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The electrocardiographic changes generated by centrally applied arachidonic acid in rats

Esra KAŞIKCI, Murat YALÇIN*

[1] **1.** Department of Physiology, Faculty of Veterinary Medicine, Bursa Uludag University, Bursa, 16059, Turkey.
Kaşıkçı E. ORCID: Orcid: 0000-0003-0155-5385; Yalçın M. ORCID: 0000-0002-5600-8162

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ABSTRACT

Arachidonic acid (AA) and its metabolites have multifunctional regulatory effects on the central nervous system. Our previous reports disclosed that centrally injected AA organized the cardiovascular system in normal or hypotensive conditions by regulating the central and peripheral mechanism. In the light of the knowledge of the potential cardiovascular effects of AA, the current study aimed to investigate the effects of intracerebroventricular (ICV) injected AA on the electrocardiography (ECG) of the anesthetized rats. The adult Sprague Dawley rats were anesthetized with ketamine and xylazine mixture (50 mg/kg and 20 mg/kg; i.m., respectively). Under the anesthesia, the guide cannula was inserted into the left lateral ventricle of the rats. The ECG traces obtained from the lead II were written by placing electrodes on the limbs of the rats. Centrally injected AA (150 µg; ICV) statistically significantly ($p<0.05$) caused to the lengthening of the ECG waves and intervals, resulting in a decrease in the heart rate of the rats without changing the ECG waveforms, the amplitude, and also the isoelectric line. The obtained results clearly show that centrally injection of AA caused the deceleration in the heart electrical activity. The deceleration in the electrical activity of the heart caused to show bradycardia in the rats by extending the duration of the ECG waves and intervals.

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Introduction

Arachidonic acid (AA), a membrane phospholipid, is abundant in central nervous system and involved in multifunction tasks (Rapoport, 2008). AA, itself, and its many biologically active cyclooxygenase (COX) and lipoxygenase (LOX) products play a crucial role in homeostasis, including synaptic signaling, neuronal firing, neurotransmitter release, nociception, neuronal gene expression, cerebral blood flow, the sleep–awake cycle, appetite (Bosetti, 2007). They are also involved in the central modulation of ion channels and the activity of many enzymes, including protein kinase A, protein kinase C, and NADPH oxidase (Katsuki and Okuda, 1995). It was reported that the hyperventilation effect with central AA injection could obtain with both central LOX (Guvenc-Bayram et al., 2020) and COX pathways (Erkan et al., 2016; 2017). Moreover, neuroendocrine effects of AA and its metabolites central injection have also been reported (Yalcin and Savci, 2004; 2007; Aydin and Yalcin, 2008; Yalcin et al., 2005a).

The central AA and its pathways are especially very active in cardiovascular modulation. Our previous report clearly showed that centrally administrated AA could produce a pressor effect by activating central COX-thromboxane A2 (TXA2) -prostaglandin (PG) D, -PGE and -PGF2α signaling pathways in normal and stimulated conditions (Erkan et al., 2016; Aydin and Yalcin, 2008; Yalcin, 2011; Yalcin and Aydin, 2009;

*Corresponding Author: Murat Yalçın
E-mail: muraty@uludag.edu.tr

2011; Altinbas et al., 2014). The centrally injected AA also generated bradycardia in normotensive animals (Erkan et al., 2016; Aydin and Yalcin, 2008; Yalcin, 2011; Altinbas et al., 2014) but tachycardia in hypotensive animals (Yalcin and Aydin, 2009; 2011). Recently we reported that intravenous (i.v.) administered AA caused bradycardia along with delay in heart electrical activity according to electrocardiography (ECG) data (Kasikci and Yalcin, 2022).

According to the previous reports, the centrally applied AA is functional in the cardiovascular system for blood pressure and heart rate, but the role of centrally applied AA on ECG reflecting the electrical activity of the heart is unknown. In the light of previous reports, the aim of the current study is to examine the role of ICV injected AA on the ECG waves as the central effect.

Materials and Methods

Ten Sprague–Dawley rats were used in the study with approving The Animal Care and Use Committee of Bursa Uludag University (2020-03/05). The animals were anesthetized by using ketamine/xylazine (50 mg/kg/20 mg/kg; i.m.) mixture. The rats were kept under anesthesia throughout the experiment. Under the anesthesia, the rats were placed in a stereotaxic frame to insert the guide cannula for ICV injection. For this reason, a burr hole was drilled through the skull 1.5 mm lateral to the midline and 1.0 mm posterior to the bregma according to the coordinates, which were taken from the atlas of Paxinos and Watson (2005). The guide cannula made of 22-gauge steel hypodermic tubing was directed through the hole towards the lateral ventricle. The cannula was lowered 4.2 mm below the surface of the skull and fixed to the skull by using acrylic cement. For the ICV injection, a hand-made injection cannula was used. The injection cannula was connected to a polyethylene tubing, which was filled with saline or saline containing the desired dose of the drug of interest in a 10 µl microsyringe. For the ICV treatment, the injection cannula was inserted through the guide cannula and 5 µl volume of saline or the drug solution was infused slowly within 60 s.

