injection of sodium nitroprusside might cause a smaller increase in HR. In fact, in the present study, the range between the upper and lower plateaus of the baroreflex curve of HR (P1) was not altered by ICV infusion of CGRP. The advantage of the present study is that we have determined the baroreceptor reflex in conscious rabbits with the direct recording of sympathetic nerve activity, since the baroreceptor reflex and sympathetic outflow are extremely modulated by the anesthesia used in the experiments.13,18 Furthermore, ICV infusions of adrenomedullin, PAMP, or CGRP did not change baseline levels of arterial pressure, HR, and RSNA in the present study, which allowed us to easily evaluate the baroreceptor reflex.

Although the present study did not clarify the exact site where adrenomedullin or CGRP acts at the central nervous system, the area postrema, nucleus of the solitary tract (NTS), and ventrolateral medulla might be candidates for the modulation of baroreceptor reflex. Allen et al19,20 showed that neurons within the area postrema were excited by bath application of adrenomedullin and that microinjection of adrenomedullin into the area postrema, not the NTS, increased arterial pressure in rats. Furthermore, in our preliminary experiment, microinjection of adrenomedullin (10 pmol) into the rostral ventrolateral medulla failed to change the arterial pressure in rats. Therefore, ICV adrenomedullin might act at the area postrema to increase arterial pressure and augment the baroreflex control of RSNA and HR, although the roles of adrenomedullin in the NTS and ventrolateral medulla have not yet been extensively examined. Furthermore, since microinjection of CGRP into the NTS failed to alter arterial pressure and baroreflex sensitivity,21 the area postrema or ventrolateral medulla might also be involved in modulation of the baroreflex sensitivities by central CGRP. The area postrema, known as a circumventricular organ, has a diminished blood-brain barrier and thus can monitor blood-borne peptides.22 Efferent fibers from the area postrema project to a number of central nervous system structures that are involved in the regulation of sympathetic outflow and cardiovascular function. Anterograde and retrograde anatomic tracing studies revealed the neuronal projection from the area postrema to the NTS.23 In addition, Wilson and Bonham24 demonstrated that the area postrema excites and inhibits sympathetic-related neurons in the rostral ventrolateral medulla of rabbits. These anatomic or electrophysiological studies might provide the hypothesis that central adrenomedullin or CGRP acts initially at the area postrema and then modulates the neuronal activities of the NTS or ventrolateral medulla. Further studies will be needed to determine the possible

### TABLE 1. Parameters and Maximum Gain of Baroreflex Control of Renal Sympathetic Nerve Activity

<table>
<thead>
<tr>
<th>Treatment</th>
<th>n</th>
<th>P1, %</th>
<th>P2</th>
<th>P3, mm Hg</th>
<th>P4, %</th>
<th>Gmax, %/mm Hg</th>
</tr>
</thead>
<tbody>
<tr>
<td>aCSF</td>
<td>6</td>
<td>251.6±12.1</td>
<td>0.127±0.008</td>
<td>91.7±2.6</td>
<td>−1.5±2.0</td>
<td>−8.0±0.7</td>
</tr>
<tr>
<td>Adrenomedullin</td>
<td>6</td>
<td>307.8±26.3*</td>
<td>0.202±0.032</td>
<td>88.2±2.9</td>
<td>−1.2±2.2</td>
<td>−14.9±1.7†</td>
</tr>
<tr>
<td>aCSF</td>
<td>6</td>
<td>258.7±12.7</td>
<td>0.137±0.009</td>
<td>82.4±2.1</td>
<td>2.0±4.7</td>
<td>9.6±0.7</td>
</tr>
<tr>
<td>PAMP</td>
<td>6</td>
<td>269.5±24.1</td>
<td>0.133±0.012</td>
<td>82.8±2.4</td>
<td>−7.6±2.7</td>
<td>−9.0±1.3</td>
</tr>
<tr>
<td>aCSF</td>
<td>6</td>
<td>280.4±18.8</td>
<td>0.130±0.009</td>
<td>75.8±2.7</td>
<td>−2.4±1.4</td>
<td>−9.2±0.9</td>
</tr>
<tr>
<td>CGRP</td>
<td>6</td>
<td>316.9±28.7</td>
<td>0.231±0.015†</td>
<td>75.0±1.9</td>
<td>0.2±1.2</td>
<td>−18.4±2.1†</td>
</tr>
</tbody>
</table>

Values are expressed as mean±SE. The baseline MAPs of adrenomedullin, PAMP, and CGRP experiments were 94.3±2.0, 85.3±1.8, and 80.0±2.3 mm Hg, respectively. P1 indicates range of RSNA; P2, slope coefficient; P3, MAP at midrange; P4, minimum RSNA; and Gmax, maximum gain of RSNA.

