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*Original Article*

# Amyloid beta peptide 22-35 induces a negative inotropic effect on isolated rat hearts

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**Abstract:** Evidences indicate that deposition of amyloid beta peptides (Aβs) plays an important role in the pathogenesis of Alzheimer disease. Aβs may influence cardiovascular system and ileum contractions. But the effect of amyloid beta peptide 22-35 ($Aβ_{22-35}$) on cardiovascular functions and contractions of ileum has not been studied. Therefore, the present study aimed to investigate the possible effects of this peptide on isolated rat heart and ileum smooth muscle. Langendorff-perfused rat heart preparations were established. The hearts were perfused under constant pressure (60 mmHg) with modified Krebs-Henseleit solution. $Aβ_{22-35}$ at doses of 1, 10 and 100 nM significantly decreased left ventricular developed pressure (LVDP; an index of cardiac contractility) and maximal rate of pressure development of left ventricle ($+dP/dt_{max}$; another index of cardiac contractility). This peptide at doses studied had no significant effect on heart rate, coronary flow, monophasic action potential amplitude (MAPamp), MAP duration at 90% repolarization ($MAP_{90}$) and ileum contractions. We suggest that $Aβ_{22-35}$ exerts a negative inotropism on isolated rat hearts with unchanged heart rate, coronary flow, MAPamp, $MAP_{90}$ and smooth muscle contractility of ileum.

**Keywords:** Amyloid beta peptide 22-35, cardiac contractility, heart rate, coronary flow, monophasic action potential, ileum contraction

## Introduction

Alzheimer's disease (AD) is a dementia form occurred among aged people [1]. AD is characterized by memory loss and a decline in cognitive function [2]. The loss of synapses and neuronal deaths in brain are characteristic events in AD [2, 3]. Patients with AD and transgenic mouse models of the disease also show motor disorders [4]. Amyloid beta peptide (Aβ) is involved in AD and this peptide is formed from the transmembrane amyloid-β precursor protein (AβPP) by proteolytic processing via the action of enzymes called beta and gama secretases [5]. Aβ is the main component of the senile plaques in the brains of patients with AD and increased Aβ production plays an important role in the pathogenesis of AD [6]. In addition, it has been reported that both soluble and fibrillar forms of Aβ exert neurotoxic effects [7].

Aβ may influence cardiovascular system and smooth muscle contraction. Aβ produces vasoconstriction on cerebral arteries and decreases resting cerebral blood flow [8]. AβPP is also synthesized in extraneuronal human tissues [9] and soluble Aβ is found in plasma and cerebrospinal fluid [10]. It has been reported that Aβ changes vascular tone and induces endothelial damage in rat coronary arteries [11]. Aβ affects the beating rate of neonatal rat cardiomyocytes [12] and also impairs contractile function of skeletal muscle in frog [3]. The onset and progression of AD are correlated with cardiovascular disorders such as hypertension, atherosclerosis and myocardial infarction [13]. Furthermore, Aβ alters myocardial calcium homeostasis and involves in arrhythmogenesis [14].

Although it has been reported that $Aβ_{22-35}$ produces toxic effect on cultured neurons taken from the rat hippocampus [15], there is not any information about the effect of $Aβ_{22-35}$ on cardiovascular parameters and ileum contractions. We hypothesized that $Aβ_{22-35}$ may play a role in the regulation of cardiovascular functions and ileum contractions. Therefore, we aimed to study the possible influence of $Aβ_{22-35}$

on cardiovascular parameters of isolated rat hearts and smooth muscle contractility of rat ileum.

