Hypertension is the most common cardiovascular disease. It affects 44% of European population aged 35-74 years (1) and is a recognized factor in the development of life-threatening pathologies such as coronary heart disease and stroke (2). Therefore nowadays, the need to effectively reduce the blood pressure does not raise any doubts. However, obtaining normal pressure (120/80 mmHg) appears to be very difficult (2).

Pathogenesis of hypertension is still not fully understood, which is the reason why administration of a single effective treatment is not possible so far. The conventional knowledge is based mostly on many years of clinical observation and large clinical trials. The fundamental goal i.e., normal blood pressure value is achieved by properly designated medications. Recommended groups of drugs used in hypertensive disease include diuretics, angiotensin converting enzyme inhibitors or angiotensin II receptor blockers, β-blockers and calcium channel blockers (2).

Results of research on the pathogenesis of hypertension revealed the mechanism of the natural development of the disease, and particularly the changes in the neuro-hormonal regulation of cardiovascular system. The most important disorder involves chronic stimulation of neuro-hormonal regulation, primarily associated with very high activity of sympathetic nervous system, leading ultimately to heart failure (3). According to published results, chronic stimulation of sympathetic fibres transmitting impulses to blood vessels originates from abnormal function of medulla centres, and particularly the rostral ventrolateral medulla (RVLM),

Abstract: Since clonidine was introduced in clinical practice, attempts are still made to obtain substances capable of centrally controlling blood pressure, however with pharmacological profile better than currently available, such as moxonidine and rilmenidine. Recently synthesized indazole derivatives exert promising action on blood pressure and heart rate in Wistar rats. In the present study, our aim was to check which of tested substituted compound exerts the best effect on basic circulatory parameters. Effects of marsanidine (M), 7-Me-marsanidine (7-Me-M), 7-Cl-marsanidine (7-Cl-M) and 7-F-marsanidine (7-F-M) on blood pressure, heart rate and diuresis were compared. Male Wistar rats were receiving i.v. tested compounds in two doses: 10 or 100 µg/kg b.w. Mean arterial pressure (MAP), heart rate (HR) and ECG were recorded continuously. Urine samples were collected before and after administration of tested imidazolines. Obtained data were filtered and subjected to statistical analysis. All tested compounds caused a profound decrease of MAP. 7-M-M reduced blood pressure to the highest extent when used in 10 µg/kg b.w. dose. 7-F-M in dose of 100 µg/kg b.w. caused the strongest drop of MAP. The weakest and the shortest effect in duration was observed after M administration. HR was reduced after administration of each compound while the strongest effect was observed after 7-M-M administration in dose of 10 µg/kg b.w. and after 7-Cl-M administered in dose of 100 µg/kg b.w. Again, the weakest and the shortest in duration effect was observed after M administration. The highest increase of diuresis was observed after 7-M-M administration. These data suggest that methyl substituent in 7 position of indazole ring is the most effective in improving hypotensive effects of newly synthesized imidazolidine derivatives.

Keywords: marsanidine derivatives, hypertension, rats, imidazoles, antihypertensive agents

Hypertension is the most common cardiovascular disease. It affects 44% of European population aged 35-74 years (1) and is a recognized factor in the development of life-threatening pathologies such as coronary heart disease and stroke (2). Therefore nowadays, the need to effectively reduce the blood pressure does not raise any doubts. However, obtaining normal pressure (120/80 mmHg) appears to be very difficult (2).

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which is directly responsible for the functioning of the heart and blood vessels (4). Hence, new drugs are needed that would change the “settings” in centres of central regulation of blood pressure.

**Imidazoline derivatives (5)**

It is believed currently that imidazoline derivatives act on I<sub>1</sub> receptors and α<sub>2</sub>-adrenoreceptor (6). Stimulation of these receptors should lead to inhibition of excitatory impulses to blood vessels which are generated by RVLM (7). So far, complete mechanism of inhibitory action in RVLM is not known (8). It is accepted that impulses from baroreceptors (the most important structures in “reading” blood pressure within the body) are transmitted to solitary tract nuclei (NTS) and subsequently, to the caudal ventrolateral medulla (CVLM), which in turn exerts inhibitory action on RVLM (7, 9). Fibres from CVLM, ending in RVLM contain α<sub>2</sub>-adrenoreceptors and I<sub>1</sub> receptors. These receptors are also present on intermediate GABA fibres in RVLM (10).

Imidazolines used in the present study were synthesized in the Department of Chemical Technology of Drugs, Medical University of Gdańsk. For experiments, imidazoline derivatives: marsanidine (M), 7-Me-marsanidine (7-Me-M), 7-Cl-marsanidine (7-Cl-M) and 7-F-marsanidine (7-F-M) were selected (Table 1).

