Effects of proadrenomedullin N-terminal 20 peptide and calcitonin on isolated perfused rat hearts

İzole perfüze sıçan kalpleri üzerinde proadrenomedullin N-terminal 20 peptit ve kalsitonin'in etkileri

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ABSTRACT

Objective: There are evidences that proadrenomedullin N-terminal 20 peptide (PAMP) and calcitonin may be involved in cardiovascular function. Therefore, we studied effects of rat PAMP and human PAMP as well as rat calcitonin and salmon calcitonin on coronary perfusion pressure, heart rate and contractile force.

Methods: Isolated rat hearts were perfused under constant flow condition and rat PAMP (1.10 and 100 nM), human PAMP (1,10 and 100 nM), rat calcitonin (10.100 and 1000 nM) or salmon calcitonin (10.100 and 1000 nM) were infused to the hearts. Coronary perfusion pressure, heart rate, left ventricular developed pressure and $+dP/dt_{max}$ were measured. Statistical analysis was performed using repeated measures ANOVA and Bonferroni posthoc tests.

Results: Rat PAMP (1.10 and 100 nM) did not alter perfusion pressure. However, it increased heart rate from 257.83 ± 23.89 to 282±24.98 beats/min ($p<0.001$), from 259.83±25.05 to 289.8±19.5 beats/min ($p<0.001$) and from 249.66±19.19 to 280.50±25.26 beats/min ($p<0.001$) for 1.10 and 100 nM, respectively. Rat PAMP decreased left ventricular developed pressure from 90.5±18.5 to 79±15.3 mmHg ($p<0.05$), from 88.00±10.12 to 73.00±12.38 mmHg ($p<0.05$) and from 79.83±8.98 to 64.83±10.12 mmHg ($p<0.05$) for 1.10 and 100 nM, respectively. The peptide also decreased $+dP/dt_{max}$ from 3710.5±370.6 to 3223.8±261.1 mmHg s^{-1} ($p<0.001$), from 3683.16±327.27 to 3040.6±423.8 mmHg s^{-1} ($p<0.01$) and from 3746.16±315.76 to 3009.83±204.64 mmHg s^{-1} ($p<0.001$) for 1.10 and 100 nM, respectively. Rat calcitonin (10.100 and 1000 nM) did not change perfusion pressure but it decreased heart rate from 269.16±22.6 to 253.6±22.84 beats/min ($p<0.05$), from 263.8±27.3 to 247.00±36.63 beats/min ($p<0.05$) and from 285.0±32.4 to 264.00±39.83 beats/min ($p<0.01$) for 10.100 and 1000 nM, respectively. Rat calcitonin did not significantly affect left ventricular developed pressure. Human PAMP or salmon calcitonin did not change perfusion pressure, heart rate and left ventricular developed pressure.

Conclusion: We conclude that rat PAMP may induce positive chronotropic and negative inotropic effect while rat calcitonin may produce a negative chronotropic effect. Human PAMP or salmon calcitonin could not alter perfusion pressure, heart rate and contractility in isolated, perfused rat hearts. *(Anadolu Kardiyol Derg 2009; 9: 176-82)*

Key words: Proadrenomedullin N-terminal 20 peptide, calcitonin, perfusion pressure, heart rate, contractility

ÖZET

Amaç: Proadrenomedullin N-terminal 20 Peptit'in (PAMP) ve kalsitoninin kardiyovasküler fonksiyonda işe karışabileceği hakkında kanıtlar vardır. Bu nedenle sıçan PAMP'ın, insan PAMP'ın, sıçan kalsitonin'in ve somon kalsitonin'in koroner perfüzyon basıncına, dakikada kalp atım sayısına ve kasılma kuvvetine etkilerini araştırdık.

Yöntemler: İzole sıçan kalpleri sabit akım durumunda perfüze edildi ve sıçan PAMP (1.10 ve 100 nM), insan PAMP (1.10 ve 100 nM), sıçan kalsitonin (10.100 ve 1000 nM) veya somon kalsitonin (10.100 ve 1000 nM) kalplere verildi. Koroner perfüzyon basıncı, dakikada kalp atım sayısı, sol ventrikülün geliştirdiği basınç ve $+dP/dt_{max}$ ölçüldü. İstatistiksel analiz tekrarlanan ölçümler için ANOVA ve Bonferroni testleri kullanılarak yapıldı.