The animals were divided into two groups which included 5 rats in each group, as the control and the experimental groups. The animals in the control group and experimental group were treated with saline (5 µL; ICV) and AA (150 µg; ICV), respectively. After the treatments, the ECG of the rats was recorded for 60 min. AA purchased from Sigma-Aldrich Co. (Deisenhofen, Germany) was freshly disolved in saline on the day of the experiment. The dose of AA was chosen from the previous study (Yalcin, 2011).

The leads II ECG of the anesthetized rats was recorded by inserting the ECG electrodes the limbs of the rats. The ECG traces were analyzed in MP36 system having AcqKnowledge software (BIOPAC Systems Inc.). The P and the T waves duration, the QRS complex duration, and the P-R, the Q-T, and the R-R intervals duration were used as ECG parameters in the present study. The heart rate (HR) of the rats was calculated by using the R-R intervals duration formula and expressed as beats per minute (bpm).

Sigma Stat 3.5 software (CA, USA) was used for the statistical analysis of data. For Statistical analysis, repeated-measures analysis of variance (ANOVA; two-way) and the post-ANOVA test of Bonferroni were preferred. The data given as mean ± standard error of the mean (SEM) in the graphs were considered significant at $p<0.05$.

Results

The basal levels of the ECG waves and intervals duration, and the basal HR of the anesthetized rats for both treatments were shown in Table 1 and Figure 1 as “0” min data, respectively. ICV injection of AA statistically significant ($p<0.05$) caused to increase in the duration of the P wave, the T wave, the QRS complex, the P-R interval, the Q-T interval, and the R-R interval compared to saline treatment (Table 1). Also, ICV injection of AA produced the bradycardia by decreasing the HR of the anesthetized rats (Figure 1) compared to the control animals.

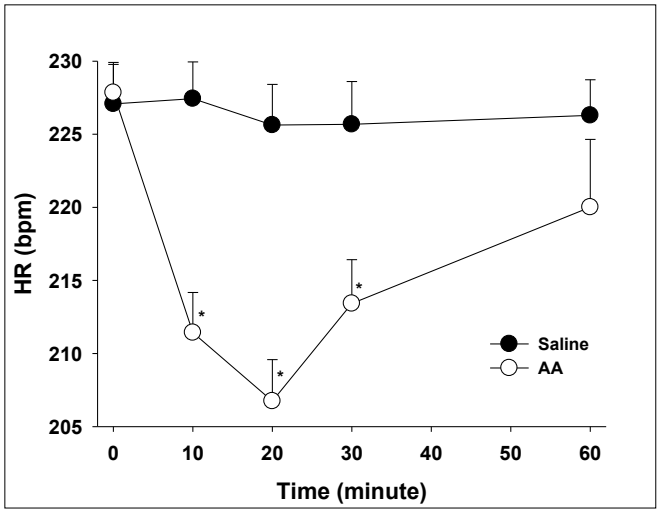

Figure 1. Effect of centrally injected AA on HR in the anesthetized rats. Saline (5 µl; ICV, n=5) or AA (150 µg; ICV, n=5) was injected to the rats. Before and 60 mins after injections, ECG was monitored for the next 60 min. HR measurements obtained from the ECG. Statistical analysis was performed using two-way RM-ANOVA with a post hoc Bonferroni test. *$p<0.05$, significantly different from the value of the saline-treated group.

Table 1. Effect of centrally injected AA on ECG waves and intervals duration in the anesthetized rats.