## Materials and methods

### *Animals, heart preparation and perfusion*

Female Sprague-Dawley rats weighing 250-300 g were used in our study. Rats were housed at 22-25°C with 12-hour light/dark cycle and fed with a standard chow diet and water ad libitum. All experiments were carried out in accordance with the "Guide to the Care and Use of Experimental Animals" by the Canadian Council of Animal Care [16]. The experimental protocols were approved by Ethical Committee of Researchs on Animals at Eskisehir Osmangazi University (30.01.2014/378). One hour after an intraperitoneal injection of 1000 IU heparin, rat's chest was opened under sodium thiopental (50 mg/kg) anesthesia. The heart was rapidly excised and immersed in ice-cold modified Krebs-Henseleit solution (mKHs). After contractions ceased, the surrounding tissues were removed. The aorta was immediately tied to a stainless steel cannula of the perfusion system. Retrograde perfusion was initiated at constant pressure (60 mmHg) using a noncirculating Langendorff technique. The pulmonary artery was excised to facilitate complete coronary drainage in the ventricles. Daily prepared mKHs with the following composition (mM/l): 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 for perfusion. mKHs was continuously gassed with carbogen (95% $O_2$ and 5% $CO_2$) and maintained at 37°C (pH 7.4 ± 0.1) throughout the experiments. The perfusion solution was filtered through a filter with a 45 μm pore size (Millipore filters, Ireland).

### *Measurement of cardiovascular parameters*

Cardiac contractile force was measured by using the previously described method by He and Downey [17]. A liquid-filled latex balloon connected to a pressure transducer (Isotec, Hugo Sachs Electronic, March-Hugstetten, Germany) was passed into the left ventricle through the mitral valve to measure peak systolic and end-diastolic pressure. Baloon was inflated with the aid of a glass syringe until baloon pressure reached a pressure of 8 mmHg and balloon pressure was maintained at this value. LVDP, a contractility index was calculated as the difference between the systolic and end-diastolic pressures. $+dP/dt_{max}$ was calculated from the left ventricular pressure by a data acquisition software (Isoheart Software, Version 1.5, Hugo Sachs Electronic, March-Hugstetten, Germany) and taken as the other contractility index. Heart rate was determined from the signals of the left ventricular pressure. The coronary flow reflecting the coronary vascular tone was obtained from the timed collection of the coronary effluent in a graduated cylinder. All of the cardiovascular parameters except coronary flow were analyzed by the Isoheart Software. MAPamp and $MAP_{90}$ were recorded by using contact electrode method [18] and MAP electrodes ($Ag/AgCl_2$) were used to record MAP. MAPamp and $MAP_{90}$ were recorded by pressing an electrode against the surface of left ventricular epicardium while other electrode touched the epicardium. A stable contact pressure was applied between the epicardium and MAP electrode.

### *Stabilization and administration of drug*

The hearts were allowed to equilibrate for 30 min with mKHs to obtain a stable baseline. Hearts were excluded if LVDP < 60 mmHg, $+dP/dt_{max}$ < 2800 mmHg $s^{-1}$, heart rate < 200 beats/min and there was an abnormal sinus rhythm. $A\beta_{22-35}$ (H-Glu-Asp-Val-Gly-Ser-Asn-Lys-Gly-Ala-Ile-Ile-Gly-Leu-Met-OH) was obtained from Abcam (Cambridge, England). $A\beta_{22-35}$ was dissolved in distilled water and stored at -20°C until used. After the stabilization period, $A\beta_{22-35}$ at the doses of 1, 10 and 100 nM was given to the hearts for 30 min. Each dose was administrated to a different group of the hearts. In the control group the hearts were perfused with mK-Hs without $\beta AP_{22-35}$.

### *Ileum preparation and measurement of contractility*

After rat anaesthetized with sodium thiopental (50 mg/kg), the abdomen was opened. The ileum was excised and placed into Tyrode solution with the following composition: (g/l) NaCl 8, KCl 0.2, $NaHCO_3$ 1, $CaCl_2$ 0.24, $MgCl_2$ 0.01, $NaH_2PO_4$ 0.05, glucose 1. The fat and connective tissue adhering to ileum were removed and ileum was cut about segments of 2 cm long. Ileum segments were suspended in isolated organ baths filled with Tyrode solution. The