Bulgular: Sıçan PAMP (1.10 ve 100 nM) perfüzyon basıncını değiştirmedi. Fakat dakikada kalp atım sayısını 1.10 ve 100 nM dozlarında sırası ile 257.83±23.89'dan 282.00±24.98 atım/dakika'ya ($p<0.001$), 259.83±25.05'dan 289.83±19.5 atım/dakika'ya ($p<0.001$) ve 249.66±19.19'dan 280.50±25.26 atım/dakika'ya ($p<0.001$) artırdı. Sıçan PAMP sol ventrikülün geliştirdiği basıncı 1.10 ve 100 nM dozlarında sırası ile 90.5±18.5'dan 79.0±15.3 mmHg'ya ($p<0.05$), 88.00±10.12'dan 73±12.38 mmHg'ya ($p<0.05$) ve 79.83±8.98'dan 64.83±10.12 mmHg'ya ($p<0.05$) azalttı. Bu peptit $+dP/dt_{max}$ değerlerini de 1.10 ve 100 nM dozlarında sırası ile 3710.5±370.6'dan 3223.8±261.1 mmHg s^{-1}'ya ($p<0.001$), 3683.16±327.27'dan 3040.6±423.8 mmHg s^{-1}'ya ($p<0.01$) ve 3746.16±315.76'dan 3009.83±204.64 mmHg s^{-1}'ya ($p<0.001$) azalttı. Sıçan kalsitonin (10,100 ve 1000 nM) perfüzyon basıncını değiştirmedi. Fakat dakikada kalp atım sayısını 10.100 ve 1000 nM dozlarında sırası ile 269.16±22.60'dan 253.60±22.84 atım/dakika'ya ($p<0.05$), 263.83±27.30'dan 247.00±36.63 atım/dakika'ya ($p<0.05$) ve 285.0±32.4'dan 264.00±39.83 atım/dakika'ya ($p<0.01$) azalttı. Sıçan kalsitonin sol ventrikülün geliştirdiği basıncı anlamlı olarak etkilemedi. İnsan PAMP veya somon kalsitonin perfüzyon basıncını, dakikada kalp atım sayısını ve sol ventrikülün geliştirdiği basıncı değiştirmedi.

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Sonuç: İzole perfüze sıçan kalplerinde sıçan PAMP pozitif kronotrop ve negatif inotrop sıçan kalsitonin ise negatif kronotrop etki yapabilir. İnsan PAMP'ın veya somon kalsitoninin perfüzyon basıncı, dakikada kalp atım sayısı ve kasılma kuvveti üzerinde etkisi olmadığı sonucuna varılmıştır. *(Anadolu Kardiyol Derg 2009; 9: 176-82)*

Anahtar kelimeler: Proadrenomedullin N-terminal 20 peptit, kalsitonin, perfüzyon basıncı, dakikada kalp atım sayısı, kasılma kuvveti

Introduction

Proadrenomedullin N-terminal 20 peptide (PAMP) is a peptide formed from preproadrenomedullin which is the precursor of adrenomedullin (1). It has been demonstrated that PAMP is secreted from rat cardiomyocytes and fibroblasts (2) and there are specific binding sites for PAMP in rat heart tissues (3). The peptide elicits inhibitory effects both on catecholamine release from cultured bovine adrenal medullary cells (4) and also on adrenocorticotropin secretion from cultured rat pituitary cells (5). The plasma concentration of PAMP is increased in patients with essential hypertension (6) and congestive heart failure (7). Rat or human PAMP contains a unique 20 amino acid sequence and rat PAMP shares 75% sequence homology with human PAMP (8). Rat PAMP increases coronary flow in isolated rat hearts (9) and human PAMP exerts a hypotensive activity in anesthetized rats (10). These data confirm that PAMP may involve in the control of coronary vascular tone. On the other hand, studies on isolated rat hearts have been showed that rat PAMP has no effect on contractile force (11) or decreases it (9).

Calcitonin is a peptide showing hormonal activity generated by calcitonin gene. It is secreted from the parafollicular cells of the mammalian thyroid gland. Calcitonin receptors are located in bone and the kidney cells (12). Calcitonin inhibits osteoclastic bone resorbtion, gastric acid secretion (13) and small intestinal motility (14). Furthermore, calcitonin exerts analgesic effects in osteoporosis (15). Salmon calcitonin is effective in the treatment of acute hypercalcemia and it may also be given to patients with congestive heart failure or azotemia (16). Rat or salmon calcitonin consists of 32 amino acids and the homology in sequence of amino acids between rat and salmon calcitonin is approximately 53% (17). Human or salmon calcitonin has no significant effects on blood pressure (18) but salmon calcitonin induces negative chronotropic and negative inotropic effects on isolated dog atrium (19), suggesting that it has a possible role in the regulation of myocardial contractile force and rate.

