

Effects of new generation triptans – frovatriptan and almotriptan – on hemodynamic parameters in intact male and female rats

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The introduction of the second generation triptans in clinical and experimental practice was a major progress in the pharmacotherapy of migraine. Frovatriptan is a second generation triptan with strong 5-HT_{1B/1D} serotonergic agonism and low 5-HT_{1A/7} receptor affinity, while almotriptan possesses not only the typical 5-HT_{1B/1D} receptor agonist activity, but shows an affinity to the 5-HT_{1F} receptor. The aim of our study was to assess the impact of frovatriptan and almotriptan on hemodynamics in male and female rats. We used a non-invasive “tail-cuff” method to measure the arterial blood pressure. Female and male Wistar rats were treated separately with high and low dosages of frovatriptan and almotriptan. Male and female rats showed reduction in all hemodynamic parameters, but only male rats showed an increase in the heart rate. In general, we could say that both almotriptan and frovatriptan potentiate cardiovascular safety.

Keywords: almotriptan, frovatriptan, rat, arterial pressure

Despite the fact that triptans were designed to exert relatively similar antimigraine activity and side-effects profile, there are some studies that show some controversies in their cardiovascular safety and sustainability. Several studies have shown that hypertension is often associated with migraine and can even lead to its chronic development. Hypertension is thought to aggravate vascular endothelial dysfunction, which negatively affects the incidence and severity of migraine attacks (1). The control of arterial pressure is closely related to the specificities of blood supply in some vascular areas. Neuronal brain activity is a major factor that could influence blood flow (2).

Serotonin (5-HT) could influence some heart functions by its receptors, the effects of which are very complex in essence. For example, 5-HT can act as a sympatholytic by activation of the 5-HT₁ receptors on sympathetic nerve terminals that inhibit the norepinephrine (NE) release. This mechanism contributes to a reduced cardiac output, which is associated with a decrease in blood pressure. On the other side, 5-HT could have some stimulating effects on the heart (3). The 5-HT receptor mechanisms in the heart are species-dependent.

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For example, the activation of 5-HT_{2A} receptors in rats, 5-HT₄ receptors in pigs and humans, and 5-HT₇ receptors in cats leads to an increase of the heart rate. The authors assume that 5-HT could both increase and decrease blood pressure by its activity in the heart (4).

The frequency of triptan-related cardiovascular side-effects in clinical trials and in practice has been extremely low. When present, they usually affect patients with cardiovascular risk or cardiovascular disease. Few cases of cardiovascular side-effects were observed among patients that do not suffer from any cardiovascular pathology. Side-effects include peripheral vasoconstriction and rise in blood pressure in the case of uncontrolled hypertension. Because of this, there is a need for careful triptan prescribing to patients at risk (5). Nowadays, there are no specific scales that could assess the efficacy of triptan treatment during a migraine attack in patients suffering from hypertension.

Tullo *et al.* (6) made a retrospective analysis of three randomized, double-blind, cross-over studies involving hypertensive and normotensive men and women aged 18–65 years suffering from migraine, with or without aura. The patients were treated with frovatriptan and other triptan agents (zolmitriptan, rizatriptan and almotriptan). Recurrent headaches have been more frequent among hypertensive patients than in normotensive patients treated with other triptans except frovatriptan, which is not surprising, having in mind the long half-life of the drug (~26 h). A conclusion that could be drawn from this is that hypertensive patients are less susceptible to triptan treatment.

In the following research, we will make a comparative analysis of efficacy after one single application of frovatriptan and almotriptan on blood pressure and heart rate in male and female rats. We use single applications of these drugs because they are used only in the treatment of acute exacerbations and not as basic antimigraine therapy.

EXPERIMENTAL

Chemicals

Saline 0.9 % NaCl was used in control groups. Frovatriptan succinate monohydrate (frova) (≥ 98 %) and almotriptan malate (almo) (≥ 98 %) were obtained from Sigma-Aldrich Chemie GmbH (Germany).

Animals

Animals used in the experiments were male and female albino Wistar rats with an approximate body mass of 180–220 g. The total number of animals used in the experiments is 32 (16 males, 16 females), each group consisting of 8 animals. Female animals were of reproductive age, not ovariectomized and not estrogen-treated. All experimental rats were housed on a 12-hour light/dark cycle under controlled temperature and lighting conditions, while food and water were provided *ad libitum*.

Exclusion criteria: underweight rats, diseased animals, not-matured enough animals, and animals that had been previously treated with other than the before-mentioned substances.