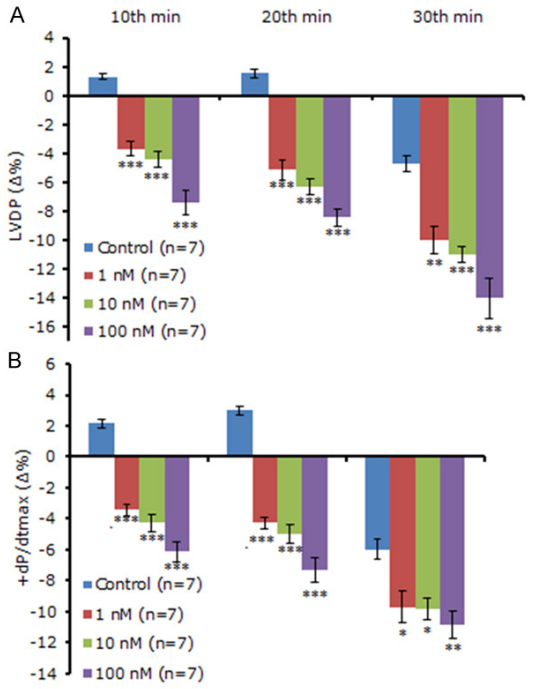


**Figure 1.** Influence of $A\beta_{22-35}$ on (A) LVDP and (B) $+dP/dt_{max}$. Δ% is percentage change from the value at 0th min in the control group and percentage change in LVDP or $+dP/dt_{max}$ from the value obtained prior to the administration of $\beta AP_{22-35}$ (the value at 0th min) in $\beta AP_{22-35}$ dose groups. +Δ% and -Δ% indicate increase and decrease, respectively. *P < 0.05, **P < 0.01 and ***P < 0.001 significantly different compared with the relevant control.

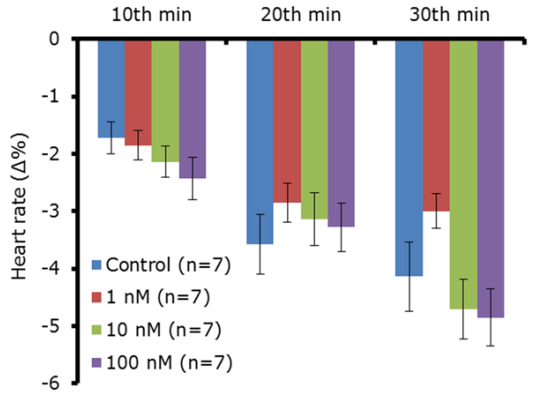


**Figure 2.** Influence of $A\beta_{22-35}$ on heart rate. Δ% is percentage change from the value at 0th min in the control group and percentage change in heart rate from the value obtained prior to the administration of $A\beta_{22-35}$ (the value at 0th min) in $A\beta_{22-35}$ dose groups. -Δ% indicates decrease.

solution was continuously gassed with a mixture of 95% $O_2$ and 5% $CO_2$ and maintained at 37°C and pH 7.4. One end of the ileum segments was tied to an isotonic transducer (FDT 10-A Biopac, Santa Barbara, CA, USA). The muscle strips of ileum were equilibrated in the Tyrode solution for 60 min. After strips were stretched to a resting tension of 1 g, dose responses to acetylcholine (10 μM) were obtained to assess the smooth muscle contractility of ileums. After that, cumulative concentration-response curves were performed for $A\beta_{22-35}$ at 1, 10, 100 nM and 1000 nM concentrations

*Statistical analysis*

The normality of data distribution was evaluated by using Kolmogorov-Smirnov test with Lilliefor's correction and Shapiro-Wilk test. The values of cardiovascular parameters and ileum smooth muscle contractions obtained after beta amyloid peptide administration were compared with control values using by one-way analysis of variance (ANOVA). Tukey-HSD multiple comparisons test was used to determine which groups significantly differ from each other. All values were expressed as mean ± SEM. A *p* value less than 0.05 was taken to be statistically significant.

## Results

Treatment with β $A\beta_{22-35}$ at the doses of 1, 10 and 100 nM significantly and dose-dependently reduced LVDP and $+dP/dt_{max}$ ($P < 0.001$). $A\beta_{22-35}$ maximally decreased LVDP from -4.71 ± 0.57% at baseline to -10 ± 0.97%, -11 ± 0.53% and -14 ± 1.4% for 1, 10 and 100 nm doses, respectively ($P < 0.01$ for 1 nM, $P < 0.001$ for 10 and 100 nM). $A\beta_{22-35}$ also caused maximal decreases in $+dP/dt_{max}$ from -6 ± 0.65% at control to -9.71 ± 1.02%, -9.85 ± 0.7% and -10.85 ± 0.88% for 1, 10 and 100 nm doses, respectively ($P < 0.05$ for 1 and 10 nM, $P < 0.01$ for 100 nM, **Figure 1**). Both LVDP and $+dP/dt_{max}$ did not return to the control values during the course of experiments and they reached the lowest level at the 30th min of the observation period.

