PHARMACOLOGICAL SCREENING REGARDING THE INFLUENCE OF SOME NEWLY SYNTHESIZED β-PHENYLETHYLAMINES ON THE SYSTOLIC AND DIASTOLIC BLOOD PRESSURE IN RATS

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Abstract

A series of newly synthesized β-phenylethylamines by the National Institute for Chemical-Pharmaceutical Research and Development – Bucharest, Romania were studied. The effects of these compounds on the systolic and diastolic blood pressure in rats were investigated. The method used for this purpose was non-invasive as the blood pressure was measured in the tail veins. The experimental results suggest that compounds noted C1 (20mg/kg), C2 (50mg/kg), C3 (100mg/kg), A1 (100mg/kg), A2 (100mg/kg), A5 (100mg/kg), A7 (100mg/kg) have an affinity for the α1 and/or β_1 adrenergic receptors. Compounds A1 and A5 especially stood out showing an increase of the systolic blood pressure by 17.43% and respectively16.86% compared to the control group. All tested compounds as well as the reference substance increased the low diastolic blood pressure resulted from administrating prazosin in a statistically significant manner compared to the control group. A possible competition between the tested compounds and prazosin for the α_1 adrenergic receptors could explain these results. However they can also be explained by a possible non-selectivity of the tested compounds resulting in the stimulation of the myocardic β_1 adrenergic receptors. Based on the obtained results we can conclude that the newly synthesized compounds have an affinity for both types of adrenergic receptors due to the β-phenylethylamine molecular structure.

Rezumat

A fost investigat efectul unor compuşi nou sintetizaţi cu nucleu beta-feniletilaminic asupra tensiunii arteriale sistolice şi diastolice la şobolan. Substanţele de testat au fost sintetizate la Institutul Naţional de Dezvoltare Chimico-Farmaceutică Bucureşti. Metoda pentru înregistrarea tensiunii arteriale sistolice şi diastolice a fost neinvazivă, presiunea arterială determinându-se la nivelul venelor cozii de şobolan. Rezultatele experimentale sugerează faptul că substanţele notate C1 (20 mg/kg), C2 (50 mg/kg), C3 (100 mg/kg), A1 (100 mg/kg), A2 (100 mg/kg), A5(100 mg/kg), A7 (100 mg/kg), prezintă o afinitate pentru receptorii alfa1 şi/sau beta1 adrenergici. S-au remarcat substanţele A1 şi A5 la care creşterea tensiunii arteriale sistolice comparativ cu lotul martor a fost de 17,43 % şi respectiv 16,86 %. Toate substanţele de testat şi substanţa de referinţă, au crescut tensiunea arterială diastolică redusă în urma administrării de prazosin,

semnificativ statistic faţă de lotul martor. Acest aspect poate fi explicat prin apariţia unei competiţii între substanţele de testat şi prazosin la nivelul receptorilor alfa1 adrenergici, dar şi printr-o oarecare neselectivitate de acţiune, respectiv stimularea receptorilor beta1 cardiaci.

Conform rezultatelor obţinute putem concluziona că substanţele nou sintetizate prezintă o afinitate pentru ambele tipuri de receptori adrenergici, datorată nucleului de beta-feniletilamină prezent în structura lor.

Keywords: adrenergic receptors, β-phenylethylamines, blood pressure

Introduction

The adrenergic receptors have been described for the first time in 1948, by Ahlquist, who proposed the existence of more than one type of receptor based on the fact that adrenaline, nor-adrenaline and other pharmacological agonists regulate various physiological functions. Many aspects of these receptors have been uncovered since their discovery, starting from the detailed molecular structure, mainly obtained by cloning [10,12] and finishing with their chemical binding ability for certain agonists and antagonists.

Two types of adrenergic receptors (α and β) were initially described [1,2,5]. Later it was revealed that there are two types of α receptors – some postsynaptic (α_1) and others presynaptic (α_2). Additional studies showed that the α_2 adrenergic receptor is not exclusively presinaptic, being possible for it to exist also in a postsynaptic or a non-synaptic location. Furthermore it was observed that the α_1 and α_2 populations are not uniform, existing at least 3 subtypes of α_1 receptors (α_{1A}, α_{1B}, α_{1C}) and 3 subtypes of α_2 receptors (α_{2A}, α_{2B}, α_{2C})[4,8,11].