We have not registered any incidents during the experiments.

Experimental design

There were two series of experiments carried out on rats, based on sex: in the 1st series we had used female rats and in the 2nd series we had used male rats. For each experiment

in the 1st and in the 2nd series, there was one control group treated subcutaneously (*s.c.*) with saline 0.9 % NaCl only (0.1 mL per 100 g b.m.) and four test groups treated subcutaneously (*s.c.*) as it follows: the 1st group was injected with frovatriptan 2.5 mg kg⁻¹ b.m.; the 2nd group was injected with frovatriptan 5 mg kg⁻¹ b.m.; the 3rd group was injected with almotriptan 3 mg kg⁻¹ b.m.; the 4th group was injected with almotriptan 6 mg kg⁻¹ b.m. Forty minutes after the drugs had been injected, animals were tested according to the indicated method. All test groups in the respective series were compared to the control group (injected with saline only). The total number of animals used in the experiment was 80: 40 males and 40 females; 8 animals in each group.

Method

The arterial blood pressure was examined non-invasively using the so-called “tail-cuff” method. All the experiments were carried out by the hemodynamic apparatus NIBP 200A (Biopac Systems Inc., USA) and its respective software package MP150. The animals were not anesthetized. All rats were initially warmed in a thermostat (Binder, Germany) up to 37 ° C. Afterwards, the animals were put into a restrainer in order to measure the arterial blood pressure using a cuff on the base of their tails. Before each measurement of the blood pressure, the rats were put into the restrainer for 5 min in order to get used to the stress levels. The values of systolic blood pressure (SBP) and diastolic blood pressure (DBP) were calculated as the means of 3 independent measurements. Their derivatives – pulse pressure (PP) and mean arterial pressure (MAP) – were calculated as follows: PP = SBP – DBP and MAP = DBP + (PP/3).

All the experimental protocols in this study were approved by the Ethical Committee of the Bulgarian Food Safety Agency with № 97/22.05.14 and were carried out following the guidelines of the European Directive 2010/63/EU.

Statistical processing

The statistical software SPSS version 19.0 was used for the multi-variant analysis. The mean and standard error of the mean (\pm SEM) were calculated for each group. A non-parametric Shapiro-Wilk test was performed to determine the level of distribution. *F*-test for variances and independent sample *t*-test, assuming equal or unequal variances, were used to compare the experimental groups with the corresponding control group. A $p < 0.05$ was considered as representing a statistically significant difference.

RESULTS AND DISCUSSION

Cardiovascular effects of frovatriptan and almotriptan in female rats

In the first series of experiments, the female group of rats treated with frovatriptan of 2.5 mg kg⁻¹ did not exhibit any significant change in hemodynamic parameters. The only group of animals, that significantly reduced DBP and MAP compared to the control group with saline, were those injected with the higher dosage of frovatriptan 5 mg kg⁻¹ (Fig. 1).

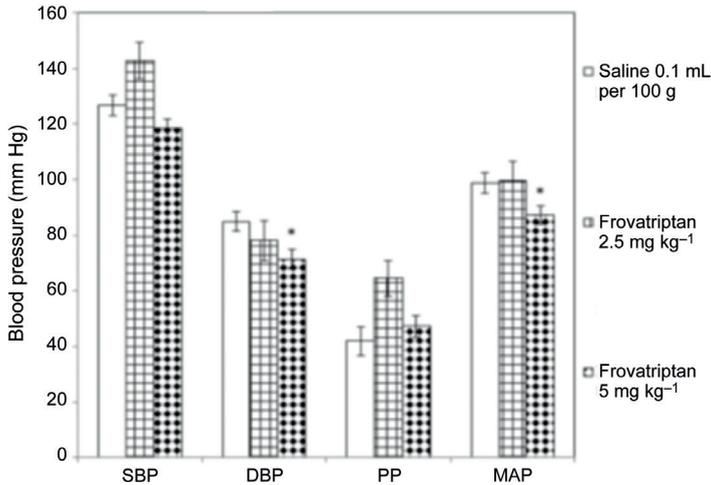


Fig. 1. Cardiovascular effects of frovatriptan after single administration in female rats (mean \pm SEM, $n = 8$). A significant difference compared to the control group: * $p < 0.05$; DBP – diastolic blood pressure, PP – pulse pressure, MAP – mean arterial pressure, SBP – systolic blood pressure.

