



## THE ANGIOTENSIN II ARRHYTHMOGENIC POTENTIAL THROUGH CENTRAL NERVOUS MECHANISM IN RATS

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**Abstract.** Angiotensin II (ATII) causes through the central nervous mechanism certain cardiovascular disorders. The participation of a central nervous component in the genesis of cardiac arrhythmia caused by Angiotensin is stated. The present paper describes the action exerted by ATII on the cardiac rhythm, after intracerebroventricular administration (icv) in rats anesthetized with ethylic urethane. The disorders of the cardiac rhythm induced by the ATII may be prevented or attenuated by blocking the AT1 receptors with an adequate antagonist, administered through icv.

**Keywords:** Angiotensin II, experimental centrogenic cardiac arrhythmias.

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### Introduction

Angiotensin II (ATII) acts on the cardiovascular system under normal and pathologic circumstances. Its intervention is produced by the participation of certain peripheral and central mechanisms (8). The central regulatory effect appears through the central rennin-angiotensin system. The ATII actions are mediated through AT<sub>1</sub> receptors.

The central nervous mechanisms through which the angiotensin participates in the regulation of arterial pressure under normal and pathological circumstances have been long known and are well documented by the literature (7, 11, 15). Although the literature provides detailed information on the participation of this peptide in the arterial hyper-

tension pathogenesis, the disorders of the cardiac rhythm caused by angiotensin and aldosterone receive only sporadic attention (6). There is no reference to the interference of a central nervous mechanism in the development of cardiac arrhythmias. That is the reason why we have undertaken the research of the effects of angiotensin II on cardiac frequency and rhythm after microinjection into the lateral cerebral ventricle in anaesthetized rats.

### Materials and Methods:

#### Laboratory animals

Experiments were conducted on white Wistar Bratislava rats, from UMF Bio-base Cluj-Napoca. We have considered male and female animals, weighing 180±20 gr. Before test rats had been kept under laboratory conditions for 24 hours, with the observance of the light-darkness daily cycle. They were provided with standard food and tap water "ad libitum." The temperature was 22±2°C. The tests were conducted between 10 am and 3 pm.

#### Analyzed Substances

Angiotensin II solution ("Angiotensin II Hu-

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mana" original solution 1mg/ml, produced by "Calbiochem", Germania) – agonist of the AT<sub>1</sub>-angiotensinergic receptors;

Candesartan pure substance (Candesartan pulvis, produced by Astra Zeneca, Swededn) – as antagonist of AT<sub>1</sub> angiotensin II receptors, as antagonist.

Angiotensin II was dissolved in physiological serum in order to obtain the utilized doses (see below). Candesartan was dissolved by adding Na<sub>2</sub>CO<sub>3</sub> 1N, the final pH of the solution being 8.

**The Experiment Protocol**

The experiment protocol was the applied in the stereotaxis laboratory of the Pharmacology Department, at UMF Cluj-Napoca (12).

For the general anesthesia of the rats we have used an intraperitoneal injection with ethylic urethane 1,25 g/kg. Then, the animals were set in the stereotaxic device "Medicor" (Hungary); after scalp sectioning and removal, the skull bones were tamponed with a perhydrol solution to obtain an optimal view of sutures. Bones were then perforated with an electric trepan in order to reach the left cerebral ventricle. It was identified by using the Szentágothai (14) atlas as follows: posterior (P)=1mm, the zero point (0) being considered the bregma, lateral (L) position from the median line=1.5mm, depth (H) = 4.5 mm under the dura mater.

The electrocardiogram (ECG), was recorded in D1 – D2 or D1-D2-D3 derivations. These parameters were written by "Bioscript BST 1", a carbon-paper mechanic writing device.

The intracerebroventricular administration (icv) of the substances was performed by means of a Hamilton microsyringe, adapted for this purpose, by modifying the needle so that it could be attached to the needle-holder of the stereotaxic device.

A control recording of the cardiac frequency and rhythm was performed before microinjecting the substances. Then, the substance was administered, the effects being continuously monitored by recording parameters.

During the first stage of the research, the doses were tested as follows:

- In the case of the angiotensin II we have injected icv progressively increasing doses (25-50-100-200 µg) in different animals, in order to identify the doses that might cause alterations of the cardiac parameters. The ECG recorded lasted 3-5 minutes.
- In the case of the candesartan, we intended to set a dose that, after its icv administration, might diminish or prevent disorders of the cardiac activity when the arrhythmogenic dose of angiotensin II was re-administered.

The next stage involved the icv injection of the substances according to the following scheme:

**Angiotensin II (arrhythmogenic dose) - Candesartan – Angiotensin II (arrhythmogenic dose)**

The agonist was re-administered 10 minutes after the candesartan microinjection.