Interesting metabolic effects [6] have been described for the β_3 adrenergic receptors such as the reduction of plasmatic insulin levels, the increase of glucose tolerance and the reduction of body weight in obese diabetic rats [7]. The major involvement of β_3 adrenergic receptors in the glucose metabolism, consequently also in the release of insulin and in obesity, has been proved in experimental non-clinical trials [3]. Studies on mice have shown that a moderate increase in fats is associated with a decrease in β_3 adrenergic receptor levels. In the case of genetic obesity in mice and rats, a reduction of RNA-messenger levels in the structure of β_3 adrenergic receptors has been highlighted [6,7,13].

The newly synthesized compounds by the National Institute of Chemical-Pharmaceutical Research and Development, Bucharest, Romania, all have a β-phenylethylamine main structure, but depending on the substituted radicals with distribution properties (-OH; $-CH_3$; $-OCH_3$), they

have a very fluctuating central and peripheral diffusion. We also underline the fact that the pharmacological pharmacophore radicals, can influence the affinity of these compounds for certain types and subtypes of adrenergic receptors, thus influencing the pharmacodynamic actions as well. For this reason the present experimental study is aiming to determine the effect of these compounds on the systolic and diastolic blood pressure in rats, in order to uncover a possible selectivity for either the α or the β adrenergic receptors.

The adrenergic receptors agonists have many different effects on all body systems and functions having multiple therapeutic uses: heart stimulation, general and local vascular constriction, bronchial dilatation, uterus relaxation and many others.

Materials and Methods

Materials:

- male white Wistar rats
- Ugo Basile – blood pressure recording machine for rats
- compounds for testing noted: C1, C2, C3, A1, A2, A5, A6, A7

The doses used for the purpose of this experiment were chosen taking into account the LD_{50} of each compound previously determined in mice and considering the therapeutic index being LD_{50}/ED_{used} and having a value higher than 10 [9]. The exact doses used are shown below:

- C1-20mg/kg-bw p.o. suspension 0,2%; C2-50mg/kg-bw p.o. suspension 0,5%;
- C3, A1, A2, A5, A6, A7-100mg/kg-bw p.o. suspension 1%; prazosin 2mg/kg-bw p.o. suspension 0.2%; ephedrine 50mg/kg-bw p.o. suspension 0.5%.

Methods:

The experiment was conducted in two steps evaluating on one hand the influence of the new compounds on the basal systolic and diastolic blood pressure and on the other hand their influence on the low blood pressure induced by prazosin. The first experiment used a population of 80 male white Wistar rats weighing 279.1g ± 26.65g (M±SD).

We used 10 rat groups that were treated with compounds C1, C2, C3, A1, A2, A5, A6, A7 administrated in the previously mentioned doses. We compared the results with a 10 rat control group that was given distilled water – 1mL/100g-bw p.o. The initial blood pressure was determined for each group and then again one hour after administrating the tested compounds and respectively the control substance (water).

The second experiment used a population of 88 male white Wistar rats weighing 276g ± 25.46g (M±SD). We used 11 rat groups. At first we determined the initial basal blood pressure; than we treated each group with prazosin 2mg/kg-bw and after one hour we measured again the blood pressure using the same method. Afterwards we administrated the new compounds, the control substance (distilled water – 1mL/100g-bw p.o.) or the reference substance (ephedrine – 50mg/kg-bw p.o.). One hour after administrating the above mentioned compounds, the blood pressure of all rat groups was measured for the third time.

The animals brought from an authorized breeding farm were kept for 7 days in the new environment having free access to special rat food provided twice a day at 8.00 in the morning and again at 17.00 in the evening. Water was provided *ad libitum* all day long.