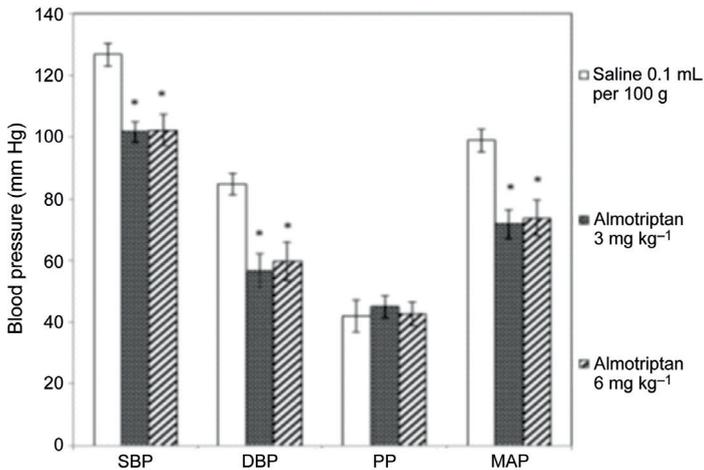


Fig. 2. Cardiovascular effects of almotriptan after single administration in female rats (mean \pm SEM, $n = 8$). A significant difference compared to the control group: * $p < 0.05$; DBP – diastolic blood pressure, PP – pulse pressure, MAP – mean arterial pressure, SBP – systolic blood pressure.

Fig. 2 shows that female groups treated with almotriptan in both dosages (3 and 6 mg kg⁻¹) showed significantly ($p < 0.05$) reduced SBP, DBP and MAP.

Heart rate was not influenced significantly in any female rat group treated with triptan (Fig. 3).

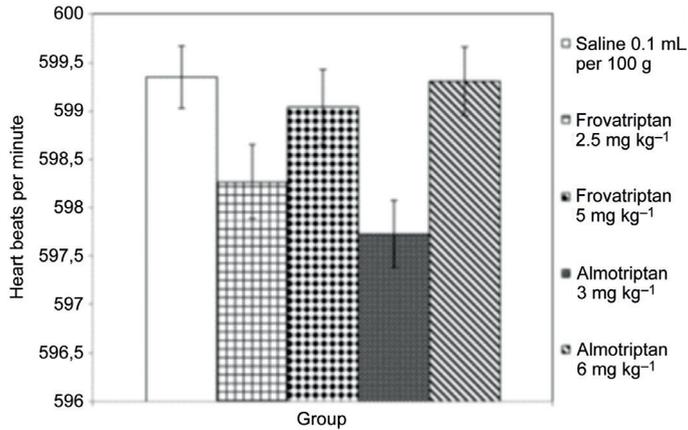


Fig. 3. Effects of frovatriptan and almotriptan on the heart rate after single administration in female rats (mean ± SEM, $n = 8$).

Cardiovascular effects of frovatriptan and almotriptan in male rats

The second series of experiments were conducted on intact male rats. The treated with frovatriptan at dosages of 2.5 and 5 mg kg⁻¹ showed a significant ($p < 0.05$) decrease in each blood pressure value (SBP, DBP, PP, MAP) compared to the control group after a single subcutaneous application (Fig. 4).

For the male rats treated with almotriptan at dosages of 3 and 6 mg kg⁻¹, a significant reduction ($p < 0.05$) of the three blood pressure values (SBP, DBP, MAP) was recorded compared to the control group. There were no significant changes for the PP (Fig. 5).

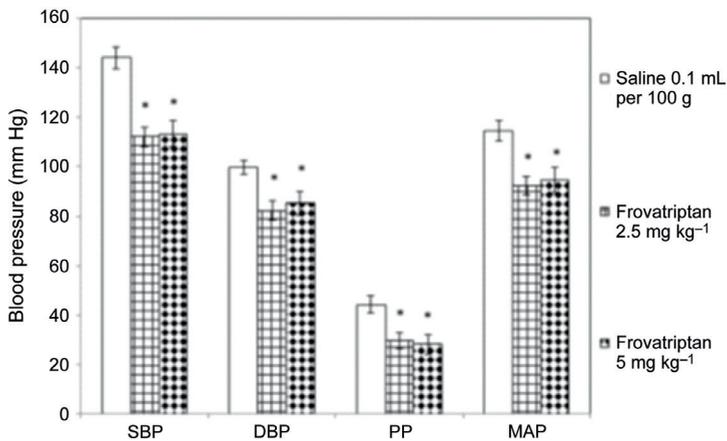


Fig. 4. Cardiovascular effects of frovatriptan after single administration in male rats (mean ± SEM, $n = 8$). A significant difference compared to the control group: * $p < 0.05$; DBP – diastolic blood pressure, PP – pulse pressure, MAP – mean arterial pressure, SBP – systolic blood pressure.

