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Effects of the Novel High-affinity 5-HT(1B/1D)-receptor Ligand Frovatriptan on the Rat Carotid Artery

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Background: In blood vessels 5-HT stimulates sympathetic nerves, the endothelium and vascular smooth muscle cells. Triptans are specific anti-migraine drugs and they activate the serotonergic 5HT_{1b/d} receptors causing vasoconstriction of the cerebral vessels.

Aim: To evaluate the effect of frovatriptan on isolated rat carotid artery.

Methods: Contractile activity of the preparations was registered isometrically. Krebs solution (pH = 7.4) was used for washing smooth muscle (SM) preparations aerated with 95% O₂ and 5% CO₂ at 37°C. The 60-minute adaptation of tone level of preparations was taken as a starting tone and the changes such as contraction or relaxation were calculated using it.

Results: Frovatriptan (1×10^{-6} mol/l - 1×10^{-5} mol/l) induced a contraction, but at higher concentrations it caused relaxation of the carotid artery. The L-norepinephrine contractile reaction was enhanced in the presence of frovatriptan. In the presence of 5-HT₂ receptor antagonist, methysergide, frovatriptan increased the relaxation. In the presence of the specific α -1 receptor antagonist, prazosin, the frovatriptan-induced relaxation decreased.

Conclusion: The observed contractile effect of frovatriptan is probably associated with the main effect of the drug – activation of the serotonergic 5HT_{1B/1D} receptors causing vasoconstriction of the cerebral vessels and their anti-migraine effect. At higher concentrations, frovatriptan, most likely via some non-specific mechanism, could activate the following intracellular chain reaction: stimulation of α_{1D} could activate eNOS which may increase in the concentration of NO which results in the final effect of relaxation.

BACKGROUND

Serotonin (5-hydroxytryptamine, 5-HT) exerts its multiplicity of physiological effects through an unsurpassed diversity of receptors. Some of these effects are mediated through actions of 5-HT in the central nervous system, whereas 5-HT also has multiple and diverse effects through direct interaction with 5-HT receptors in different parts of the cardiovascular system.¹ In blood vessels 5-HT stimulates sympathetic nerves, the endothelium and vascular smooth muscle cells.²

Triptans are specific anti-migraine drugs gener-

ally recommended to patients whose conditions are refractory to analgesics. The therapeutic effect of triptans appears to be induced by activating the serotonergic 5-HT_{1B/1D} receptors in the trigeminovascular system causing vasoconstriction of the cerebral vessels and neuronal inhibition. Despite their relative selectivity, the vasoconstrictive activity of triptans also affects peripheral arteries, although to a lesser extent.³

Triptans differ markedly in terms of their pharmacokinetic and pharmacodynamic properties. Some triptans, such as sumatriptan, are considered to be

fast-acting while also possessing greater risk of adverse events. Frovatriptan is one of the newest triptans and its distinct pharmacokinetic and pharmacodynamics profile reveals a clinical potential due to the long duration of action (half-life is 26h) and low incidence of side effects and drug interactions. The elimination half-life of frovatriptan is five times greater than that of other triptans, while the time to maximum concentration is similar to that of other triptans when given orally.⁴ It has high affinity to 5-HT_{1B/1D} receptors and moderate affinity to 5-HT₇ receptors. It does not inhibit or induce cytochrome P450, associate to a low rate binding to plasma proteins, which in turn leads to a lower risk of drug interactions.⁵

AIM

To evaluate the effect of frovatriptan on isolated rat carotid artery.

MATERIALS AND METHODS

Male Wistar rats with initial body weight in the range of 220 to 280 g were provided by the Animal House of Medical University Plovdiv, Bulgaria. The rats were housed in standard laboratory conditions (23-25°C, 50-55% humidity and 12/12h light/dark cycle) and fed with standard commercial food and given water *ad libitum*. At the beginning of the experiments the animals were euthanized by overdose anesthesia (ketamine and xylazine). Smooth muscle preparations of *a. carotis communis* 0.8-1.0 mm wide and 15 mm long were cut. All experiments were carried out according to the guidelines of using laboratory animals in EU- guidelines/EEC Directive of 1986.

