PRACA ORYGINALNA

Influence of chronically-administered amitriptyline on cardiovascular reactivity in rats

Wpływ przewlekle podawanej amitryptyliny na reaktywność układu krążenia u szczurów

Jerzy Jochem[1], Karolina Walas[1], Katarzyna Chojnacka[1], Agata Mitera[1], Adam Krawiec[1], Anna Stasiak[2], Alicja Kasperska-Zając[3]

[1]Katedra i Zakład Podstawowych Nauk Medycznych, Wydziału Zdrowia Publicznego Śląskiego Uniwersytetu Medycznego w Katowicach
[2]Zakład Biochemii Hormonów, Katedra Diagnostyki Obrazowej i Biochemii Hormonów Uniwersytetu Medycznego w Łodzi
[3]Katedra i Klinika Chorób Wewnętrznych, Alergologii i Immunologii Klinicznej Wydziału Lekarskiego z Oddziałem Lekarsko-Dentystycznym w Zabrzu Śląskiego Uniwersytetu Medycznego w Katowicach

ADRES DO KORESPONDENCJI:
Prof. dr hab. n. med. Jerzy Jochem
Katedra i Zakład Podstawowych Nauk Medycznych
Wydziału Zdrowia Publicznego
Śląskiego Uniwersytetu Medycznego
w Katowicach
ul. Piekarska 18
41-902 Bytom
tel. +48 32 397 65 42
fax +48 32 397 65 37
e-mail: jjochem@poczta.onet.pl

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ABSTRACT

INTRODUCTION
Amitriptyline, a tricyclic antidepressant used in the treatment of many psychiatric, neurological and gastrointestinal diseases, affects cardiovascular system functions. The aim of the present study was to examine the influence of chronically administered amitriptyline on cardiovascular responsiveness to a histamine vasodilatatory stimulus.

MATERIAL AND METHODS
The studies were carried out on male Wistar rats pre-treated with amitriptyline (2.7 and 5.4 mg/kg, subcutaneously for 7 days).

RESULTS
Histamine (5.0 and 10.0 μg/kg) administered intravenously in a bolus injection evoked dose-dependent decreases in systolic and diastolic blood pressure as well as in renal (RBF) and skeletal muscle microcirculatory flow (SMMF), with no significant changes in heart rate (HR) in the control, saline pre-treated group. Amitriptyline prevented histamine-evoked decreases in blood pressure (2.7 and 5.4 mg/kg) and produced increases in RBF and SMMF (5.4 mg/kg), but did not influence HR dynamics.

CONCLUSION
We demonstrate for the first time that chronically administered amitriptyline to rats influences the cardiovascular responsiveness to histamine with an effect on peripheral perfusion.

KEY WORDS
amitriptyline, cardiovascular responsiveness, histamine

STRESZCZENIE

WSTĘP

Amitryptylina to trójpierścieniowy lek przeciwdepresyjny stosowany w leczeniu wielu schorzeń psychicznych, neurologicznych oraz przewodu pokarmowego, wpływający także na czynność układu krążenia. Celem pracy było zbadanie wpływu przewlekle stosowanej amitryptyliny na reaktywność układu krążenia na działanie bodźca hipotensyjnego – histaminy.

MATERIAŁ I METODY

Badania przeprowadzono na samcach szczurów szczepu Wistar, którym podawano amitryptylinę (2,7 i 5,4 mg/kg) podskórnie przez 7 dni.

WYNIKI

Histamina (5,0 i 10,0 μg/kg) podawana dożylnie (bolus) w grupie kontrolnej wywoływała zależne od dawki spadki ciśnienia tętniczego skurczowego i rozkurczowego oraz przepływu w tętnicy nerkowej (RBF) i w mikrokrążeniu mięśnia szkieletowego (SMMF), nie wpływając na częstość rytmu serca (HR). Amitryptylina zapobiegała wywoływanym przez histaminę spadkom ciśnienia tętniczego krwi (2,7 i 5,4 mg/kg), powodowała wzrost RBF i SMMF (5,4 mg/kg), nie wpływając na HR.