The temperature was maintained between 21-24ºC, while the humidity oscillated between 45-60%. All researches were conducted in accordance with The European Directive 86/609/EEC/24.11.1986 and The Romanian Government Ordinance 37/30.01.2002 regarding the protection of animals used for experimental and other scientific purposes.

Results and Discussion

The statistical evaluation of the results was performed using a special software – GraphPad Prism version 5.00 (www.graphpad.com). This software analyses two group populations, either with normal distribution using the Student t test, either with abnormal distribution using the Mann-Whitney test for 2 unpaired groups or the Wilcoxon test for 2 paired groups. The D`Agostino – Pearson test was used to determine if the population is distributed normally or abnormally. The Microsoft Office Excel 2003 application provided the technical support for all graphs used to illustrate the results of this experiment.

The statistical significance of the results regarding the evolution of the basal arterial blood pressure compared to the initial moment and to the control group is shown in table I. The effect of the tested compounds on the systolic and diastolic arterial blood pressure compared to the initial moment of each group and to the control group is also showed in the tables I-II.

I Experimental results regarding the effect of the new compounds on the basal arterial blood pressure.

One hour after the administration of the researched compounds, the systolic and diastolic arterial blood pressure within the control group did not change significantly (table I). In the case of compounds C1, C2, C3, A1, A5, A7, a statistically significant increase of the systolic arterial blood pressure

compared to the initial moment has been observed, while this was not the case for compounds A2 and A6 (table I). C1, C2 and A1 induced a slight increase of the diastolic arterial blood pressure compared to the initial moment (table I, figure 1), while C3, A2, A5, A6, A7 lowered slightly this parameter without any statistical significance (table I). Some of the tested compounds increased significantly the systolic arterial blood pressure compared to the control group (figure 1) as it is showed below: C2: 7.39% (p=0.0442*) A1: 17.43% (p=0.0043**); A2: 13.43% (p=0.0364*); A5: 16.86% (p=0.0038**); A6: 8.82% (p=0.0357*). No statistically significant variation of the diastolic arterial blood pressure compared to the control group was observed (table I).

Table I

The systolic and diastolic arterial blood pressure variation (%) for the tested compounds and the statistical significance of the results (compared to the initial moment and to the control group)

	Group / BP	Mean/Group								
		Control	**C1**	**C2**	**C3**	**A1**	**A2**	**A5**	**A6**	**A7**
Basal	**SBP (mmHg) ± SD**	**113 ±9.843**	**113.4 ±12.1**	**113.9 ±9.643**	**112.3 ±5.579**	**119.3 ±10.3**	**117.4 ±12.4**	**112 ±9.22**	**113.4 ±8.017**	**113.8 ±9.45**
	Normal distribution	yes	no	no	yes	yes	yes	yes	yes	yes
	DBP (mmHg) ± SD	**79.5 ±0.527**	**79.3 ±0.483**	**79.5 ±0.527**	**79.6 ±0.5164**	**78.63 ±3.114**	**80 ±0**	**79.75 ±0.7071**	**79.63 ±0.5175**	**79.75 ±0.4629**
	Normal distribution	no	yes	no	no	no	-	no	yes	yes
One hour after the administration	**SBP (mmHg) ± SD**	**111.4 ±9.107**	**118.3 ±12.58**	**120.7 ±12.33**	**116.1 ±6.402**	**138.4 ±23.0**	**131.5 ±22.1**	**129.3 ±12.4**	**121.8 ±10.58**	**122.1 ±15.1**
	Normal distribution	no	yes	yes	yes	yes	yes	yes	yes	yes
	DBP (mmHg) ± SD	**79.5 ±0.7071**	**79.8 ±0.4216**	**79.8 ±0.4216**	**79.7 ±0.483**	**79.63 ±0.744**	**80 ±0**	**79 ±0.9258**	**79.5 ±0.5345**	**79.5 ±0.7559**
	Normal distribution	yes	yes	yes	yes	no	-	yes	no	da
Variation % versus basal	**SBP (%)**	**-1.42**	**4.32**	**5.97**	**3.38**	**16.01**	**12.01**	**15.45**	**7.41**	**7.29**
	Wilcoxon (p)	0.5527 ns	0.0057 **	0.0058 **	-	-	-	-	-	-
	Student t test (p)	-	-	-	0.0066 **	0.0345 *	0.056 ns	0.0039 **	0.105 ns	0.0445 *
	DBP (%)	**0.00**	**0.63**	**0.38**	**0.13**	**1.27**	**0.00**	**-0.94**	**-0.16**	**-0.31**
	Wilcoxon (p)	1 ns	-	0.2986 ns	0.7656 ns	0.75 ns	-	0.1875	1 ns	-
	Student t test (p)		0.0522	-	-	-	-	-	-	0.3506 ns
Effect % compared to control group	**SBP (%)**		**5.74**	**7.39**	**4.80**	**17.43**	**13.43**	**16.86**	**8.82**	**8.71**
	Mann Whitney (p)		0.1382ns	0.0442 *	0.0623 ns	0.0043 **	0.0364 *	0.0038 **	0.0357 *	0.1418 ns
	Student t test (p)		-	-	-	-	-	-	-	-
	DBP (%)		**0.63**	**0.38**	**0.13**	**1.27**	**0.00**	**-0.94**	**-0.16**	**-0.31**
	Mann Whitney (p)		-	-	-	-	-	-	0.8794	-
	Student t test (p)		0.2643 ns	0.2643 ns	0.4697 ns	0.6311 ns	-	0.2116 ns	-	1 ns