Contractile activity of the preparations was registered isometrically using Tenzo detectors (Swema, Sweden). The initial mechanical stress of the preparations reached by the stretch tensosystem is a value corresponding to the tensile force of 10 mN. Krebs solution (pH = 7.4) used for washing the SM preparations was continuously aerated with a gas mixture of 95% O₂ and 5% CO₂ at 37°C. A 60-minute adaptation of tone level of preparations was taken as a starting tone and changes such as contraction or relaxation were compared to it. During the adaptation the Krebs solution changed several times. The drug-caused reactivity of the SM preparations was measured and registered by

gain stage Microtechna (the Czech Republic) and recorded using paper recorder (Linseis, Germany).

CHEMICALS AND SOLUTIONS

The following chemicals were used: acetylcholine (Dispersa Baeschlin, Germany); DMSO (Sigma), frovatriptan succinate monohydrate (Sigma), methysergide maleate salt (Sigma), L-norepinephrine hydrochloride (Sigma), prazosin hydrochloride (Sigma).

The Krebs solution had the following composition (mM): NaCl 120; KCl 5.9; CaCl₂ 2.5; MgCl₂ 1.2; NaH₂PO₄ 1.2; NaHCO₃ 15.4 and glucose 11.5 (Merck).

STATISTICS

The data obtained were expressed as mean ± standard error of the mean (SEM). The number of tissue preparations used in each experiment is indicated by n. Statistical differences were tested using the Student's t-test, and P<0.05 was considered significant. All statistical analyses were performed using a specialized software SPSS, version 17.0 (SPSS Inc. Chicago, IL).

RESULTS

EFFECTS OF FROVATRIPTAN ON THE CONTRACTILE ACTIVITY OF SM PREPARATION OF CAROTID ARTERY.

At the concentration of 1×10^{-6} mol/l and 1×10^{-5} mol/l, frovatriptan induced contraction in the SM preparations of carotid artery (Fig. 1).

Figure 1. Changes in the contractile activity of isolated SM preparations of carotid artery after application of frovatriptan at concentration of 1×10^{-6} mol/l (1), 5×10^{-6} mol/l (2), 1×10^{-5} mol/l (3), and 5×10^{-5} mol/l (4) and 1×10^{-4} mol/l (5), (n=9).

At concentrations of 5×10^{-5} mol/l and higher frovatriptan provoked significant relaxation (Fig. 2).

Figure 2. Frovatriptan isometric record of smooth muscle activation changes from isolated preparations of carotid artery (n=6).

EFFECTS OF 1×10^{-5} MOL/L L-NOREPINEPHRINE ON THE CONTRACTILE ACTIVITY OF SM PREPARATIONS OF CAROTID ARTERY IN PRESENCE OF FROVATRIPTAN.

In the presence of frovatriptan at a concentration of 5×10^{-5} mol/l we observed a significant increase of the contractile reaction of L-norepinephrine (1×10^{-5} mol/l) compared to the control reaction (**Fig. 3**).

Figure 3. Changes in the tone of isolated SM preparations induced by L-norepinephrine (1×10^{-5} mol/l (1)) and L-norepinephrine (2) in the presence of frovatriptan (5×10^{-5} mol/l) (n=7). Statistically significant effects are indicated by * ($P < 0.05$).

EFFECTS OF 5×10^{-5} MOL/L OF FROVATRIPTAN ON THE CONTRACTILE ACTIVITY OF SM PREPARATIONS OF CAROTID ARTERY IN PRESENCE OF METHYSERGIDE.

Frovatriptan (5×10^{-5} mol/l) in the presence of the 5-HT₂ receptor antagonist methysergide (1×10^{-5} mol/l) significantly increased the relaxation com-

pared to the control reaction exerted by frovatriptan alone (**Fig. 4**).