WNIOSEK

Przewlekle stosowana amitryptylina wpływa na reaktywność układu krążenia i przepływy obwodowe w odpowiedzi na dożylne podanie histaminy u szczurów.

SŁOWA KLUCZOWE

amitryptylina, reaktywność układu krążenia, histamina

INTRODUCTION

Amitriptyline is a tricyclic antidepressant (TCA) used in the treatment of many neurological, psychiatric and gastrointestinal diseases, including depressive disorders, bipolar disorder, anxiety, migraine, eating disorders, insomnia, neuropathic pain and abdominal pain-related functional gastrointestinal disorders [1, 2, 3]. Primarily, it acts as a serotonin-noradrenaline reuptake inhibitor, however, it is also an antagonist of 5-HT_{2A}, 5-HT_{2C}, 5-HT_3, 5-HT_6, 5-HT_7 receptors, nicotinic acetylcholine receptors, α_1-adrenergic and histamine H_1 receptors [4, 5]. Moreover, it is able to activate σ_1 and neurotrophic tyrosine kinase type 1 (TrkA) and type 2 (TrkB) receptors [6, 7] and block sodium, potassium and calcium channels [8, 9, 10].

In addition to the nervous system, amitriptyline also influences the cardiovascular system functions. Initial experimental studies by Thorstrand [11] demonstrated that intravenously injected amitriptyline (0.5–2 mg/kg) induces a decrease in the mean arterial pressure (MAP) and heart rate (HR) with a significant prolongation of QRS and PQ duration in rats. In further placebo-controlled double blind studies in humans, amitriptyline given in a single oral dose (100 mg or 150 mg) caused a decrease in blood pressure, particularly, in the upright position, an elongation of electromechanical systole, an increase in HR, especially under orthostatic stress and a reduction in total peripheral resistance [12]. Interestingly, the study by Kopera [13] demonstrated amitriptyline-induced postural hypotension in humans, which may suggest its effect on cardiovascular responsiveness.

There are many pharmacological methods used to study cardiovascular responsiveness *in vivo*. Usually, endogenous or exogenous vasodilatatory/vasoconstrictive agents are administered intravenously and then, direct

and reflex-induced changes in cardiovascular parameters, such as HR, MAP, systolic (SBP) and diastolic blood pressure (DBP) as well as peripheral blood flows, are measured [14]. In our previous experimental studies concerning cardiovascular responsiveness with a vasodilatatory stimulus histamine, we showed that a single dose of amitriptyline and the selective serotonin reuptake inhibitor citalopram, reduce the histamine-induced influence on MAP and pulse pressure (PP), and this effect was greater in amitriptyline- than in citalopram-pre-treated animals [15]. In contrast to arterial pressure changes, pre-treatment with both antidepressants did not influence HR [15]. Since amitriptyline is usually used chronically, the aim of the present study was to examine the effect of amitriptyline given for 7 days on the cardiovascular responsiveness to histamine in rats.

MATERIAL AND METHODS

The studies were carried out on male Wistar rats weighing 247–293 g (5–6 months old). The animals were housed individually in cages in the animal colony, under controlled conditions of temperature (20–22°C), humidity (60–70%) and a 12 h light/dark cycle. Throughout the experiment, the rats were provided with food and water *ad libitum*. All the studied groups consisted of five animals. All the procedures were performed in accordance to EU directives, after acceptance by the Local Ethics Committee, Katowice, Poland (notifications No 7/2011, 70/2011, 71/2011 and 27/2012).