ns – not significant

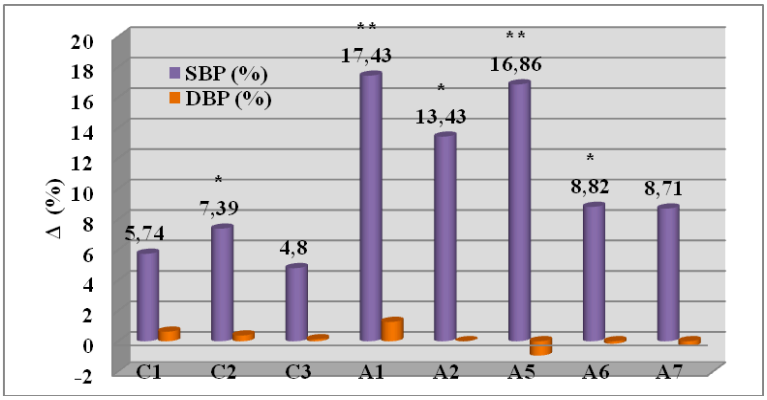

Figure 1

The influence of C1, C2, C3, A1, A2, A5, A6, A7 on the arterial systolic and diastolic blood pressure compared to the control group

The experimental results suggest that compounds C2, A1, A2, A5, A6, in the tested doses, have an affinity for the α_1 and/or β_1 adrenergic receptors. The increase in systolic blood pressure induced by these compounds could be due to the activation of the α_1 adrenergic receptors (increase in vascular resistance) and/or the stimulation of the β_1 adrenergic receptors (increase in heart flow by stimulating the myocardic contraction and excitation)

The diastolic arterial blood pressure does not change significantly. Experimental results sustain the hypothesis that the slight lowering of the diastolic arterial blood pressure caused by compounds A5, A6, A7 may be due to an affinity for the vascular β_2 adrenergic receptors. In the case of C1, C2, C3, A1, A2, the slight increase of the diastolic arterial blood pressure is explainable by an affinity for the α_1 adrenergic receptors.

Compounds A1 and A5 demonstrated the highest effect, increasing the systolic arterial blood pressure by 17.43% and respectively 16.86%. Correlating all experimental results, we can conclude that compound A5, increases the systolic arterial blood pressure by cardiac stimulation (affinity for the β_1 adrenergic receptors) and lowers the diastolic arterial blood pressure by activating the vascular β_2 adrenergic receptors. Compound A1may increase the systolic and diastolic arterial blood pressure by α_1 activation, but also by cardiac stimulation. The same mechanism deduced for compound A1, could also occur for compounds A2, C1, C2, C3.