It is known that PAMP and adrenomedullin are formed from preproadrenomedullin by enzymatic cleavage (20). The structures of the N-terminal 6-7 amino acid ring and C-terminal amid are common for adrenomedullin and calcitonin. These structures are essential for biological activity of the peptides (21) Since the carboxy terminus of PAMP is also amidated (10), there is a structural similarity between PAMP and calcitonin. Therefore, we studied cardiac effects of PAMP and calcitonin in the present study. Since salmon calcitonin is more active than human calcitonin in humans (12), we also studied salmon calcitonin. There is a need of more extensive studies on the effects of rat and human PAMP as well as rat and salmon calcitonin. There are conflicting studies related to contractile action of rat PAMP and the effect of human PAMP on heart rate and contractile force has not been examined. Furthermore, the effect of rat calcitonin on coronary perfusion pressure and heart rate has not been studied and little is known on the effect of rat calcitonin on contractile force. Isolated rat hearts, the effect of salmon calcitonin on perfusion pressure, heart rate and contractile force has not also been studied.

So, in this study we aimed to investigate the possible effects of PAMP and calcitonin forms on perfusion pressure, heart rate and contractile force in isolated perfused rat hearts.

Methods

Isolated heart preparation and perfusion

Sprague-Dawley rats of either sex weighing 300-400 g were used. All procedures were applied in accordance to the "Guide to the Care and Use of Experimental Animals" by the Canadian Council of Animal Care (22).

The rats were fed with a standard rat chew and housed in cages with a 12 hour light/dark cycle and temperature of 20-25^{o}C. One hour after the administration of 1000 IU heparin i.p., the chest was opened under light ether anesthesia. The heart was excised rapidly and placed into an ice-cold modified Krebs-Henseleit solution until contractions ceased. After the heart was cleaned of surrounding tissues, the aorta was immediately tied to a stainless steel cannula of the perfusion system, and the heart was perfused retrogradely by noncirculating Langendorff technique (23). The pulmonary artery was incised to facilitate a complete coronary drainage in the ventricles. Daily prepared modified Krebs-Henseleit solution with the following composition (mM): NaCl 118, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 25 and glucose 11 was used as the perfusion solution. The solution was continuously oxygenated with 95 % O_2 and 5% CO_2 and pH of the solution was 7.4. The temperature was maintained at 37^{o}C. The hearts were perfused under constant flow condition (12 ml/min) by using a peristaltic pump (Ismatec Reglo, Hugo Sachs Electronic, March-Hugstetten, Germany). Our preliminary studies indicated that vehicle infusions did not change cardiac and coronary variables over the time.

Measurement of hemodynamic variables

Coronary perfusion pressure was measured by attaching the side arm of the aortic cannula to a pressure transducer (Isotec, Hugo Sachs Electronic, March-Hugstetten, Germany). Myocardial contractile force was measured according to the method described by He and Downey (24). A liquid-filled latex balloon was connected to a pressure transducer (Isotec, Hugo Sachs Electronic, March-Hugstetten, Germany) and inserted into the left ventricle via the mitral valve. Peak systolic pressure and end diastolic pressure were measured. Diastolic balloon pressure was maintained at 8 mmHg. Left ventricular developed pressure was calculated as the difference between the systolic and diastolic pressures and this pressure was accepted as

contractile force. Heart rate was calculated from the signals of the left ventricular pressure. Furthermore, the maximum rate of increase of left ventricular pressure ($+dP/dt_{max}$) was determined. All of the hemodynamic parameters were analyzed by a data acquisition and analysis system (Plugsys Transducer Amplifier Modules and Isoheart Software, Hugo Sachs Elektronic, March-Hugstetten, Germany). The hearts were equilibrated for 30 minutes (min) to establish a stable baseline.