The animals divided into groups were subcutaneously (*sc*) pre-treated with amitriptyline (2.7 and 5.4 mg/kg) or saline (0.2 ml) for 7 days. On the 8th day, after the induction of a general anaesthesia with ketamine/xylazine (100 mg/kg + 10 mg/kg intraperitoneally [*ip*], supplemented if required), the rats were implanted with catheters filled with heparinised saline (100 IU/ml) in the right femoral artery and vein. SBP, DBP and HR were measured using a TAM-A transducer amplifier module and ECGA amplifier (Hugo Sachs Elektronik, Germany), respectively. A electromagnetic perivascular probe (type 1RB, Hugo Sachs Elektronik, Germany) was implanted around the right renal artery to monitor renal blood flow (RBF) using a TTFM transit time flow-meter module (Transonic Systems Inc., USA). Skeletal muscle microcirculatory flow was measured after the implantation of a needle electrode (OxyFlo XP probe 19, Oxford Optronix Ltd., United Kingdom) in the middle part of the right femoral muscle using an Oxylab Microvascular Perfusion Monitor (Oxford Optronix Ltd., United Kingdom). Microcirculatory blood flow was expressed in relative blood perfusion units (RBPU). All the measurements were started after a 30 min adaptation period to avoid the influences of probe implantation. The doses of amitriptyline and histamine were taken from the literature [14, 15]. The haemodynamic parameters were measured after intravenous (*iv*) injections of histamine (5.0 and 10.0 μg/kg). The rats were given both doses of histamine, allowing enough time for the measured parameters to return to baseline levels between each injection. A similar protocol was used previously to study cardiovascular responsiveness in rats [16]. All the experiments were performed between 8:00 am and 2:00 pm.

The following drugs were used: amitriptyline hydrochloride, histamine dihydrochloride (Sigma-Aldrich, USA), ketamine hydrochloride, xylazine (Biowet Sp. z o.o., Poland) and heparin (Polfa, Poland). All the drug solutions were freshly prepared on the day of the experiment.

All the values are given as means ± the standard deviation (SD) with $p < 0.05$ considered as the level of significance. The statistical evaluation was performed by analysis of variance (ANOVA) and the post-ANOVA test of Student-Newman-Keuls.

RESULTS

There were no differences in the initial (before histamine treatment) SBP, DBP and HR between the groups, the values being in the control saline-pre-treated group: 115.4 ± 9.5 mmHg, 70.4 ± 4.5 mmHg and 274 ± 24 beats/min, respectively. Similarly, the RBF and SMBF did not differ between the groups before the histamine injections (in the control saline-pre-treated group: 4.9 ± 0.7 ml/min and 710 ± 96 RBPU, respectively).

In the control saline pre-treated group, the bolus injection of histamine (5.0, 10 μg/kg) induced decreases in SBP up to 77.8 ± 6.3 and 69.2 ± 5.8 mmHg, respectively, and in DBP up

to 44.2 ± 3.9 and 38,6 ± 3,5 mmHg, respectively, with no influence on HR (fig. 1A–C). The effects were accompanied by decreases in RBF from 4.9 ± 0.7 to 1.92 ± 0.61 ml/min and from 4.64 ± 0.51 to 1.52 ± 0.6 ml/min, respectively, as well as in SMMF from 710 ± 96 to 398 ± 125 RBPU and from 617 ± 157 to 359 ± 75 RBPU, respectively (fig. 2A–B).