II. Experimental results regarding the effect of the new compounds on the low arterial blood pressure induced by prazosin.

The systolic and diastolic arterial blood pressure of the control group is significantly lowered compared to the basal values, one hour after

administrating prazosin (figure 2). This trend continues after administrating the distilled water for at least another hour (SBP – 10.68%, p<0.0001 ***; dBP – 1.88% p= 0.012 *). This reduction in blood pressure is due to the vascular effect of prazosin that blocks the α adrenergic receptors. The same initial decrease in blood pressure is induced by administrating 2mg/kg-bw of prazosin p.o to all groups (figure 2).

The administration of 50mg/kg-bw ephedrine p.o, has lead to a statistically significant rise in the systolic and diastolic arterial blood pressure compared to the basal values of each group. This effect was evident one hour after the administration and is caused by the cardiac stimulation as a result of ephedrine activating the β_1 adrenergic receptors.

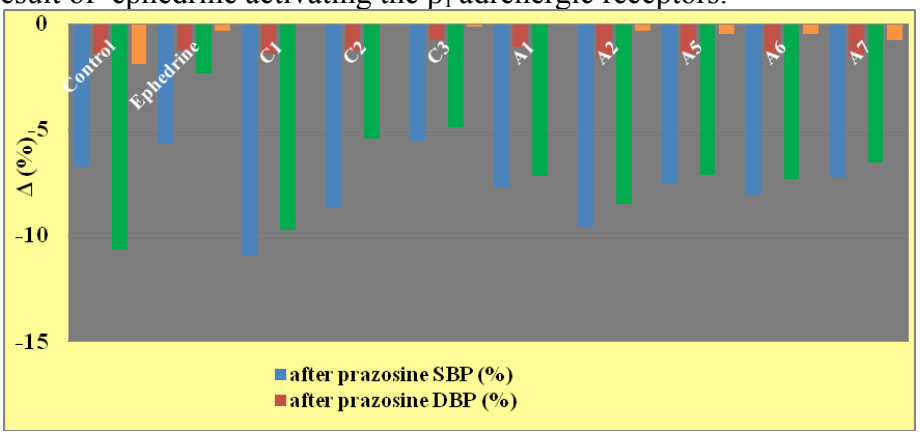

Figure 2

The systolic and diastolic arterial blood pressure variation, one hour after the administration of prazosin.

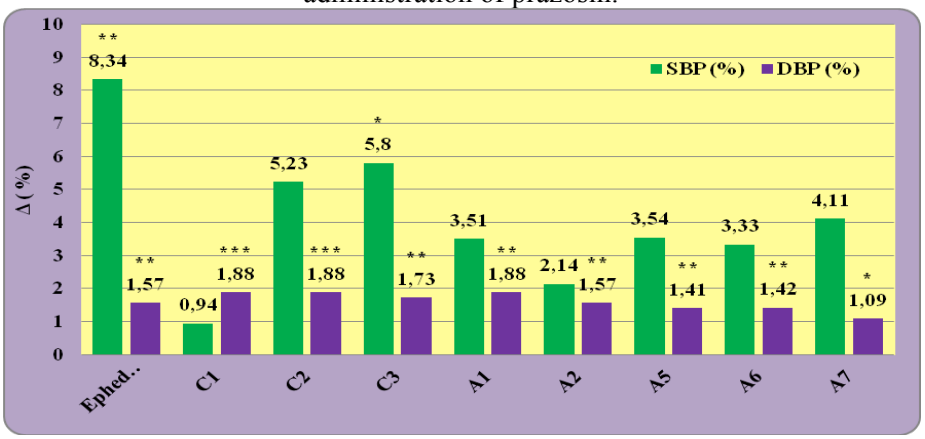

Figure 3

The systolic and diastolic arterial blood pressure variation, one hour after the administration of the tested compounds and respectively two hours after the administration of prazosin compared to the control group