Figure 4. Changes in the tone of SM preparations of frovatriptan (5×10^{-5} mol/l) (1) and of frovatriptan combined with methysergide (1×10^{-5} mol/l) (2) (n=7). Statistically significant effects are indicated by * ($P < 0.05$).

EFFECTS OF 5×10^{-5} MOL/L FROVATRIPTAN ON THE CONTRACTILE ACTIVITY OF SM PREPARATIONS OF CAROTID ARTERY IN PRESENCE OF PRAZOSIN.

In the presence of the specific alpha-1 receptor antagonist, prazosin (1×10^{-6} mol/l), the relaxation induced by frovatriptan (5×10^{-5} mol/l) significantly decreased compared to that in the controls by frovatriptan alone (**Fig. 5**).

Figure 5. Changes in the tone of isolated SM preparation, induced by (1) frovatriptan (5×10^{-5} mol/l) and (2) frovatriptan in the presence of prazosin (1×10^{-6} mol/l) (n=7). Statistically significant effects are indicated by * ($P < 0.05$).

DISCUSSION

Studies using isolated blood vessels provide a robust quantitative pharmacological comparison of drugs and may identify subtle differences between triptans. These differences are of scientific interest since, in theory, all triptans belong to the same

class (i.e. 5-HT_{1B/1D} receptor agonists) and therefore would be expected to have similar pharmacological actions. It is important to remember that any differences detected using *in vitro* studies should not be extrapolated and used as predictors or comparators of drug effects when administered to man.

5-HT was recognized as a substance isolated from blood serum (sero-) that could modify the tone of smooth muscles (-tonin).⁶⁻⁸

Frovatriptan, which belongs to the group of triptans, may act by constricting cranial vessels through 5-HT_{1B} receptors, by inhibiting peripheral trigeminal nerve afferents that innervate the vessels and pain-producing dura mater through 5-HT_{1D} receptors, or by inhibiting central trigeminal neuronal traffic through 5-HT_{1D} receptors, or by a combination of these mechanisms.⁹

Our results shows that at concentrations of 1×10^{-6} mol/l and 1×10^{-5} mol/l frovatriptan induces contractions in SM preparations of carotid artery. This effect is probably related to the drug's main action – activation of serotonergic 5-HT_{1B/1D} receptors causing vasoconstriction of the cerebral vessels and their anti-migraine effect. It is well known that α -adrenoceptors play an important role in the regulation of vascular tone and blood pressure and that stimulation of the receptors mediate constriction of the isolated carotid artery.¹⁰

There are 3 α -1 adrenergic receptor subtypes: α 1A, α 1B, and α 1D, all of which signal through the Gq/11 family of G-proteins and different subtypes show different patterns of activation. They activate mitogenic responses and regulate growth and proliferation of many cells. Catecholamines like norepinephrine (noradrenaline) and epinephrine (adrenaline) influence signaling pathways through the α ₁-adrenergic receptor in the central and peripheral nervous systems. α _{1A}- and α _{1B}-ARs localized to the plasma membrane while α _{1D}-ARs accumulate intracellularly.¹¹

There is evidence in the literature that the activation of α ₁-adrenoceptors induces relaxation in rabbit bronchial artery¹² and rat pulmonary arteries, in a process involving the endothelial production and release of nitric oxide (NO). In the rat carotid artery, phenylephrine produce an endothelium-dependent relaxation mediated by α _{1D} adrenoceptors and involving the endothelial NO synthase (eNOS).¹³

Frovatriptan possesses higher 5-HT_{1B} agonist affinity and less affinity to α _{1D} adrenoceptors. Our experiment showed that in its lower concentration

frovatriptan occupied by priority 5-HT_{1B} receptors, and only then the NO activation pathway of α _{1D} receptors could be possible. After all this a smooth muscle contraction is observed in the 1×10^{-6} mol/l and 1×10^{-5} mol/l drug concentrations.