Infusion of peptides and experimental protocols

Since rat PAMP at the doses of 10 pM, 100 pM, 1 nM and 10 nM decreased left ventricular pressure and $+dP/dt_{max}$ (9), we selected the doses of 1, 10 and 100 nM of PAMP. The effect of human and salmon calcitonin at the doses of 10 pM, 100 pM, 1 nM and 10 nM on the perfusion pressure of rabbit isolated mesenteric vasculature has been investigated (25). In addition, the effect of rat calcitonin at a dose 1000 nM on the contractility of rat isolated atrium has been studied (26). Therefore, we used the doses of 10, 100 and 1000 nM of calcitonin. After stabilization period, rat PAMP (1, 10 and 100 nM), human PAMP (1, 10 and 100 nM), rat calcitonin (10, 100 and 1000 nM) or salmon calcitonin (10, 100 and 1000 nM) were infused by using an infusion pump (Graseby Medical, Model 3400, Watford Herts). Each dose was applied to different groups of the hearts. Rat PAMP and rat calcitonin were infused for 30 min while human PAMP and salmon calcitonin were infused for 10 min because of the absence of response. The infusion rate was 0.1 ml/min in all of experiments.

Materials

Rat PAMP, rat calcitonin and salmon calcitonin were obtained from AnaSpec Inc (San Jose, CA, USA). Human PAMP was purchased from the Sigma Chemical Company (St. Louis, MO, USA). All peptides were dissolved in distilled water, stored -20°C and diluted with modified Krebs-Henseleit solution just before the infusions.

Statistical analysis

The values obtained prior to the addition of the drugs were taken as controls. Statistical analysis was performed using SPSS for Windows (version 13.0, SPSS Inc, Chicago IL, USA). The normality of data distribution was assessed using the Kolmogorov-Smirnov test with Lilliefor's correction. Data were analyzed by repeated measures ANOVA and time- dependent effects of different doses of the peptides were evaluated by Bonferroni test. Values are given as mean±SD and a p value less than 0.05 was considered as statistically significant.

Results

The effect of PAMP on hemodynamic parameters

While rat PAMP (1, 10 and 100 nM) did not alter perfusion pressure (Table 1), it significantly increased heart rate ($p<0.001$). The peak increase in heart rate occurred 8 min after the administration of the peptide and heart rate remained high during the observation period of 30 min (Fig. 1). Rat PAMP significantly decreased the developed pressure ($p<0.05$). The maximum effect was seen between in 8 to 15 min after the treatments and afterwards developed pressure remained low (Fig. 2). Rat PAMP also decreased +dP/dtmax ($p<0.001$, Fig. 3). After washout of rat PAMP, heart rate, developed pressure and $+dP/dt_{max}$ returned to the control level. Human PAMP (1, 10 and 100 nM) had no significant effect on perfusion pressure, heart rate and developed pressure (Table 2).

The effect of calcitonin on hemodynamic parameters

Different doses of rat calcitonin had no influence on perfusion pressure (Table 3). As illustrated in Fig. 4, rat calcitonin (10, 100

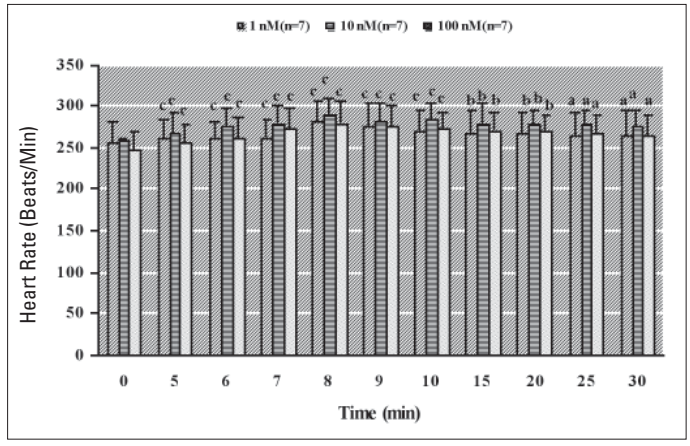

Figure 1. Effect of rat PAMP on heart rate. After a stabilization period, the peptide was infused for 30 min. Time 0 represents control values. Data are presented as mean ± SD and vertical bars show SD. Rat PAMP increased heart rate

a - $p<0.05$, b - $p<0.01$, c - $p<0.001$ - significantly different from the respective control by repeated measures ANOVA and Bonferroni tests