All these compounds have a common chemical structure of 1-[(imidazolidin-2-yl)imino]indazole. Marsanidine, as a compound with no substituent in position 7 of imidazoline ring represents a basic structure for other substances. Despite similarities in their chemical structure, the substances possess different affinity to the α<sub>2</sub>-adrenoreceptor and to the I<sub>1</sub> imidazoline receptor (Table 1).

In preliminary studies, all presented substances were tested in Wistar rats in dose of 100 μg/kg b.w. In these experiments marsanidine caused a decrease of MAP and HR, while effects of 7-Me-marsanidine were even more pronounced (11). 7-Cl-marsanidine yielded results similar to 7-Me-marsanidine [unpublished data] and suppression of MAP after 7-F-marsanidine was even stronger (12). 7-Me-marsanidine was tested also in dose of 10 μg/kg b.w., which also resulted in substantial decrease of MAP and HR (13). Action of marsanidine and 7-Me-marsanidine was assessed after vagotomy, which confirms that action of these compounds was not only related to their affinity for α<sub>2</sub>-adrenoreceptor (13).

With respect to above data, it seemed reasonable to assess which of previously tested imidazoline

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<th>Name</th>
<th>Structure</th>
<th>α&lt;sub&gt;2&lt;/sub&gt; IC&lt;sub&gt;50&lt;/sub&gt; (nM)</th>
<th>I&lt;sub&gt;1&lt;/sub&gt; IC&lt;sub&gt;50&lt;/sub&gt; (nM)</th>
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derivatives could be the best candidate for an antihypertensive drug. To this end, in the present study effects of M, 7-Me-M, 7-Cl-M and 7-F-M on MAP, HR and diuresis were compared.

EXPERIMENTAL

The following chemicals and drugs were used: thiopental, Sandoz, Austria; isotonic saline, Fresenius Kabi, Poland; Heparinum, POLFA, Warszawa, Poland, RX 821002, Sigma-Aldrich USA. M, 7-Me-M, 7-Cl-M and 7-F-M (imidazoline derivatives) were synthesized in the Department of Chemical Technology of Drugs, Faculty of Pharmacy, Medical University of Gdańsk, Poland.

Male Wistar rats weighing 200–250 g were purchased from the Animal House of the Medical University of Gdańsk, Poland. All experiments were approved by the Local Ethical Committee on Animal Experiments. The animals were fed a commercial rodent chow (Labofeed-B, Poland). Tap water was available ad libitum. Rats were anesthetized by i.p. injection of thiopental at a dose of 70 mg/kg b.w. The animals were placed on a heated table and body temperature was maintained between 36 and 37°C. Tracheostomy was performed and catheters were inserted into the carotid artery for blood pressure and heart rate monitoring, into a jugular vein for infusions, and into the bladder for free diuresis and collecting urine samples. Urine samples were collected between –20 and 0, 0 and 20, 20 and 40, 40 and 60 min of experiment. Following all surgical procedures, a 40 min recovery period was allowed to establish steady state. The rats were infused with iso-

Figure 1. ΔMAP changes after administration of tested compounds. (A) Dose 10 mg/kg b.w.; (B) Dose 100 mg/kg b.w. Tested compounds were administered i.v. at time 0
tonic saline supplemented with thiopental (to maintain anesthesia dose 30 µg/kg b.w./min) and heparinum at the rate of 1.2 mL/h.

Tested compounds were administered as a 100 µL bolus through the venous catheter. The time of injection of tested compounds was considered as time “0”. During single experiment only one dose of tested compounds was used. The compounds were tested in two doses: 10 µg/kg b.w. (M – n = 7 rats; 7-M-M n = 7; 7-Cl-M n = 5; 7-F-M n = 5) and 100 µg/kg b.w. (M – n = 5 rats; 7-M-M n = 7; 7-Cl-M n = 5; 7-F-M n = 4).

Arterial blood pressure and heart rate were monitored directly and sampled continuously at 100 Hz, using Biopac Systems, Inc., Model MP 100 (Goleta, CA, USA). Obtained results were processed using ACQKnowledge (Goleta, CA, USA) measurement system and were selected, scaled and filtered to remove accidental signal disturbances. The recorded time domain transient data were presented as graphs using Excel (Microsoft, USA).

To directly compare the responses to treatment between examined groups, ANOVA test was used to evaluate ∆MAP and ∆HR calculated as the differences between MAP and HR and their baseline values (“time 0”) as described previously (15).

Data were analyzed by ANOVA with repeated measurements, using Statistica StatSoft software (StatSoft, Inc., Tulsa, OK, USA). For all statistically significant effects, post hoc comparisons were performed using Fisher’s test. A value of p < 0.05 was considered statistically significant.