As shown in **Figures 2-4**, all tested doses of $A\beta_{22-35}$ did not change heart rate, coronary flow, MAPamp and $MAP_{90}$. Furthermore, although there was a trend towards a dose-dependent inhibition, $A\beta_{22-35}$ at doses of 1 nM-1000 nM had no significant effect on the smooth muscle contractions of ileum (**Figure 5**).

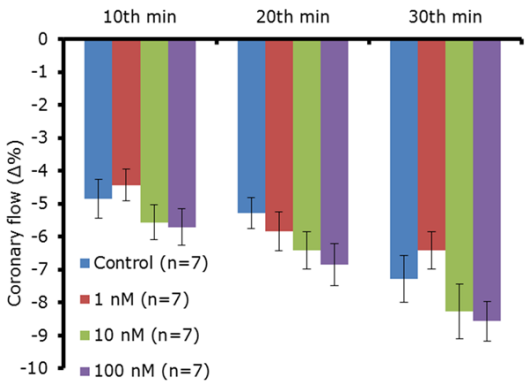


**Figure 3.** Effect of $A\beta_{22-35}$ on coronary flow. Δ% is percentage change from the value at 0th min in the control group and percentage change in coronary flow from the value obtained prior to the administration of $A\beta_{22-35}$ (the value at 0th min) in $\beta_{22-35}$ dose groups. -Δ% indicates decrease.

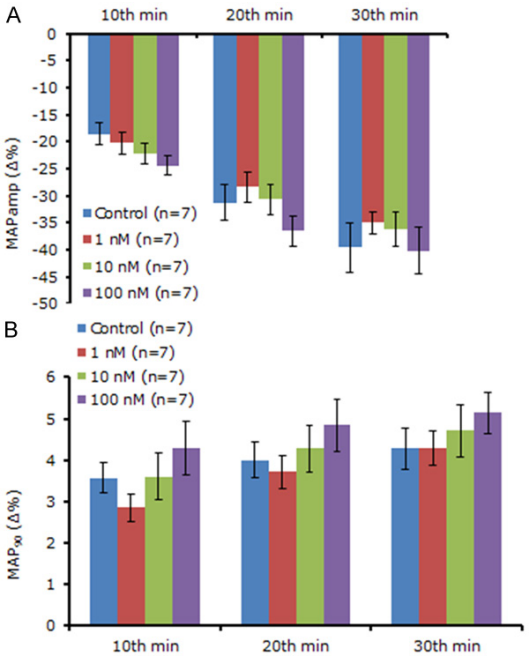


**Figure 4.** Effect of $A\beta_{22-35}$ on (A) MAPamp and (B) $MAP_{90}$. Δ% is percentage change from the value at 0th min in the control group and percentage change MAPamp or $MAP_{90}$ from the value obtained prior to the administration of $A\beta_{22-35}$ (the value at 0th min) in $A\beta_{22-35}$ dose groups. +Δ% and -Δ% indicate increase and decrease, respectively.

## Discussion

The results in the present study indicate that $A\beta_{22-35}$ exerts a negative inotropic effect. The mechanisms underlying the negative inotropic action induced by $\beta AP_{22-35}$ remain unclear. It has been found that $A\beta_{22-35}$ decreases phosphorylated phospholamban (p-PLB), p-PLB/PLB ratio, intracellular $Ca^{2+}$ transient ($Ca^{2+}_i$), $Ca^{2+}$ store of sarcoplasmic reticulum (SR), L-type $Ca^{2+}$ channel expressions and L-type $Ca^{2+}$ current in pulmonary vein cardiomyocytes of rabbit [14]. The reduction in p-PLB and p-PLB/PLB ratio result in a decrease in SR calcium pool, which may lead to the diminished $Ca^{2+}_i$. The reduction in the L-type $Ca^{2+}$ current may also decrease $Ca^{2+}_i$ [14]. Furthermore, skeletal muscle from transgenic mice overexpressing AβPP exerts $Ca^{2+}$ transient with small amplitude and develops less contractile force [19]. It has been reported that $Ca^{2+}$ signaling is decreased in AD [20] and it is well known that decreased $Ca^{2+}_i$ causes a reduced myocardial contractility. Therefore, the decrease in $Ca^{2+}_i$ may be responsible for the negative inotropic effects of $A\beta_{22-35}$ observed in the present study. Moreover, p38 mitogen-activated protein kinases (MAPK) are activated in AD and p38 MAPK pathway involves in cardiomyocyte contractile dysfunction [21]. Thus, $A\beta_{22-35}$-induced negative inotropic action may also be mediated by p38 MAPK pathway. Further studies are needed to clearly explain the effect of $A\beta_{22-35}$ on myocardial contractile force.