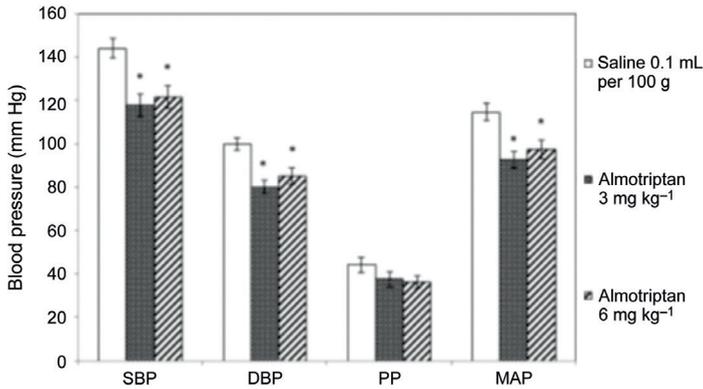


Fig. 5. Cardiovascular effects of almotriptan after single administration in male rats (mean \pm SEM, $n = 8$). A significant difference compared to the control group: * $p < 0.05$; DBP – diastolic blood pressure, PP – pulse pressure, MAP – mean arterial pressure, SBP – systolic blood pressure.

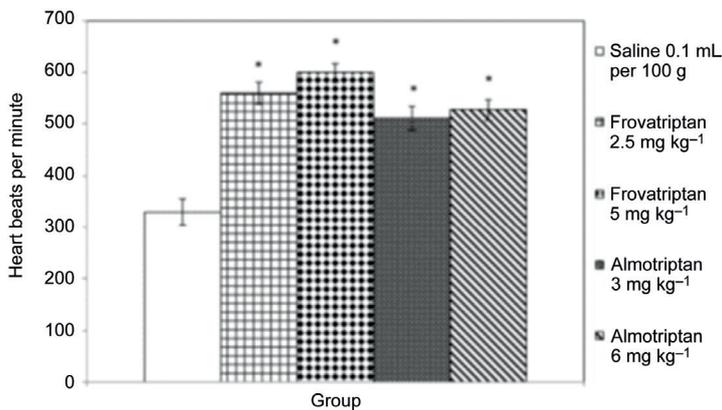


Fig. 6. Effects of frovatriptan and almotriptan on the heart rate after single administration in male rats. (mean \pm SEM, $n = 8$). A significant difference compared to the control group: * $p < 0.05$.

The heart rate was significantly increased in all groups of male rats treated with both triptans compared to the controls (Fig. 6).

Discussion of the results

In the experiments on intact male and female rats, both dosages of almotriptan decreased SBP, DBP and MAP, whereas frovatriptan did not show similar results in both sexes. Heart rate was not affected by any of the triptans tested on intact female rats. Almotriptan and frovatriptan generally showed hypotensive effects in both male and female rats. Keeping in mind that these drugs are serotonergic agonists, it should be mentioned that the effects of serotonin on the cardiovascular system are very complex and sophisti-

cated. These may result either in bradycardia or tachycardia, hypotension or hypertension, vasoconstriction or vasodilatation, and all these effects are primarily mediated by 5-HT₁, 5-HT₂ and 5-HT₃ receptors (3).

Watts and Davis (7) reported that 5-HT_{1B/1D} receptors are actively involved in cardiovascular effects and alter the physiological parameters of blood pressure and heart rate. In their review, they emphasize the role of 5-HT in reducing systemic blood pressure after single or multiple administrations of serotonin in rats. This effect is mainly due to interaction with 5-HT_{1B/1D} receptors. The activation of these receptors results also in decreased release of norepinephrine (NE) from noradrenergic neurons thus leading to a reduction in the contractile tone without resulting in vasodilation, which leads to hypotension (7). This effect is a possible explanation for the results in our experiments with almotriptan and frovatriptan on intact animals. Reborado *et al.* (8) suggest that 5-HT_{1B/1D} receptors are extremely expressed in mesenteric arteries and can mediate strong vasoconstriction effects (8).