The duration (Second)		Time (Minute)				
		0	10	20	30	60
P	Saline	$0.0252 \pm 2.0x10^{-4}$	$0.0248 \pm 3.2x10^{-4}$	$0.0248 \pm 4.0x10^{-4}$	$0.0250 \pm 2.0x10^{-4}$	$0.0248 \pm 4.0x10^{-4}$
	AA	$0.0252 \pm 1.7x10^{-4}$	$0.0261 \pm 4.0x10^{-4}$ *	$0.0269 \pm 4.7x10^{-4}$ *	$0.0258 \pm 5.0x10^{-4}$ *	$0.0254 \pm 3.2x10^{-4}$ *
T	Saline	$0.0520 \pm 3.1x10^{-4}$	$0.0510 \pm 3.8x10^{-4}$	$0.0514 \pm 3.4x10^{-4}$	$0.0518 \pm 3.6x10^{-4}$	$0.0508 \pm 3.2x10^{-4}$
	AA	$0.0520 \pm 2.8x10^{-4}$	$0.0618 \pm 2.4x10^{-4}$ *	$0.0686 \pm 3.1x10^{-4}$ *	$0.0604 \pm 2.9x10^{-4}$ *	$0.0568 \pm 2.4x10^{-4}$ *
QRS	Saline	$0.0220 \pm 1.3x10^{-4}$	$0.0219 \pm 4.0x10^{-4}$	$0.0219 \pm 4.0x10^{-4}$	$0.0222 \pm 4.9x10^{-4}$	$0.0218 \pm 4.4x10^{-4}$
	AA	$0.0220 \pm 1.5x10^{-4}$	$0.0239 \pm 6.8x10^{-4}$ *	$0.0244 \pm 4.5x10^{-4}$ *	$0.0236 \pm 4.5x10^{-4}$ *	$0.0234 \pm 4.3x10^{-4}$ *
P-R	Saline	$0.0516 \pm 4.0x10^{-4}$	$0.0515 \pm 4.9x10^{-4}$	$0.0513 \pm 4.0x10^{-4}$	$0.0514 \pm 4.5x10^{-4}$	$0.0516 \pm 4.1x10^{-4}$
	AA	$0.0516 \pm 3.6x10^{-4}$	$0.0526 \pm 4.1x10^{-4}$ *	$0.0529 \pm 4.3x10^{-4}$ *	$0.0520 \pm 5.5x10^{-4}$ *	$0.0524 \pm 3.9x10^{-4}$ *
Q-T	Saline	$0.0700 \pm 4.2x10^{-4}$	$0.0690 \pm 3.3x10^{-4}$	$0.0700 \pm 4.2x10^{-4}$	$0.0690 \pm 4.0x10^{-4}$	$0.0710 \pm 3.5x10^{-4}$
	AA	$0.0700 \pm 2.4x10^{-4}$	$0.0810 \pm 2.4x10^{-4}$ *	$0.0850 \pm 2.9x10^{-4}$ *	$0.0790 \pm 2.7x10^{-4}$ *	$0.0750 \pm 2.6x10^{-4}$ *
R-R	Saline	$0.2900 \pm 1.2x10^{-3}$	$0.2980 \pm 1.4\ x10^{-3}$	$0.2960 \pm 1.3\ x10^{-3}$	$0.2980 \pm 1.4\ x10^{-3}$	$0.2960 \pm 1.5\ x10^{-3}$
	AA	$0.2900 \pm 1.0x10^{-3}$	$0.3120 \pm 0.9\ x10^{-3}$ *	$0.3300 \pm 0.9x10^{-3}$ *	$0.3260 \pm 1.2x10^{-3}$ *	$0.3180 \pm 1.4x10^{-3}$ *

Saline (5 µl; ICV, n=5) or AA (150 µg; ICV, n=5) was injected to the rats. Before and 60 mins after injections, ECG was monitored. The duration of the P wave, the T wave, the QRS complex, the P-R interval, the Q-T interval, and the R-R interval measurements were obtained from the ECG. Statistical analysis was performed using two-way RM-ANOVA with a post hoc Bonferroni test. *p<0.05, significantly different from the value of the saline-treated group.

It was observed that the delay in the duration of the ECG traces and resulting the bradycardia, which was produced by ICV injection of AA, started just after the injection and lasted 60 mins (Table 1, Figure 1). The most potent effects in the ECG traces and the HR were observed 20 min after the AA injection (Table 1, Figure 1). Although ICV injected AA caused to lengthen the rate of the electrical activity of the heart, it did not alter the ECG waveforms, amplitude, and isoelectric line.

Discussion

The current findings demonstrated that ICV administered AA let to bradycardia by prolonging the duration of the ECG waves and intervals without changing the ECG waveforms, amplitude as well as the isoelectric line.