PAMP - proadrenomedullin N-terminal 20 peptide

Table 1. Effect of rat PAMP on perfusion pressure

Perfusion Pressure, mmHg											
	Min										
Doses	**0**	**5**	**6**	**7**	**8**	**9**	**10**	**15**	**20**	**25**	**30**
1 nM n=7	41.90±7.24	43.50±7.25	43.00±7.62	43.46±7.66	43.11±7.38	43.15±7.60	43.0±7.6	43.10±7.49	43.2±7.6	42.15±7.30	42.13±7.32
10 nM n=7	45.83±10.40	48.83±11.90	48.00±11.6	48.0±11.5	47.0±10.2	48.6±10.5	49.0±11.6	48.00±7.55	48.2±10.6	48.00±5.98	48.3±9.9
100 nM n=7	46.00±8.92	49.16 ± 7.73	48.83±7.62	48.83±6.82	49.5±6.5	48.16±8.80	49.16±7.08	48.50±8.31	48.50±8.33	48.83±8.42	48.83±8.58

After a stabilization period, the peptide was infused for 30 min. Time 0 represents control values. Data are presented as mean ± SD. Rat PAMP had no effect on perfusion pressure by repeated measures ANOVA and Bonferroni tests

PAMP - proadrenomedullin N-terminal 20 peptide

Table 2. Effect of human PAMP on perfusion pressure, heart rate and developed pressure

Perfusion Pressure, mmHg					
Min					
Doses	**0**	**4**	**6**	**8**	**10**
1 nM n=7	60.94±12.74	58.20±11.37	59.64±13.10	62.44±16.64	65.66±13.42
10 nM n=6	54.45±9.90	63.61±11.10	66.15±12.50	67.98±13.60	68.24±15.04
100 nM n=6	51.56±9.62	64.26±11.23	67.13±13.60	73.23±17.04	72.87±17.25
Heart Rate, Beats/min					
Min					
Doses	**0**	**4**	**6**	**8**	**10**
1 nM n=7	247.14±44.62	229.14±39.34	223.00±43.74	229.42±43.53	217.2±49.3
10 nM n=7	224.71±41.27	205.71±39.98	220.71±41.02	216.28±44.82	209.10±45.18
100 nM n=7	251.14±35.78	241.42±40.36	236.28±40.76	237.00±43.55	240.38±39.56
Developed Pressure, mmHg					
Min					
Doses	**0**	**4**	**6**	**8**	**10**
1 nM n=7	55.55±5.98	60.57±11.42	59.90±10.41	59.08±7.46	58.79±10.00
10 nM n=9	65.06±15.00	67.18±6.78	64.77±15.60	66.97±15.70	66.20±15.10
100 nM n=7	63.55±12.85	73.87±17.00	69.44±16.14	74.11±17.20	73.25±14.90

After a stabilization period, the peptide was infused for 10 min. Time 0 represents control values. Data were presented as mean ± SD. Rat PAMP had no effect on perfusion pressure, heart rate and developed pressure by repeated measures ANOVA and Bonferroni tests
PAMP - proadrenomedullin N-terminal 20 peptide

Table 3. Effect of rat calcitonin on perfusion pressure

Perfusion Pressure, mmHg											
Min											
Doses	0	5	6	7	8	9	10	15	20	25	30
10 nM n=7	45.80±7.36	46.83±7.44	47.00±7.37	47.33±7.17	47.50±7.36	48.0±7.4	47.83±7.30	48.16±7.27	47.50±7.28	47.66±7.58	47.83±7.38
100 nM n=7	49.0±9.5	49.16±9.10	50.33±9.60	50.33±9.60	50.3±9.6	50.50±9.56	49.83±7.78	49.66±7.76	50.00±7.66	50.10±7.66	50.20±7.66
1000 nM n=7	48.16 ±7.33	47.8±8.0	48.1±8.0	48.33±7.78	48.16±8.30	48.16±7.93	49.00±8.64	49.16±8.50	49.33±6.94	48.33±9.89	48.66±9.47

After a stabilization period, the peptide was infused for 30 min. Time 0 represents control values. Data are presented as mean ± SD. Rat calcitonin had no effect on perfusion pressure by repeated measures ANOVA and Bonferroni tests