*P<0.05, †P<0.01 vs aCSF by paired t test.

### TABLE 2. Parameters and Maximum Gain of Baroreflex Control of Heart Rate

<table>
<thead>
<tr>
<th>Treatment</th>
<th>n</th>
<th>P1, bpm</th>
<th>P2</th>
<th>P3, mm Hg</th>
<th>P4, bpm</th>
<th>Gmax, bpm/mm Hg</th>
</tr>
</thead>
<tbody>
<tr>
<td>aCSF</td>
<td>6</td>
<td>210.9±19.9</td>
<td>0.099±0.011</td>
<td>96.5±3.3</td>
<td>139.2±12.1</td>
<td>−5.1±0.5</td>
</tr>
<tr>
<td>Adrenomedullin</td>
<td>6</td>
<td>189.2±11.0</td>
<td>0.172±0.016*</td>
<td>93.0±2.4</td>
<td>152.6±9.5</td>
<td>−8.1±0.8*</td>
</tr>
<tr>
<td>aCSF</td>
<td>6</td>
<td>166.3±17.2</td>
<td>0.134±0.016*</td>
<td>80.5±2.1</td>
<td>169.6±12.2</td>
<td>−5.4±0.5</td>
</tr>
<tr>
<td>PAMP</td>
<td>6</td>
<td>146.4±7.3</td>
<td>0.153±0.009</td>
<td>81.1±2.2</td>
<td>176.7±7.9</td>
<td>−5.6±0.5</td>
</tr>
<tr>
<td>aCSF</td>
<td>6</td>
<td>159.7±9.7</td>
<td>0.160±0.014</td>
<td>75.3±2.9</td>
<td>163.2±12.1</td>
<td>−6.3±0.4</td>
</tr>
<tr>
<td>CGRP</td>
<td>6</td>
<td>175.0±13.0</td>
<td>0.259±0.026*</td>
<td>75.2±2.3</td>
<td>160.0±12.9</td>
<td>−11.0±0.9*</td>
</tr>
</tbody>
</table>

Values are expressed as mean±SE. The baseline MAPs of adrenomedullin, PAMP, and CGRP experiments were 94.3±2.0, 85.3±1.8, and 80.0±2.3 mm Hg, respectively. P1 indicates range of HR; P2, slope coefficient; P3, MAP at midrange; P4, minimum HR; and Gmax, maximum gain of HR.

*P<0.01 vs aCSF by paired t test.
Adrenomedullin is structurally homologous to CGRP. CGRP(8-37) is a putative antagonist of CGRP or adrenomedullin. The blocking effects of CGRP(8-37) on the central action of adrenomedullin have been controversial. Saita et al.\(^4\) reported that pretreatment with CGRP(8-37) suppressed the central effect of adrenomedullin; conversely, Samson et al.\(^11\) did not have the same result. The results of the present study did not determine whether ICV adrenomedullin and CGRP act at the same receptors in the central nervous system to modulate the baroreceptor reflex. This seems difficult to establish in the present study because adrenomedullin or CGRP was continuously infused intracerebroventricularly to steadily supplement both peptides. Specific receptor antagonist of adrenomedullin or CGRP will be needed to determine the receptor at which ICV adrenomedullin or CGRP acts to modulate the baroreceptor reflex.

Recently, Samson et al.\(^11\) demonstrated that 1 or 0.1 nmol of ICV injection of PAMP caused an increase in arterial pressure in conscious rats. In our preliminary experiment, however, even a higher dose of PAMP (30 nmol) did not change arterial pressure and RSNA in conscious rabbits. These different responses might be solely attributed to the species used in the experiments. In the present study, however, any evidence that central PAMP participates in cardiovascular regulation and modulation of baroreceptor reflex was not observed. PAMP is distributed not only in plasma but also in brain tissue.\(^25\) However, distributions of PAMP in the medulla oblongata, which play an important role in the regulation of arterial pressure and the sympathetic nervous system, may be different between rats and rabbits. Further evaluations will be required to determine whether central PAMP plays a role in the regulation of the cardiovascular system.

In conclusion, central adrenomedullin exerts a pressor response mediated by enhanced sympathetic outflow. Central adrenomedullin and CGRP augment the baroreflex control of RSNA and HR in conscious rabbits, while central PAMP fails to change the baroreflex control of RSNA and HR. Adrenomedullin and CGRP, but not PAMP, participate in cardiovascular regulation not only by their direct effects on vascular smooth muscle but also through their actions in the central nervous system.

**References**


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