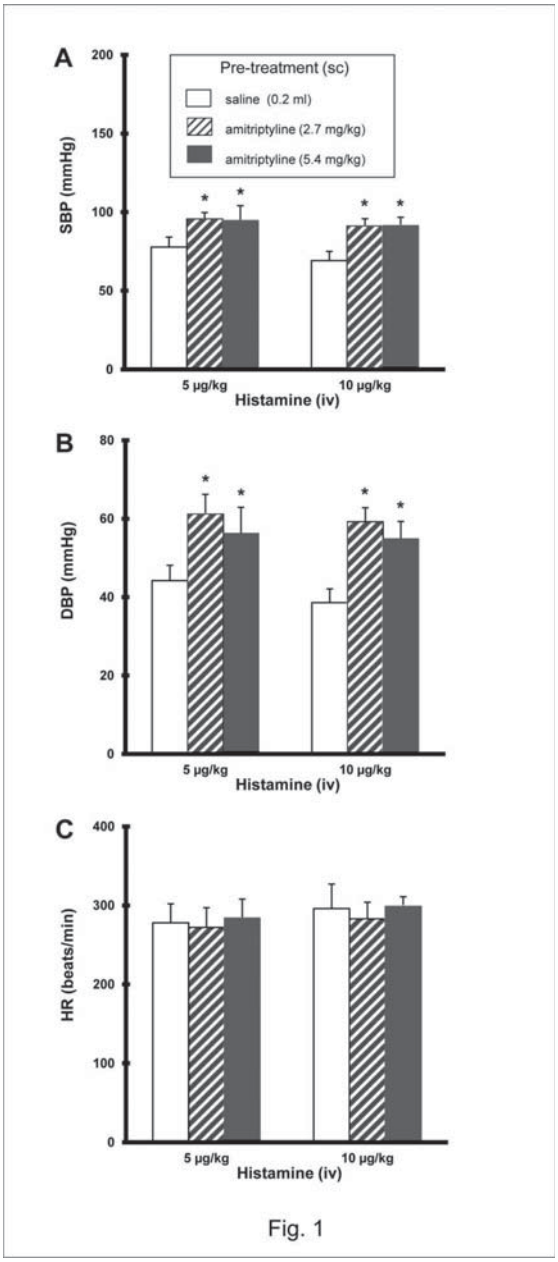

Fig. 1

Fig. 1. Minimal SBP (A), DBP (B) and maximal HR (C) after histamine (5.0 and 10.0 µg/kg) bolus injection in rats pre-treated with saline (0.2 ml) and amitriptyline (2.7 and 5.4 mg/kg) for 7 days; means ± SD; five animals per group; * $p < 0.05$ vs. saline-pre-treated control group.

Ryc. 1. Najniższe wartości SBP (A), DBP (B) oraz najwyższe wartości HR (C) po dożylnym podaniu histaminy (5,0 i 10 µg/kg) u szczurów, którym przez 7 dni podawano 0,9% roztwór NaCl bądź amitryptylinę (2,7 i 5,4 mg/kg); wyniki przedstawiono jako średnie ± SD; n = 5; * $p < 0,05$ w porównaniu z grupą, w której podawano w 0,9% roztwór NaCl.