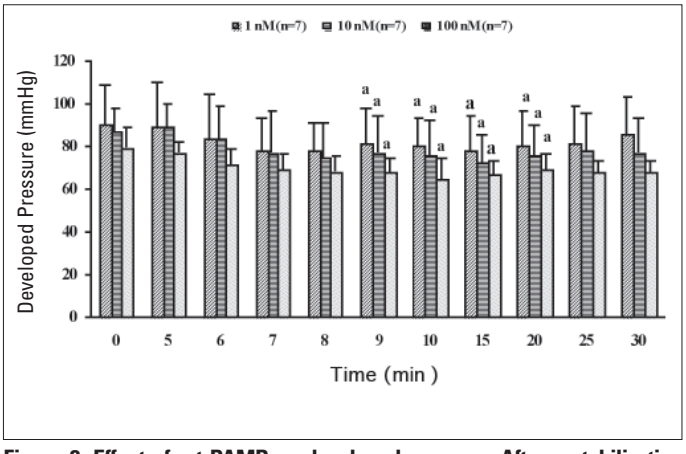

Figure 2. Effect of rat PAMP on developed pressure. After a stabilization period, the peptide was infused for 30 min. Time 0 represents control values. Data are presented as mean ± SD and vertical bars show SD. Rat PAMP decreased developed pressure

a - p<0.05 - significantly different from the respective control by repeated measures ANOVA and Bonferroni tests
PAMP - proadrenomedullin N-terminal 20 peptide

and 1000 nM) significantly reduced heart rate ($p<0.01$). The maximum decrease in heart rate was reached 7 to 9 min after the infusion of the peptide. Afterwards heart rate did not return to control values during the infusion. After an infusion period of 30 min, heart rate increased to the control values. Also this peptide did not alter developed pressure (Table 4). Salmon calcitonin (10, 100 and 1000 nM) did not significantly affect cardiovascular parameters including perfusion pressure, heart rate and developed pressure (Table 5).

Discussion

We found that rat PAMP caused to an increase in heart rate ($p<0.001$). In accordance with our results, Fujisawa et al. (27) demonstrated that rat PAMP (200 nM/kg) increased heart rate in conscious rats. We observed that rat PAMP caused to a decrease in developed pressure ($p<0.05$) and $+dP/dt_{max}$ ($p<0.001$). Similarly Yang et al. (9) reported that rat PAMP (10 pM-10 nM) decreased contractile force. In contrast to our results, Szokodi

Table 4. Effect of rat calcitonin on developed pressure

Developed Pressure, mmHg											
Min											
Doses	**0**	**5**	**6**	**7**	**8**	**9**	**10**	**15**	**20**	**25**	**30**
10 nM n=7	94.6±13.4	95.16±16.2	94.16±15.3	94.66±15.7	94.16±16.3	93.5±16.1	92.33±14.6	92±14.8	94.16±18.4	92.66±19.5	90.66±17.8
100 nM n=7	101.5±21.4	102.0±21.3	102.5±23.5	101.0±16.8	99.83±18.6	97.83±16.50	98.16±17.3	96.33±16.30	95.16±17.8	93.5±19.2	93.0±18.1
1000 nM n=7	95.33±14	95.0±14.8	95±16.1	96.66±13.40	96±14	93.66±15.10	91.6±14.0	94.33±13.3	93.83±13.70	92.66±13.60	90.5±14.1

After a stabilization period, the peptide was infused for 30 min. Time 0 represents control values. Data are presented as mean ± SD. Rat calcitonin had no effect on developed pressure by repeated measures ANOVA and Bonferroni tests

Table 5. Effect of salmon calcitonin on perfusion pressure, heart rate and developed pressure

Perfusion Pressure, mmHg					
Min					
Doses	**0**	**4**	**6**	**8**	**10**
10 nM n=6	42.98±2.75	45.68±5.88	45.63±11.23	45.78±8.37	45.86±8.74
100 nM n=9	47.08±8.90	49.13±9.26	49.31±8.32	50.07±8.08	50.09±8.18
1000 nM n=7	43.70±12.05	47.91±13.65	48.60±13.52	50.12±12.23	52.09±12.78
Heart Rate, Beats/Min					
Min					
Doses	**0**	**4**	**6**	**8**	**10**
10 nM n=9	226.66±40.46	217.00±46.50	216.33±47.70	215.22±47.10	222.31±46.80
100 nM n=9	266.77±30.93	269.33±28.50	257.11±29.90	255.00±29.32	251.43±31.10
1000 nM n=9	240.88±41.87	249.44±41.60	250.88±38.16	240.33±37.68	246.17±35.32
Developed Pressure, mmHg					
Min					
Doses	**0**	**4**	**6**	**8**	**10**
10 nM n=8	62.25±9.55	58.50±11.36	58.77±11.50	59.22±13.02	58.05±13.60
100 nM n=8	68.81±15.58	68.10±14.82	70.12±14.81	70.40±12.75	70.86±12.14
1000 nM n=8	63.52±13.89	66.37±15.27	66.20±14.50	64.81±15.54	66.65±14.65