ECG is a simple technic but presents important knowledge about the myocardium's functional and structural characteristics by reflecting the heart's electrical activity. Thus, the ECG gives information about the heart's work with the progression of the action potential produced in the sinoatrial node, which is a natural pacemaker along the atria and ventricles. As a result, an ECG recording shows the P wave during atrial depolarization, the QRS complex during ventricular depolarization, and the T wave during ventricular repolarization (Hall, 2011; Wagner et al., 2009). The current findings have shown that ICV AA administration increases the duration of ECG waves and intervals, resulting in bradycardia. The heart has sympathetic and vagal nerves effects to provide heart rate homeostasis (Zhang and Anderson, 2014). The current findings showing ICV injected AA-induced bradycardia with are consistent with previous reports (Erkan et al., 2016; Aydin and Yalcin, 2008; Yalcin, 2011; Altinbas et al., 2014). The bradycardia and delay in ECG waves observed after the ICV applied AA may be due to the baroreflex response developed as a result of the increase in blood pressure in response to the application of central AA. Because central AA injection causes an increase in plasma catecholamine, vasopressin, and angiotensin levels (Aydin and Yalcin, 2008), which cause an increase in blood pressure and peripheral resistance in normotensive animals (Erkan et al., 2016; Aydin and Yalcin, 2008; Yalcin, 2011; Altinbas et al., 2014), and may mediate the activation of the baroreflex mechanism as a homeostatic mechanism. Moreover, we recently reported that IV injected AA caused bradycardia with delay in ECG waves and intervals in similar way to the current findings (Kasikci and Yalcin, 2022). This effect of IV administered AA may also have exerted a central effect by crossing the blood-brain barrier. Because it is well known that AA can easily cross the blood-brain barrier bi-directly (Pifferi et al., 2021). In addition, it was reported that centrally applied TXA2 mimetic stimulated cardiac vagal afferent fibers to elicit reflex changes in HR resulting the bradycardia (Wacker et al., 2002). This report confirms the bradycardia response with the delay in ECG waves

obtained in the current study. Because centrally administered AA may cause bradycardia by slowing down the electrical activity of the heart by stimulating the afferent fibers of the vagal nerve, similar to the effect of TXA2.

AA, a polyunsaturated phospholipid of the cell membrane, is abundant in the central nervous system (Rapoport, 2008). AA itself is involved in many physiological adjustments, particularly in central cardiovascular regulation (Rapoport, 2008; Bosetti, 2007). Previously we reported that ICV applied AA causes to increase in blood pressure by increasing plasma adrenaline, noradrenaline, and vasopressin levels, and renin activity in normotensive (Erkan et al., 2016; Aydin and Yalcin, 2008; Yalcin, 2011; Altinbas et al., 2014) and hemorrhaged hypotensive rats (Yalcin and Aydin, 2009; 2011). Again, we showed that centrally injected TXA2, one of the AA metabolites, can increase blood pressure in normal conditions and reverse hypotension in hemorrhagic shock conditions by activating brain TXA2 receptors (Yalcin and Savci, 2004; Yalcin et al., 2005a; 2005b; 2006). The activation of peripheral catecholaminergic, vasopressinergic, and renin–angiotensin systems mediates these cardiovascular responses to TXA2 (Yalcin and Savci, 2004). Additionally, our previous report demonstrated that centrally administered melittin, as a phospholipase A2 activator, affects the cardiovascular system and increases blood pressure in both normal (Yalcin et al., 2006; Yalcin and Erturk, 2007) and hypotensive conditions (Yalcin and Savci, 2007). The activation of central TXA2 (Yalcin et al., 2006) or cholinergic nicotinic receptors (Yalcin and Erturk, 2007) is partially involved in these effects of melittin, and the increase in plasma catecholamine, vasopressin, and renin activity mediates the cardiovascular responses to melittin in both conditions (Yalcin and Savci, 2007). Moreover, peripherally injected melittin also causes a pressor effect by activating the central cyclooxygenase (COX) pathway and cholinergic nicotinic receptors (Yalcin et al., 2009). This is because while pretreatment with central indomethacin, a nonselective COX inhibitor, completely blocked the cardiovascular effects evoked by intraperitoneally injected melittin, pretreatment with mecamylamine, a nicotinic receptor antagonist, did so only partially (Yalcin et al., 2009). Central PGD, PGE and PGF2α, AA metabolites (Erkan et al., 2017), and the central lipoxygenase pathway (Guvenc-Bayram et al., 2020) are also involved in the AA produced pressor effect. Centrally administered AA causes an increase in blood pressure in normotensive animals but to decrease in heart rate as similar to the current findings (Erkan et al., 2016; Aydin and Yalcin, 2008; Yalcin, 2011; Altinbas et al., 2014). These studies collectively suggest that the central AA cascade plays a very important role in the central regulation of the cardiovascular system. Consistent with the results of the current study, AA, which plays a role in central cardiovascular regulation, also may direct the work of the heart by affecting the electrical activity of the heart.

Conclusion

In summary, the present findings suggest that ICV administration of AA generates bradycardia by prolonging the rate of the electrical activity of the heart. The similar increase in the duration of the ECG waveforms and intervals might mean that centrally injected AA activates the nervous influence on the heart. The nervous effect on the heart may have occurred directly over the entire heart or through the sinoatrial node. It is possible that the neural effect may have been secondary to the baroreflex response. The fact that the amplitude of the ECG waves and the isoelectric line were not affected strengthens this possibility.

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