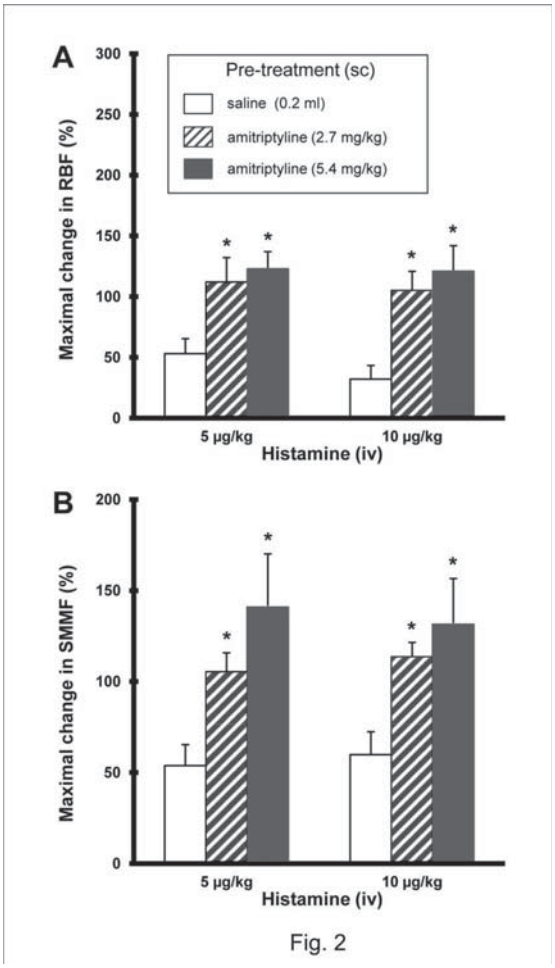

Fig. 2

Fig. 2. Maximal changes in RBF (A) and SMMF (B) after histamine (5.0 and 10.0 µg/kg) bolus injection in rats pre-treated with saline (0.2 ml) and amitriptyline (2.7 and 5.4 mg/kg) for 7 days; means ± SD; five animals per group; in all groups, initial values of RBF and SMMF were taken as 100%; in the control saline-pre-treated group, basal RBF and SMMF were 4.9 ± 0.7 ml/min and 710 ± 96 RBPU, respectively; * $p < 0.05$ vs. saline-pre-treated control group.

Ryc. 2. Maksymalne zmiany RBF (A) i SMMF (B) po dożylnym podaniu histaminy (5,0 i 10,0 mg/kg) u szczurów, którym przez 7 dni podawano 0,9% roztwór NaCl bądź amitryptylinę (2,7 i 5,4 mg/kg); wyniki przedstawiono jako średnie ± SD; n = 5; we wszystkich grupach za wartości początkowe RBF i SMMF przyjęto 100%; w grupie kontrolnej wyjściowe wartości RBF i SMMF wynosiły odpowiednio 4,9 ± 0,7 ml/min i 710 ± 96 RBPU; * $p < 0,05$ w porównaniu z grupą, w której podawano w 0,9% roztwór NaCl.

Pre-treatment with amitriptyline (2.7 and 5.4 mg/kg) reduced the histamine-induced effects on SBP and DBP (Fig. 1A-B), with no influence on HR (fig. 1C). Interestingly, amitriptyline-treated rats (5.4 mg/kg) showed significant

increases in RBF after a histamine (5.0, 10 μg/kg) injection from 4.42 ± 0.52 up to maximally 5.4 ± 0.45 ml/min and from 5.04 ± 0.48 up to 6.08 ± 0.76 ml/min, respectively. A similar effect was observed in respect to SMMF, the values before and after histamine treatment being 689 ± 156 vs. 951 ± 146 RBPU and 726 ± 121 vs. 942 ± 135 RBPU, respectively. Fig. 2 demonstrates the maximal changes in RBF and SMMF after histamine (5.0, 10 μg/kg) injection in the studied groups.

DISCUSSION

In the present study, we demonstrate for the first time that chronic treatment (7 days) with amitriptyline influences the cardiovascular responsiveness to histamine in rats. Furthermore, we show that amitriptyline significantly affects peripheral perfusion in response to histamine.

The present paper is a continuation of our previous studies concerning the influence of tricyclic antidepressants on the cardiovascular system [15]. Using the *in vivo* model, direct and reflex-induced changes in cardiovascular parameters (arterial pressure, HR, peripheral vascular blood flows and resistances) can be measured after the injection of vasoconstrictive/vasodilatatory agents [16]. As in the earlier studies, we used histamine which acting peripherally *via* H_1 and H_2 receptors, induces a direct vasodilatatory effect [15]. Our present results confirm that under normal conditions, histamine given in a bolus *iv* injection evokes a prompt short-lasting decrease in SBP and DBP associated with a reduction in RBF and SMMF. The decreases in peripheral perfusion after histamine treatment can be explained by a dramatic drop in blood pressure due to general vasodilatation, despite a decrease in peripheral vascular resistance in the studied region. The lack of significant changes in HR after histamine injection is in accordance with our earlier results [15]. We suggest that HR dynamics can be a result of a simultaneously occurring (1) reflex-induced increase in the sympathetic nervous system activity and (2) direct action of histamine on cardiac H_1 and H_2 receptors.