After a stabilization period, the peptide was infused for 10 min. Time 0 represents control values. Data are presented as mean ± SD. Salmon calcitonin had no effect on perfusion pressure, heart rate and developed pressure by repeated measures ANOVA and Bonferroni tests

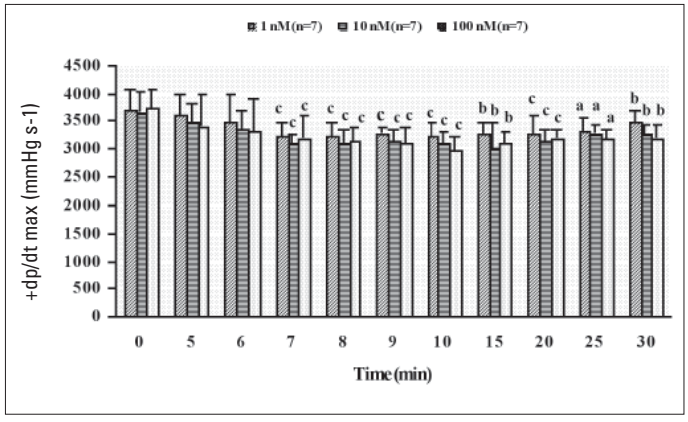

Figure 3. Effect of rat PAMP on +dP/dtmax. After a stabilization period, the peptide was infused for 30 min. Time 0 represents control values. Data are presented as mean ± SD and vertical bars show SD. Rat PAMP decreased +dP/dtmax.

a -p<0.05, b - p<0.01, c -p<0.001 - significantly different from the respective control by repeated measures ANOVA and Bonferroni tests

PAMP - proadrenomedullin N-terminal 20 peptide

et al. (11) demonstrated that rat PAMP (10-100 nM) had no effect on contractile force in isolated rat hearts. Here, we need further studies to explain the differences of the results. It has been reported that PAMP did not alter cAMP content in isolated rat hearts (28) but it is reported that the peptide inhibited the voltage-gated Ca^{2+} channel current in rat pheochromocytoma-derived PC 12 cells (29). It is known that Ca^{2+} influx through voltage-gated Ca^{2+} channels triggers the Ca^{2+} release from sarcoplasmic reticulum (30) and increased intracellular Ca^{2+} concentration increases contractility. Therefore, it is possible to say that the inhibition of Ca^{2+} current induced by PAMP may be responsible for the decrease in contractile force observed in our study. Our results suggest that rat PAMP may function as an endogenous modulator of heart rate and contractile force. We also found that the infusions of rat PAMP did not change perfusion pressure (p>0.05). Similarly, it has been reported that rat PAMP (200 nM/kg) had no vasodilatory effect in conscious rats (27). Furthermore, in rat aorta PAMP had no effect on the production of nitric oxide (31) which is a mediator of endothelium-dependent vasodilation (32). However, our finding is different

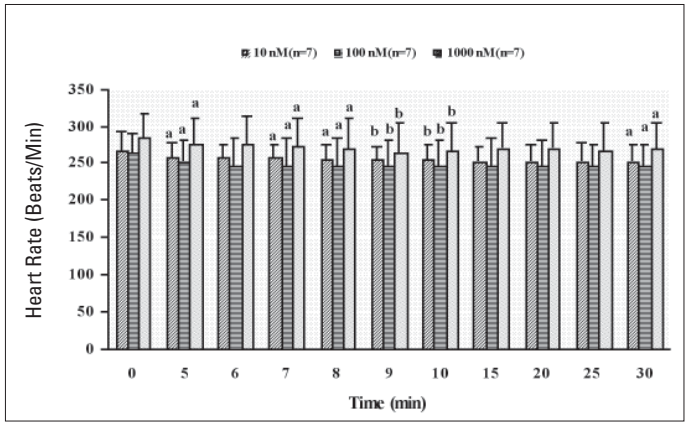

Figure 4. Effect of rat calcitonin on heart rate. After a stabilization period, the peptide was infused for 30 min. Time 0 represents control values. Data are presented as mean ± SD and vertical bars show SD. Rat calcitonin reduced heart rate.

a - p<0.05, b - p<0.01 - significantly different from the respective control by repeated measures ANOVA and Bonferroni tests

from those of Yang et al. (9), who observed that rat PAMP (10 pM-10 nM) produced a vasodilator effect in isolated rat hearts. It is not possible to explain reasons for this divergent result because our doses and experimental protocols are very similar to those of these investigators. Our data reproducibly indicated that rat PAMP had no vasodilatory activity and the explanation of the different results requires additional studies.