RESULTS

Effects of tested compounds on blood pressure and heart rate are shown in Figures 1–3. Intravenous injection of presented compounds caused a profound

![Figure 2. ∆HR changes after administration of tested compounds. (A) Dose 10 mg/kg b.w.; (B) Dose 100 mg/kg b.w. Tested compounds were administered i.v. at time 0](image)
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decrease in ∆MAP (Fig. 1) although a short-time elevation of MAP was observed after administration of each tested compound.

The strongest effect was observed after administration of 7-F-M in dose of 100 µg/kg b.w. (Figs. 1B and 3A). With a dose of 10 µg/kg b.w. the most profound effect was observed after administration of 7-Me-M (Figs. 1A and 3A). The shortest duration of ∆MAP decrease was observed for M given in dose of 10 µg/kg b.w. (Fig. 1A). This substance exerted also the weakest effect on ∆MAP (Fig. 3A).

Also, as presented in Figures 2 and 3B, all tested compounds decreased the ∆HR. The most profound decrease of heart rate was observed after administration of 7-Cl-M in dose of 100 µg/kg b.w. (Figs. 2B and 3B). When administered in dose of 10 µg/kg b.w., the most potent action of tested compounds was observed for 7-Me-M (Figs. 2A and 3B). Again, the weakest effect on HR was observed after administration of M in dose 100 µg/kg b.w. Also, as compared to other substances, duration of the effect exerted by M was the shortest one.

Additionally, influence of tested compounds given in dose of 100 µg/kg b.w. on diuresis was assessed. Obtained results are presented in Figure 4. The most noticeable increase of diuresis was observed after administration of 7-Me-M, and the maximum was reached 20 min after administration of tested compound (Fig. 4).

**DISCUSSION AND CONCLUSIONS**

The tested substances caused rapid and profound decrease of ∆MAP (Fig. 1A and B) and ∆HR
Direct comparison of mean effect of tested compounds (Fig. 3A and B) lets to conclusion that with reference to blood pressure regulation, the effect of 7-Me-M given in dose 10 mg/kg b.w. is the most promising. The decrease of ΔMAP after 7-Me-M administration in dose of 10 µg/kg b.w. is not only significantly stronger as compared to other substances given in this dose, but also is not significantly weaker than the effects observed after administration of tested compounds in dose of 100 µg/kg b.w. (Fig. 3A). On the other hand, the weakest hypotensive (Fig. 3A) and the shortest in duration (Fig. 1A and B) effects were exerted by M. These observations suggest that each of tested substituents in position 7 of imidazoline ring of marsanidine improves the hypotensive effect of the compound.

A short-time elevation of blood pressure observed after administration of each tested compound could be related to activation of different subtypes of α₂-adrenoreceptors. Stimulation of α₂B receptors seems to be responsible for initial increase of blood pressure whereas the long lasting hypotensive effects result from activation of α₂A receptor (16).

Also, however to the lesser extent, ΔHR changes observed after administration of tested compounds support the observation that the effects of 7-Me-M are the most profound. The strongest reduction of ΔHR was observed after administration of 7-Cl-M in dose 100 µg/kg b.w. However, 7-Cl-M given in dose of 10 µg/kg b.w. was not able to reduce ΔHR significantly more than 7-Me-M given in the same dose (Fig. 3B). Similarly to changes observed in ΔMAP, the weakest effect on ΔHR was observed after both doses of M.

Analysis of changes in diuresis during the experiment also indicates the preferable properties of 7-M-M. During the in vivo experiments, diuresis was estimated based on four 20 min urine collections, one before and three after administration of investigated compounds. We observed markedly increased volume of excreted urine within 40 min after the administration of M, 7-Me-M and 7-Cl-M. However, between 20 and 40 min of experiment, diuresis induced by M was markedly lower than in the previous collection and significantly lower than in the 7-Me-M and 7-Cl-M groups. Comparison of diuresis increases observed after administration of 7-Me-M and 7-Cl-M indicates that the influence of 7-Me-M on diuresis is more potent (Fig. 4). An increase of diuresis could be additional advantage of 7-M-M since this leads to reduction of the whole blood volume which is a factor contributing to the regulation of blood pressure.

Moxonidine and rilmenidine, currently commercially available imidazoline drugs, act in CNS by decreasing the sympathetic tone, reducing total peripheral resistance with maintaining baroreflex, and reducing the blood pressure variability (17, 18). Furthermore, these drugs reduce hypertrophy of heart and vessel walls, which further contributes to their effects on the reduction of risk factors for cardiovascular disease (19, 20). The impact of moxonidine and rilmenidine on metabolism should be also mentioned. Acting on pancreas, both these drugs ele-
vate insulin while reduce glucagon secretion (21). They decrease elevated levels of cholesterol (22) and they affect the water and electrolyte balance by increasing sodium and water excretion (23). This effect is probably due to the reduction in sympathetic tone at spinal cord (3). However, it should be noted that imidazolines may also influence the higher parts of CNS, limiting appetite for sodium (24). Thus, future experiments should be designed in order to assess the influence of tested compounds, and particularly, of 7-Me-M on cardiovascular risk factors. Up till now, elevated natriuresis after 7-Me-M administration in Wistar rats has been described (25).

