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

Izole perfüze çıkan kalpler 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/dtmax 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 282.01±24.98 beats/min (p<0.01), from 298.88±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 79.0±15.3 mmHg (p<0.05), from 79.83±8.98 to 64.83±10.12 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/dtmax from 3710.5±370.6 to 3223.8±261.1 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 247.0±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 100 nM, respectively. Rat calcitonin (10.100 and 1000 nM) did not change perfusion pressure, heart rate and 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 and positive inotropic effect.

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 fonksiyonunda işe karışabileceği hakkında kanıtlar vardır. Bu nedenle çıkan PAMP’in, insan PAMP’in, çıkan kalsitonin’in ve somon kalsitonin’in koroner perfüzyon basıncına, dakikada kalp atım sayısına ve kasılma kuvvetine etkileri araştırıldı.

Yöntemler: İzole perfüze çıkan kalpler sabit akım durumunda perfüze edildi ve çıkan PAMP (1.10 ve 100 nM), insan PAMP (1.10 ve 100 nM), çıkan kalsitonin (10.100 ve 1000 nM) veya somon kalsitonin (10.100 ve 1000 nM) kalplere verildi. Koroner perfüzyon basıncı, dakikada kalp atımı, sol ventrikülün gelişirdiği basınç ve +dP/dtmax ölçüldü. İstatistiksel analiz tek kritaryan ölçüm değerleri için ANOVA ve Bonferroni testleri kullanılarak yapıldı.

Bulgular: çıkan PAMP (1.10 ve 100 nM) perfüzyon basıncını değiştirdi. Fakat dakikada kalp atımı sayısının 1.10 ve 100 nM dozlarında sırası ile 257.83±23.89 dan 282.01±24.98 atm/dakika’ya (p<0.001), 258.83±25.05 dan 289.83±19.5 atm/dakika’ya (p<0.001) ve 249.66±19.19 dan 280.50±25.26 atm/dakika’ya (p<0.001) arttırdı. çıkan PAMP sol ventrikülün gelişirdiği basınç 1.10 ve 100 nM dozlarında sırası ile 79.83±8.98 dan 64.83±10.12 atm/mmHg’ya (p<0.05), 79.83±8.98 dan 64.83±10.12 atm/mmHg’ya (p<0.05) azalttı. Bu peptit +dP/dtmax 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) azalttı. çıkan kalsitonin sol ventrikülün gelişirdiği basınç anlamlı olarak etkilemedi. İnsan PAMP veya somon kalsitoninin perfüzyon basıncını, dakikada kalp atımı sayısını ve sol ventrikülün gelişirdiği basınçını değişirdi.
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 adrenocorticotropic 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 resorption, 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 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°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 noncircuiting 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, CaCl2 2.5, MgSO4 1.2, KH2PO4 1.2, NaHCO3 25 and glucose 11 was used as the perfusion solution. The solution was continuously oxygenated with 95 % O2 and 5% CO2 and pH of the solution was 7.4. The temperature was maintained at 37°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/dtmax) 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 Elektronik, 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/dtmax (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 has been studied (26). Therefore, we used the doses of 1, 10 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 Lilliefors'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/dtmax 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

<table>
<thead>
<tr>
<th>Table 1. Effect of rat PAMP on perfusion pressure</th>
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<tr>
<td><strong>Perfusion Pressure, mmHg</strong></td>
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<td><strong>Min</strong></td>
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<tr>
<td><strong>Doses</strong></td>
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<tr>
<td>0 nM</td>
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<tr>
<td>1 nM</td>
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<td>10 nM</td>
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<td>100 nM</td>
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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**

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. 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.
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/dtmax (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
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

### Table 4. Effect of rat calcitonin on developed pressure

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<tr>
<th>Doses</th>
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<tbody>
<tr>
<td>10 nM</td>
<td>94.6±13.4</td>
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<td>94.16±15.3</td>
<td>94.66±15.7</td>
<td>94.16±16.3</td>
<td>93.5±16.1</td>
<td>92.33±14.6</td>
<td>92±14.8</td>
<td>94.16±18.4</td>
<td>92.66±19.5</td>
<td>90.66±17.8</td>
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<tr>
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<td>102.0±21.3</td>
<td>102.5±23.5</td>
<td>101.0±16.8</td>
<td>99.83±18.6</td>
<td>97.83±16.50</td>
<td>98.16±17.3</td>
<td>96.33±16.30</td>
<td>95.16±17.8</td>
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<tr>
<td>1000 nM</td>
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<td>95±16.1</td>
<td>96.66±13.40</td>
<td>96±14</td>
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Table 4. Effect of salmon calcitonin on developed pressure, heart rate and developed pressure

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<td>10 nM</td>
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<td>45.63±11.23</td>
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<td>100 nM</td>
<td>47.08±8.90</td>
<td>49.13±9.26</td>
<td>49.31±8.32</td>
<td>50.07±8.08</td>
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<tr>
<td>1000 nM</td>
<td>43.70±12.05</td>
<td>47.91±13.65</td>
<td>48.60±13.52</td>
<td>50.12±12.23</td>
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Table 5. Effect of salmon calcitonin on developed pressure, heart rate and developed pressure

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<tr>
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<td>269.33±28.50</td>
<td>257.11±29.90</td>
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<tr>
<td>1000 nM</td>
<td>240.88±41.87</td>
<td>249.44±41.60</td>
<td>250.88±38.16</td>
<td>240.33±37.68</td>
<td>246.17±35.32</td>
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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.

### Table 5. Effect of salmon calcitonin on developed pressure

<table>
<thead>
<tr>
<th>Doses</th>
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<tbody>
<tr>
<td>10 nM</td>
<td>62.25±9.55</td>
<td>58.50±11.36</td>
<td>58.77±11.50</td>
<td>59.22±13.02</td>
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<tr>
<td>100 nM</td>
<td>68.81±15.58</td>
<td>68.10±14.82</td>
<td>70.12±14.81</td>
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<tr>
<td>1000 nM</td>
<td>63.52±13.89</td>
<td>66.37±15.27</td>
<td>66.20±14.50</td>
<td>64.81±15.54</td>
<td>66.65±14.65</td>
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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.

Figure 3. Effect of rat PAMP on +dp/dt max. 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/dt max.

- 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
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 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 involved 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.
Acknowledgments
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