Despite the drug's lower α ₁-adrenoceptor affinity in higher concentrations, it could be balanced under the following intracellular pathway: activation of α _{1D}-ARs – NO synthesis – guanylate cyclase activation – increasing of internal cGMP – activation of protein kinase G (PKG). On the one hand PKG could influence directly the contractile machinery of smooth muscles¹⁴, on the other hand it could block L-type Ca²⁺ channels (in terms of Ca²⁺ influx reduction, necessary for muscle contraction)¹⁵ and on third hand PKG could activate calcium pumps which also leads to reduction of intracellular calcium. The overall processes cumulation leads to 5-HT_{1B} and α _{1D} activation and subsequent smooth muscle relaxation at a concentration of 5×10^{-5} mol/l of frovatriptan.

Such a hypothesis is supported by the results of experiments conducted with methysergide (specific inhibitor (5HT) receptor) and prazosin (specific inhibitor of alpha-1 receptors). Our data shows that the block 5-HT receptors by methysergide is probably due to activation of the α _{1D} receptors by frovatriptan.

The blockade of α ₁ receptors by prazosin reduced significantly the frovatriptan-induced relaxation which confirms the probable involvement of α _{1D} receptors in the realization of its effects. As a result of experiments conducted in the presence of frovatriptan (5×10^{-5} mol/l), the contractile response to L-norepinephrine was significantly increased compared to control reaction on the carotid artery. Our results also suggest that frovatriptan predominantly at higher concentrations probably by non-specific mechanism, which could activate the following intracellular chain reaction: stimulation on α _{1D} → activation of eNOS → increase in the concentration of NO¹⁶ and subsequent relaxation, which is atypical for the triptans.

In conclusion, frovatriptan in concentrations of 1×10^{-6} mol/l, 1×10^{-5} mol/l induces contractions in the carotid artery which is due to the activation of 5-HT_{1B/1D} receptors. At higher concentrations (5×10^{-5} mol/l) frovatriptan induces relaxation atypical for triptans. This effect could be a result from the activation of α _{1D} receptors. On the other hand, at a concentration of 5×10^{-5} mol/l frovatriptan potentiates the effect of L-norepinephrine which in turn

can activate the following intracellular cascade: stimulation of α_{1D} receptors followed by activation of eNOS and subsequently increased concentration of NO with the final effect of relaxation.

Apparently frovatriptan initiates very complicated and balanced pathways via 5-HT_{1B} and α_{1D} receptors, which, depending on its concentration, could lead to contraction or relaxation of the carotid artery.