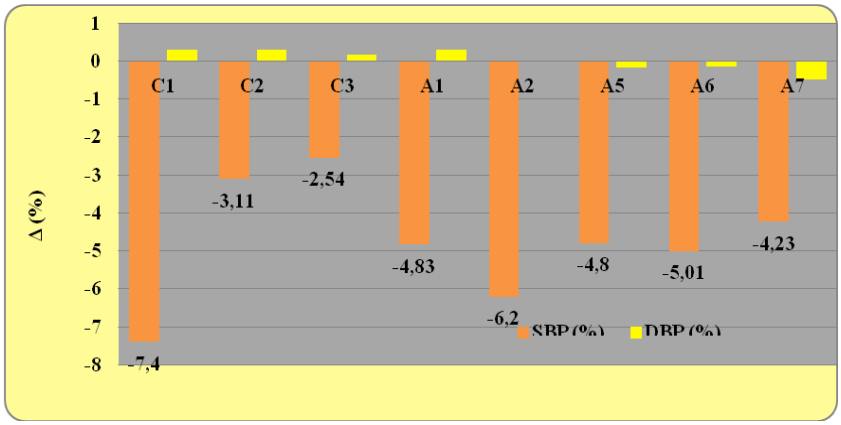

Figure 4

The systolic and diastolic arterial blood pressure variation, one hour after the administration of the tested compounds and respectively two hours after the administration of prazosin compared to the reference group

All tested compounds as well as the reference substance have increased in a statistically significant manner the systolic arterial blood pressure compared to the control group (figure 3). However, as it is shown in figure 1, the increase of the systolic arterial blood pressure after the hypotension induced by prazosin does not reach the initial basal values under the influence of the tested compounds or the reference substance. A competition for the α_1 adrenergic receptors between prazosin and the tested compounds could explain this phenomenon. A possible β_1 activation, in other words a lack of selectivity on the part of the tested compounds, could also account for the above mentioned observations.

Ephedrine is known by the literature [1,2] as a nonselective adrenergic agonist thus activating both types of receptors α and β [3]. It probably increases the diastolic arterial blood pressure by activating the α_1 adrenergic receptors in the vascular muscles. A similar effect was also observed in the case of compounds C1, C2, A1 that increase the diastolic arterial blood pressure by up to 1.88% compared to the control group.

The affinity of the new compounds for the α_1 adrenergic receptors can be explained by the structural similarities they share with ephedrine (all being β-phenylethylamines) [4,5]. Compounds C3, A5, A6 and A7 also showed statistically significant rises in the diastolic arterial blood pressure compared to the control group (figure 3)

Compound C3 increased the systolic arterial blood pressure by 5.8%, while A2 only by 2.14% but both results were statistically significant. The possible mechanism involved is cardiac β1 adrenergic stimulation.

Comparing the effect of the new compounds on the systolic blood pressure with that of ephedrine, we can state that the tested compounds have the same type of effect as ephedrine but less intense (figure 4), consequently they have some affinity for the cardiac β1 adrenergic receptors, but not as much as ephedrine has. Compounds C1, C2, C3, A1 have a greater affinity for the vascular α_1 adrenergic receptors, inducing a higher increase of the diastolic arterial blood pressure than ephedrine (figure 4).

Finally we can establish a scale in order to illustrate how the intensity of the rise in systolic blood pressure decreases among the studied compounds compared to the control group:

Ephedrine > C3 > C2 > A7 > A5 > A1 > A6 > A2 > C1

A similar scale, for the rise in diastolic blood pressure is envisioned below: C1 = C2 = A1 > C3 > A2 = Ephedrine > A6 > A5 > A7

Conclusions

The experimental results regarding the influence of the new β-phenylethylamines on systolic and diastolic blood pressure have proven that these compounds have an affinity for the α and β adrenergic receptors. The rise in systolic arterial blood pressure can be attributed to the increase in cardiac blood flow as a result of β_1 stimulation, while the rise in diastolic arterial blood pressure may be due to vascular constriction as a result of α_1 stimulation. The rise in blood pressure caused by the tested compounds after having administrated prazosin suggests a possible competition for the α_1 receptors.

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