Our findings demonsrate that $A\beta_{22-35}$ did not change heart rate and coronary flow. In neonatal rat cardiomyocytes, Haase et al. [12] observed that $A\beta_{25-35}$ and $A\beta_{10-35}$ increases heart rate, whereas $A\beta_{1-15}$, $A\beta_{1-40}$ and $A\beta_{1-42}$ decreases this parameter. They suggested that the different resuls may be depend on the difference of amino acid sequence of amyloid beta peptides (Aβs). On the other hand, Haase et al. [12] also observed that $A\beta_{25-35}$ produces a coronary vasoconstrictory action in Langendorrf-perfused rat hearts. $A\beta_{1-40}$ and $A\beta_{1-42}$ also enhances endothelin-1-induced vasoconstriction in rat aorta [22] and vasoconstriction decreases coronary flow [23]. Moreover, $A\beta_{-40}$ reduces vasodilator response induced by acetylcholine in the rat aorta [24]. The variation in the amino acid sequence of Aβs may be responsible for the divergent results. Further studies are necessary for explanation of the effect of $A\beta_{22-35}$ on heart rate and coronary flow.

The MAP method is a very beneficial for understanding the cardiac electrophysiology in physiological and clinical studies [25] and MAP is a reflection of the myocardial transmembrane

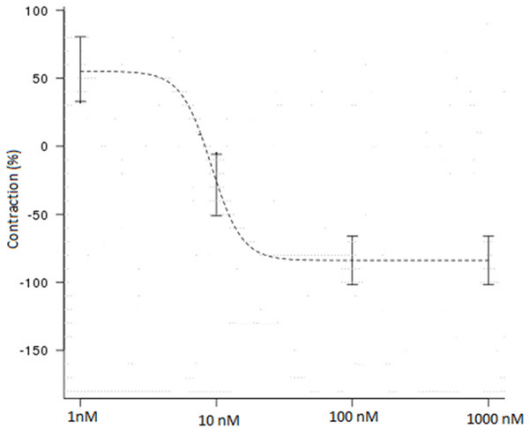


**Figure 5.** Effect of the doses of $A\beta_{22-35}$ on isolated rat ileum (n=9). Bars indicate SD values.

MAP recorded from outside of the cell [26]. MAPamp is determined by inward sodium current during the upstroke of MAP [27]. On the other hand, MAP duration shortens as heart rate enhances under physiological conditions. The elevations in outward repolarization currents and/or reductions in inward depolarization currents involve in frequence dependence of MAP duration [28]. In the present study, $A\beta_{22-35}$ did not affect MAPamp and $MAP_{90}$. Our results indicate that $A\beta_{22-35}$ does not changes the currents, which contribute to MAPamp and $MAP_{90}$ formation.

We observed that $A\beta_{22-35}$ did not influence ileum contractility. Shimohigashi et al. [29] reported that $A\beta_{21-35}$ at the doses range of 10 nM-100 μM induces contractions of smooth muscle of guinea pig ileum. In agreement with our results, they also reported that $A\beta_{25-35}$ which is a shorter peptide does not affect ileum conractility of guinea pig at 10 nM-100 μM doses. Further studies are needed to define the action of $A\beta_{22-35}$ on smooth muscle contraction of ileum.

In conclusion, we present first time that $A\beta_{22-35}$ induces a decrease in cardiac contractility. In addition, our findings also suggest that $A\beta_{22-35}$ does not alter heart rate, coronary flow, MAPamp, $MAP_{90}$ and ileum contractility. Furthermore, $A\beta_{22-35}$ accummulation in AD may cause decrease in myocardial contractility.

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## Disclosure of conflict of interest

None.

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