Our study indicated that human PAMP did not significantly alter the coronary perfusion pressure, heart rate and contractile force (p>0.05). The reason for the absence of significant effect dealing with heart rate and contractile force is not clear. It is evident that the peptide exerts species-specific effects. The effects might be greatly reduced or cancelled by a single amino acid variation in peptide chain. The amino acid sequence of human PAMP was identical with that of rat PAMP except for three amino acids (8). These insignificant effects on heart rate and contractile force may be attributed to the amino acid variations of human PAMP compared to that of rat PAMP. Our results suggest that human PAMP has no significant role in the regulation of coronary vascular tone, contractile force and rate in isolated rat hearts.

We demonstrated that rat calcitonin did not affect perfusion pressure (p>0.05) in any of the given doses while it decreased heart rate (p<0.01). Rat calcitonin did not cause any significant change in force of contraction (p>0.05). Our finding was similar to the result of Piao et al. (26) who demonstrated that rat calcitonin (1 μM) did not significantly change atrium contractility. The mechanisms by which rat calcitonin can modify the heart rate are not known. Possible mechanisms which decrease the discharge rate of sinoatrial (SA) node cells may be the increase of K^+ conductance in SA node cells and the slowness of the opening of Ca^{2+} channels (33). Additional studies are necessary to clarify the effects of rat calcitonin. Our result suggests that rat calcitonin may play a role in the regulation of heart rate by inducing a bradycardia effect. Our findings suggest that rat calcitonin may induce a modest bradycardic effect and we do not conclude that rat calcitonin may induce a physiologically relevant negative chronotropic effect especially on human.

We observed that salmon calcitonin did not significantly change coronary perfusion pressure, heart rate or contractile force (p>0.05). In agreement with our findings, experiments on isolated rat mesenteric vasculature had shown that salmon calcitonin (30 pM-10 nM) did not induce a significant change in perfusion pressure (25). Salmon calcitonin (1 μM) did not cause a significant contractility response on isolated rat atrium (26) and also it (<or = 100 nM) had no significant effect on contractile force of the rat ventricular myocytes (34). Additionally it has been reported that salmon calcitonin has no significant effect on blood pressure of the rats and concluded that salmon calcitonin does not produce acute systemic cardiovascular effects (18). According to our results, salmon calcitonin has no significant role in the regulation of cardiovascular functions. Salmon calcitonin is more potent than human calcitonin in humans (12) and it did not produce significant effects on the rat hearts. The absence of the significant effects of salmon calcitonin on the rat hearts may be related to species-specific effects of the peptide. Meanwhile, our results are not in agreement with the study showing negative chronotropic and negative inotropic effects of salmon calcitonin (0.005-0.16 units) in isolated dog atrium preparations (19). The discrepancy may depend on to the difference in species used in the experiments. In addition, we observed differences in means of the effects of rat calcitonin and salmon calcitonin. In comparison of amino acid sequence of rat calcitonin with salmon calcitonin rat calcitonin has fifteen different amino acids from that of salmon calcitonin (17). It is possible that even the presence of one different amino acid in the sequence may change or prevent the peptide action. Therefore, the different effects may be due to the amino acid variations.

Limitations of the study

We have no facility to study molecular mechanisms involve in the effect of PAMP and calcitonin on perfusion pressure, heart rate and contractile force. It is possible that PAMP or calcitonin may exert same effects on intact hearts but rat PAMP or rat calcitonin may not produce same effects on humans due to their strong species-specific actions. It is very difficult to extrapolate our results to humans and the clinical relevance questionable. Further studies are needed to determine clinical importance of these peptides.

Conclusion

We suggest that rat PAMP may produce positive chronotropic and negative inotropic effects, while rat calcitonin may cause a negative chronotropic effect. The rat forms of PAMP and calcitonin may play a role in the control of cardiovascular function. Our findings are not sufficient to conclude that rat calcitonin or rat PAMP may produce a physiologically relevant negative chronotropic effect especially on human. Further studies are necessary about cardiac effects of these peptides on humans. Furthermore, human PAMP or salmon calcitonin has no effect on coronary vascular tone, heart rate and contractile force of the isolated, perfused rat hearts.

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