The result evaluation criteria were: (a) cardiac frequency (FC) – expressed by the number of cardiac beats per minute, (b) arrhythmogenic activity, evaluated through the calculation of the arrhythmogenic index (IA), which expresses the relative frequency of ectopic beats on a three-minute interval (3). As this does not take into account the nature of rhythm disorders, they were also described.

**Statistic Processing of Results Obtained**

The values of the same parameters (e.g. FC or IA) obtained before/after the treatment on a group of animals were compared by means of the Excel „t-test paired two sample for means". The statistical significance threshold was p=0.05.

**Results**

After the "per se" administration of the substances we have obtained the following data:

I.1. ATII administered in doses of 25 and 50 µg did not cause significant changes in the parameters (table 1).

No. Obs.	CONTROL		AFTER ATII		Statistical comparison of effects (x,y)	
	CF average/min (x)	ARRHYTHMIAS (3 min)	CF average/min (y)	ARRHYTHMIAS (3 min)	AI	P
10	386±57.38	0	377±54.98	0	0	0.362219

**Table 1:** ATII action after icv microinjection of 25 and 50µg doses on the cardiac frequency and rhythm: Legend CF-cardiacfrequency, AI- arrhythmogenic index

No. Obs.	CONTROL		AFTER ATII		Statistical comparison of effects (x,y)	
	CF average/min (x)	ARRHYTHMIAS (3 min)	CF average/min (y)	ARRHYTHMIAS (3 min)	AI	p
10		0		4.18±4.95	1.09±1.49	0.002536

**Table 2:** ATII action after icv microinjection of a 100µg dose on the cardiac frequency and rhythm:

Legend CF-cardiac frequency, AI- arrhythmogenic index

The microinjection of a dose of 100 µg, caused bradycardia, bradyarrhythmia, sinus pauses, and an increase in the R-R interval; ventricular extrasystoles were less frequent. The changes caused by this dose are shown in table 2

The conjoined administration of the substances, according to the scheme: AGONIST – ANTAGONIST – AGONIST has yielded the following results:

I.1. The icv administration of a 100 µg ATII dose caused certain changes in the parameters, which were similar to those described above at A.I.1.

I.2. After the candesartan treatment, the re-administration of the initial angiotensin dose was followed by a significant decrease in the number of occurrences of cardiac rhythm disorders. The aspects presented above are described in table 3 and figure 2.

**Interpretation of results**

Although the literature provides detailed information on the influence ATII exerts on arterial pressure, the disorders of the cardiac rhythm caused by this neuropeptide receive only sporadic attention (6). There is no reference in the literature to the interference of a central nervous mechanism in the development of cardiac arrhythmias induced by ATII.

Before actually discussing our results, we should note that years ago an experiment was conducted on dogs; angiotensin was injected through an icv perfusion in small doses (5-7 µg/kg) in dogs and it did not cause any changes in the electrocardiogram (2). In our research, by administering icv gradually increasing doses of angiotensin, we have identified its arrhythmogenic potential when dosage was of 100 and 200 µg. On the other hand, by administering angiotensin and candesartan, antagonist of the AT<sub>1</sub> receptors it was possible to prevent or attenuate cardiac rhythm disorders, when the initial (arrhythmogenic) dose of ATII was re-administered. Our findings are evidence to the intervention of an angiotensinergic mechanism in the development of cardiac rhythm disorders through centrogenic mechanism.

For a better understanding of our results, we should also consider the fact that, besides its own intervention, in the development of complex cardiovascular action of the ATII, of great importance is its interaction with other neurotransmitters (catecholamine, acetylcholine, adenosine, glutamate, etc.) and hormones (vasopressin) (4, 9, 10). Therefore, we intend to continue our research in the future on the influence of certain neurotransmitters, especially the glutamate, on the arrhythmogenic potential of ATII through central nervous mechanism.

No. Obs.	CONTROL			AFTER ATII			ATII AFTER CANDESARTAN		
	CF average/min (x)	AR (3min)	IA	CF average/min (y)	AR (3 min)	AI	CF average/min (z)	AR (3min)	AI
10	320±75.71	0	0	241.85±82.96	4.28±3.35	0.70±0.62	262.66±80.11	0	0
<b>Statistical comparison of effects</b>				(x,y);(x,z)	p<0.05		(x,z)	p= 0.2509	

**Table 3.** ATII action after icv microinjection of a 100µg dose on the cardiac frequency and rhythm before and after the icv microinjection of the Candesartan:

Legend CF-cardiac frequency, AI- arrhythmogenic index

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