In our previous study we demonstrated that amitriptyline and citalopram given alone did not affect the cardiovascular parameters [15]. In contrast, a single-dose pre-treatment with any of these antidepressants (15 min or 1 h prior to histamine) leads to significant inhibition of cardiovascular responsiveness to histamine [15]. The present results also demonstrate that chronic pre-treatment with amitriptyline reduces the decreases in SBP and DBP evoked by histamine. Similar to the previous studies, we did not find significant changes in HR.

The mechanism of the histamine-induced depressor effect is related to a decrease in the total peripheral vascular resistance, despite local vasoconstrictive action in the coronary circulation [17]. We demonstrate here that a bolus injection of histamine evokes a critical decrease in renal and skeletal muscle perfusion. It can be hypothesized that the effect results from pronounced systemic hypotension, despite the decrease in peripheral vascular resistance in the local vascular beds. Interestingly, we show for the first time that amitriptyline (at a dose 5.4 mg/kg) completely reverses peripheral perfusion changes evoked by histamine: it induced increases in RBF and SMMF. These results are in line with a previous *in vitro* study, which showed that amitriptyline and citalopram inhibit histamine-induced contractions of an isolated porcine coronary artery [18].

The direct, vascular mechanisms of amitriptyline action can be explained by (1) H_1 receptors antagonism, (2) the influence on histamine degrading enzymes and (3) post-receptor influences. The first pathway can be related to the well-known antagonistic properties of amitriptyline to the H_1 receptor demonstrated *in vitro* [19]. On the other hand, amitriptyline and citalopram reduce an increase in plasma histamine levels after its bolus injection [15, 20]. These findings support the hypothesis concerning the changes in histamine degrading enzymes activity. Indeed, Rajtar and Irman-Florjanc [21] demonstrated that amitriptyline enhances the activity of diamine oxidase and histamine-N-methyltransferase, the two main enzymes responsible for histamine catabolism in mammals. The restriction of the increase in plasma histamine concentrations may be responsible for the inhibited cardiovascular responsiveness to histamine, however, we have to take into consideration the earlier suggested amitriptyline-induced functional modulation of the structure of cellular membranes and post-receptor changes [15,20].

Additionally, we cannot exclude other possible pathways involved in the amitriptyline influence on the cardiovascular system function,

since it easily crosses the blood-brain barrier. To verify the hypothesis concerning the centrally-mediated effects of amitriptyline on the cardiovascular centre neurons, we previously injected *icv* amitriptyline and citalopram into rats [22]. We have demonstrated that centrally acting amitriptyline, but not citalopram, influences the vascular resistance regulatory mechanisms and reduces histamine-induced MAP and PP changes [22]. These effects were, however, significantly lower in comparison to those observed after peripheral pre-treatment with both antidepressants [15].

Finally, we demonstrated previously the influence of amitriptyline on the central histamine-induced resuscitating effect in haemorrhage-shocked rats [23]. We showed that histamine administered *icv* to haemorrhage-shocked rats evokes long-lasting rises in MAP and HR, with an increase in the survival rate [23]. Pre-treatment with amitriptyline increases central histamine-induced rises in MAP and HR, with no influence on survival. Interestingly, amitriptyline given alone (10, 20 nmol; *icv*) also evokes rises in MAP and HR, with increases in the survival rates in rats subjected to haemorrhagic shock [23]. On the other hand, the study by Richardson and Chiu shows that a high dose (0.9 µmol) of amitriptyline given *icv* evokes a hypotensive effect in anaesthetised rats, which is probably related to the inhibition of noradrenaline re-uptake and its action in the anterior hypothalamus [24]. Thus, we summarized that amitriptyline affects the reactivity of the cardiovascular centre to hypotensive stimuli and influences the central histamine-induced resuscitating effect.

In conclusion, our present results demonstrate for the first time that chronically administered amitriptyline influences cardiovascular responsiveness and peripheral perfusion after histamine injection in rats.

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