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REFERENCES

1. Kaumann JA, Levy FO. 5-hydroxytryptamine receptors in the human cardiovascular system. *Pharmacol Ther* 2006;111(3):674-706.
2. Geerts IS, Matthys KE, Herman AG, et al. Involvement of 5-HT_{1B} receptors in collar-induced hypersensitivity to 5-hydroxytryptamine of the rabbit carotid artery. *Br J Pharmacol* 1999;127(6):1327-36.
3. Roberto G, Piccinni C, D'Alessandro R, et al. Triptans and serious adverse vascular events: Data mining of the FDA Adverse Event Reporting System database. *Cephalalgia* 2014;34(1):5-13.
4. Tullo V, Valguarnera F, Barbanti P, et al. Comparison of frovatriptan plus dextketoprofen (25mg or 37.5 mg) with frovatriptan alone in the treatment of migraine attacks with or without aura: A randomized study. *Cephalalgia* 2014;34(6):434-45.
5. Savi L, Mogavero S, Egan CG. Efficacy and pharmacokinetic activity of frovatriptan compared to rizatriptan in patients with moderate-to-severe migraine. *Drug Des Devel Ther* 2014;8:983-92.
6. Rapport MM, Green AA, Page IH. Serum vasoconstrictor, serotonin; isolation and characterization. *J Biol Chem* 1948;176(3):1243-51.
7. Erspamer V, Asero B. Identification of enteramine, the specific hormone of the enterochromaffin cell system, as 5-hydroxytryptamine. *Nature* 1952;169(4306):800-1.
8. Page IH, McCubbin JW. Modification of vascular response to serotonin by drugs. *Am J Physiol* 1953;174(3):436-44.
9. Goadsby PJ. Serotonin 5-HT_{1B/1D} receptor agonists in migraine comparative pharmacology and its therapeutic implications. *CNS Drugs* 1998;10(4):271-86.
10. Willems EW, Heiligers JPC, De Vries P, et al. Alpha1-adrenoceptor subtypes mediating vasoconstriction in the carotid circulation of anaesthetized pigs: possible avenues for antimigraine drug development. *Cephalalgia* 2001;21:110-9.
11. McCune DF, Edelmann SE, Olges JR, et al. Regulation of the cellular localization and signaling properties of the alpha(1B)- and alpha(1D)-adrenoceptors by agonists and inverse agonists. *Mol Pharmacol* 2000;57(4):659-66.
12. Zschauer AO, Sielczak MW, Smith DA, et al. Norepinephrine-induced contraction of isolated rabbit bronchial artery: role of alpha 1- and alpha 2-adrenoceptor activation. *J Appl Physiol* 1997;82(6):1918-25.
13. de Andrade CR, Fukada SY, Olivon VC, et al. Alpha1 D-adrenoceptor-induced relaxation on rat carotid artery is impaired during the endothelial dysfunction evoked in the early stages of hyperhomocysteinemia. *Eur J Pharmacol* 2006;543(1-3):83-91.
14. Lee MR, Li L, Kitazawa T. Cyclic GMP causes Ca²⁺ desensitization in vascular smooth muscle by activating the myosin light chain phosphatase. *J Biol Chem* 1997;272(8):5063-8.
15. Carvajal JA, Germain AM, Huidobro-Toro JP, et al. Molecular mechanism of cGMP-mediated smooth muscle relaxation. *J Cell Physiol* 2000;184(3):409-20.
16. Lowry JL, Brovkovich V, Zhang Y, et al. Endothelial nitric-oxide synthase activation generates an inducible nitric-oxide synthase-like output of nitric oxide in inflamed endothelium. *J Biol Chem* 2013;288(6):4174-93.

Эффект воздействия нового высокоэффективного 5-HT(1B/1D)-рецептора лиганда фроватрипана на сонную артерию крысы

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Введение: В кровеносных сосудах 5-HT стимулирует симпатические нервы, эндотелий и клетки гладких мышц сосудов. Триптаны являются специфическими антимигренозными медикаментами, которые активируют серотонинэргические 5HT_{1B/1D} рецепторы, вызывая вазоконстрикции сосудов головного мозга.

Цель: Оценить эффект воздействия фроватрипана на изолированную сонную артерию крысы.

Методы: Сократительная активность препаратов зарегистрирована в изометрическом режиме. Раствор Кребса (pH = 7.4) использован для отмывания препаратов гладких мышц (ГМ), аэрированных 95% O₂ и 5% CO₂ при 37°C. 60-минутная адаптация уровня цвета препаратов взята в качестве контрольного цвета и изменения в виде сокращения и релаксации измерены в соответствии с ним.

Результаты: Фроватрипан (1×10⁻⁶ мол/л - 1×10⁻⁵ мол/л) индуцирует сокращение, но в более высоких концентрациях вызывает релаксацию сонной артерии. L-норэпинефриновая сократительная реакция была усилена наличием фроватрипана. При наличии 5-HT₂ рецептора антагониста, метисергида, фроватрипан усиливает проявление релаксации. При наличии специфического α-1 рецептора антагониста, празосина, фроватрипан-индуцированная релаксация понижается.

Заключение: Установленный сократительный эффект фроватрипана вероятно связан с основным эффектом препарата – активацией серотонинэргических 5HT_{1B/1D} рецепторов, вызывающих вазоконстрикции сосудов головного мозга и их антимигренозный эффект. В более высоких концентрациях фроватрипан, вероятнее всего неизвестным неспецифическим механизмом может активировать следующий интрацеллюлярный каскад реакций: стимуляция α1D может активировать eAOC (эндотелиальную синтазу оксида азота), которая может повыситься в концентрации АО, что приводит к конечному эффекту релаксации.