In other studies, zolmitriptan is suggested to provoke a short-term increase in the arterial blood pressure (3). This effect is due to the contraction of the cranial blood vessels. Alternatively, vessels in the periphery and mainly in the vascular mesenteric vessels are also involved in this process. The acute parenteral administration of zolmitriptan and its short-term hypertension could be followed by long-term peripheral hypotension mainly because of venous pressure is also being decreased. The authors believe that this is one of the key mechanisms of action involved in the complex cardiovascular effects of zolmitriptan. In addition to these mechanisms of the peripheral blood pressure regulation, there is also available evidence for the central regulation. For example, Watts *et al.* (3) describe that central serotonergic effects on the blood pressure are due to the stimulation of serotonin receptors located in the wide intraneuronal network that defines both sympathetic and vagal regulation.

We could explain the tachycardia observed in male rats relying on the classical principle of the baroreceptor reflex mechanism and the normal functions of the sinus node mediated by the afferent stimulation to the vasomotor center in the brain. Conversely, migraine could provoke some vagal effects with its cerebral ischemia that may result in a subsequent hypotension (9).

There are similar outcomes to our results, but perceived from three double-blinded, randomized, crossover studies with frovatriptan, rizatriptan, almotriptan and zolmitriptan led on hypertensive and normotensive patients (6). These outcomes claim that there was no increase or even decrease in the blood pressure during their trials, and no further changes in the antihypertensive therapy were applied. The heart rate (HR) was not changed, nor a higher incidence of adverse drugs' reactions was observed in hypertensive patients. The only exception was that some of the treated subjects were less susceptible to triptan therapy, which, we believe, is important for the overall cardiovascular profile of these drugs.

Having in mind the pharmacokinetics of frovatriptan and its moderate affinity to 5-HT₇ receptor ($pK_i/IC_{50} = 6.7 \text{ nmol L}^{-1}$), we suggest that this receptor and its influence are involved in some hemodynamic parameters (10). There is sufficient data proving that 5-HT₇ receptors (located in *nucleus tractus solitarii*) are involved in the central regulation of arterial pressure and the heart rate.

Expression of 5-HT₇ receptors, apart from the CNS and peripheral nervous system, is also found in smooth muscle cells and blood vessels of the cardiovascular system, where

they mediate relaxation in arteries and veins (11). These are G-protein-coupled receptors (Gs) that increase the intracellular adenylate cyclase activity and cAMP levels (12). It is well known that the increase of cAMP and the following activation of protein kinase A (PKA) lead to sustained SM relaxation. This relaxation exactly supports our hypothesis about the decrease in DBP and MAP in female rats treated with frovatriptan 5 mg kg⁻¹.

In the literature, there is data that frovatriptan and almotriptan possess a moderate affinity to the 5-HT_{1A} receptor (13, 14). Having this mind, we may explain why male rats treated with frovatriptan in both dosages decrease all four hemodynamic parameters SBP, DBP, PP and MAP. According to Ramage (15), the central stimulation of 5-HT_{1A}-receptors could reduce the vasoconstrictor sympathetic nerve activity and increase the cardiac vagal nerve activity, both leading to a decrease in blood pressure. This hypothesis is confirmed by van den Buuse and Wegener (16). In their experiment, they found a significant decrease in the blood pressure after applying intravenously 5-HT_{1A} receptor agonist 8-OH-DPAT to rats.

On the other hand, Pagniez *et al.* (17) had previously established hypotension and bradycardia on anesthetized rats treated with sumatriptan and rizatriptan. Furthermore, the authors prove 5-HT_{1B/1D} receptors to be poorly involved in these effects by co-administration of GR127935, a potent and specific 5-HT_{1B/1D} antagonist. They do not exclude the possibility for a secondary reduced sympathetic vascular tone.

The sympathetic varicosities of the nerves innervating small arteries and arterioles express 5-HT_{1B/1D} receptors. Stimulation of these receptors results in the inhibition of NE release and the reduction of nerve-stimulated contraction. Thus, there are multiple ways in which 5-HT interacts with arteries, promoting both vasoconstriction and vasorelaxation (7). Based on this hypothesis, we could explain the fluctuations of the blood pressure in our results in frovatriptan and almotriptan treated male and female rats.

CONCLUSIONS

Our experiment was conducted with selective serotonergic agonists frovatriptan and almotriptan. We had examined the effects on intact male and female rats and came to the conclusion that these effects were beneficial to the cardiovascular system of female rats. The receptors' affinity of frovatriptan and almotriptan to the specific subtypes of 5-HT receptors could explain some of the haemodynamic fluctuations.

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