Described above pharmacological class of the drugs is generally well tolerated by patients, and the most common side effect of moxonidine is dry mouth. Other side effects that may occur are similar to these observed with ACE inhibitors such as enalapril (18). It should be noted, however, that some of the advantages are associated with certain problems. Rilmenidine administration, for example, results in temporary reduction in cardiac output. In contrast, the use of both rilmenidine and moxonidine, induces bradycardia associated with baroreceptor reflex (26). In the initial phase of treatment with rilmenidine and moxonidine, pancreas starts to secrete more glucagon and less insulin, which results in temporary worsening of glucose tolerance (21).

The side effects of imidazoline drugs are most likely related to their affinity for $\alpha_2$-adrenergic receptors. Therefore, one may presume that 7-Me-M, displaying the most promising effect in cardiovascular system, with the lowest affinity for $\alpha_2$-adrenoreceptors, will exert the weakest side effects in comparison to other tested compounds (Tab. 1) (8). AGN 192403, a compound possessing a 500-fold higher affinity for $I_1$ receptor than for $\alpha_2$-adrenoreceptor, administered intravenously in rats and monkeys had no effect on blood pressure (27). Moreover, LNP509, an imidazoline compound lacking affinity for $\alpha_2$-adrenoreceptor, decreased blood pressure in mice and rabbit but its action was augmented by concomitant administration of $\alpha_2$-adrenergic agonist $\alpha$-methylnoradrenaline ($\alpha$-MNA) (28). Also, our previous experiments are in agreement with above observations and indicate that hypotensive action of 7-Me-M is weaker in the presence of $\alpha_2$-adrenergic blockade (13). Therefore, imidazoline based drugs should possess high affinity for $I_1$ receptors and also, however lower, for $\alpha_2$-adrenoreceptors. Nevertheless, the most favorable $\alpha_2/I_1$ ratio has not been established yet (8). From this point of view, presented herein substances can be characterized as follows: M acts rather as $\alpha_2$ agonist with only low affinity for $I_1$ receptors, similarly to 7-M-Cl, while 7-M-F and 7-Me-M, with apparent domination of 7-Me-M, could be described as mixed $\alpha_2/I_1$ agonists. Since 7-Me-M had the strongest influence on blood pressure, heart action and diuresis, our results support the hypothesis that imidazoline agonist most suitable for development of new antihypertensive drugs should possess relatively low affinity for $\alpha_2$ and much higher for $I_1$ receptors.

In summary, tested in this study newly synthesized imidazoline derivatives differ in their affinities for $I_1$ receptor, although they share the similar structure (Table 1). Among them, 7-Me-M possesses the highest affinity for $I_1$ receptor and the lowest affinity for $\alpha_2$-adrenoreceptor. Affinity profile of 7-Me-M confirms the mentioned above hypothesis that substances with high affinity for $I_1$ receptors with only low affinity for $\alpha_2$-adrenoreceptors display the most preferable effect in cardiovascular system. 7-CI-M, which showed the second strongest effect on MAP (Fig. 1) and HR (Fig. 2), has much lower affinity for $I_1$ receptor and several-fold higher affinity for $\alpha_2$-adrenoreceptor (Tab. 1). 7-F-M has higher than 7-CI-M affinity for $I_1$ receptor but similar affinity for $\alpha_2$-adrenoreceptor, and its effect is even weaker than this of 7-CI-M (Figs. 1 and 2). In this set of substances M appears to be an $\alpha_2$-adrenoreceptor agonist rather than any form of mixed type $\alpha_2/I_1$ agonist. Its affinity for $\alpha_2$-adrenoreceptors is significantly higher than of any other presented herein substances and affinity of M for $I_1$ receptor is the lowest one (Tab. 1).

Thus, taken together our observations indicate that 7-Me-M should be considered as a potential candidate for developing a new hypotensive drug. 

Acknowledgments

This study was supported by the Medical University of Gdansk grants W-78, ST-54 and by the Ministry of Science and Higher Education of the Republic of Poland, from the quality-promoting subsidy, under the Leading National Research Centre (KNOW) programme for the years 2012–2017. The authors would like to thank dr. Barbara Lewko for her assistance in preparing the manuscript.

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Received: 4. 03. 2016