Agmatine facilitates sympathetic neurotransmission in frog myocardium via an action on alfa 2-adrenergic receptors

Agmatin alfa-2 adrenerjik reseptörler aracılığı ile kurbağa kalbinde sempatik nörotransmisyonu kolaylaştırmaktadır

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ABSTRACT

Objective: In this study, the effect of agmatine was studied on sympathetic neurotransmission in the frog isolated ventricular strips.

Methods: Ventricular strips were prepared from the heart of the pitched frog. Each strip was mounted vertically in an organ bath. Muscle contractions were recorded isometrically by a force displacement transducer and displayed on a polygraph.

Results: Concentration-response relationships to noradrenaline were obtained on contractility of frog ventricular strips evoked by electrical stimulation. The responses of noradrenaline were re-obtained in presence of agmatine ($3X10^{-4}$ M). Agmatine was found to be ineffective on contractile responses of noradrenaline in electrically driven ventricular strips of frog heart. Transient additional stimulations (TAS) induced contractions. The contractions induced by TAS were re-obtained in presence of agmatine, idazoxan + agmatine and yohimbine + agmatine. Agmatine significantly increased the positive inotropic responses of TAS. The effect of agmatine on contractile responses of TAS was not changed by idazoxan, indicating that imidazoline receptors have not functions in this response. The effect of agmatine on the contractile responses to TAS was reversed by yohimbine, indicating involvement of α2 adrenoceptors in this response. Agmatine did not change the contractile responses of ventricular strips to exogenous noradrenaline, indicating that agmatine does not affect postjunctional adrenoceptors.

Conclusion: These results suggest that agmatine facilitates sympathetic neurotransmission in frog myocardium via an action on prejunctional α2 adrenergic receptors located on sympathetic nerve terminals. *(Anadolu Kardiyol Derg 2006; 6: 34-8)*

Key words: Agmatine, α2 adrenoceptors, idazoxan, yohimbine, frog myocardium, sympathetic nerve activity

ÖZET

Amaç: Bu çalışmada, agmatin'in kurbağa kalbinden izole edilen ventriküler striplerdeki sempatik nörotransmisyonlar üzerine olan etkisi araştırıldı.

Yöntemler: Kurbağa kalbinden ventriküler stripler hazırlandı. Her bir strip vertikal olarak bir organ banyosuna asıldı. Kas kontraksiyonları bir transdüser ve poligraf kullanılarak izometrik olarak kaydedildi.

Bulgular: Noradrenaline bağlı konsantrasyon-cevap ilişkisi elektriksel stimülasyolar aracılığı ile kurbağa ventrikül striplerinde kasılma olarak gözlendi. Noradrenalin cevapları agmatin ($3X10^{-4}$ M) varlığında yeniden ortaya çıktı. Agmatin kurbağa kalbinin ventriküler striplerinin elektriksel uyarılarında, noradrenalinin kasılma cevapları üzerinde etkisiz bulundu. Uygulanan geçici ilave stimülasyonlar kontraksiyonlara neden oldu. Geçici ilave stimülasyonların neden olduğu kontraksiyonlar agmatin, agmatin +idazoksan, agmatin + yohimbin varlığında yeniden ortaya çıktı. Agmatin geçici ilave stimülasyonların pozitif inotropik cevaplarını anlamlı olarak artırdı. Geçici ilave stimülasyonların kasılma cevaplarında agmatinin etkisi idazoksan tarafından değiştirilemedi, bu durum imidazolin reseptörlerinin bu cevapta fonksiyonları olmadığını göstermektedir. Geçici ilave stimülasyonların kasılma cevaplarında agmatinin etkisi yohimbin tarafından tersine çevrildi, Bu duruma oluşan bu cevapta α2 adrenoreseptörlerin etkilerinin olduğunu göstermektedir. Agmatin'in ventriküler striplerdeki kasılma cevapları ekzojenik noradrenalinle değişmedi, böylece ``postjunctional`` adrenoreseptörler üzerine etki etmediği gösterildi.

Sonuç: Bu sonuçlar agmatin'in sempatik sinir terminallerinde lokalize olan ``prejunctional`` α2 adrenoreseptörlerin etkisine bağlı olarak kurbağa kalbinde sempatik nörotransmisyonu kolaylaştırdığını ortaya koymaktadır. *(Anadolu Kardiyol Derg 2006; 6: 34-8)*

Anahtar kelimeler: Agmatin, α2-adrenoreseptörler, idazoxan, yohimbin, kurbağa miyokardı, sempatik aktivite

Introduction

Agmatine is a polycationic amine synthesized from L-arginine, by the enzyme arginine decarboxylase (ADC). Agmatine and ADC have recently been identified in a number of mammalian tissues including brain, stomach, intestine and aorta (1). The highest concentration was found in stomach, aorta, and small intestine, followed by smaller levels in spleen, adrenal, aorta, skeletal muscle and brain (2). Agmatine, with its ability to bind at alpha 2-adrenoceptors and imidazoline binding sites (IBS) of

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both I1- and I2-subclasses, has a substantial diversity of regulatory functions on many visceral organs systems (3). Agmatine, while having no direct action upon vascular smooth muscle, can inhibit the neurogenically mediated contraction of rat tail artery by activating prejunctional alpha 2-adrenoceptors (4). Agmatine also prevented the development of tolerance to the substance dependence on morphine in guinea pig ileum in vitro by activation of imidazoline receptors (5).

The functions of agmatine mediated by α2-or I-receptors are uncertain and are not fully understood, however, agmatine may gain interest as a potential inotropic agent?

In this study, we investigated the effect of agmatine on sympathetic neurotransmission in the frog isolated ventricular preparation. To investigate whether α2 or I receptors have role on the effects of agmatine, we preferred to study idazoxan, as an imidazoline receptor blocker, and yohimbine, as an α2 receptor blocker.

Methods

An experimental animal study was conducted. Animal care complied with the "Principles of Laboratory Animal Care" as formulated by the National Society for Medical Research and the Guide for the Care and Use of Laboratory Animals (NIH publication No. 85-23, revised 1996). All studies were approved by the Animal Care and Use Committee of Medical Faculty of the Cumhuriyet State University.

Tissue preparation

Ventricular strips prepared from the heart of the pitched frog Rana temporaria (25-75 g) were used (n=16). Each strip was mounted vertically in an organ bath containing 10 ml of glucose-free, frog Ringer's solution of the following composition (mM): NaCl, 111.0; KCl, 1.9; $CaCl_2$, 1.1; $NaHCO_3$, 2.4; NaH_2PO_4, 0.07. The temperature of the solution was kept at room temperature and aerated with O_2. Each tissue was maintained at 0.5 g resting tension and the preparation was allowed to stabilize for 1 hour, the bathing fluid being replaced every 15 minute. Muscle contractions were recorded isometrically by a force displacement transducer (Grass FT 03) and displayed on a polygraph (Grass 7 B).

Experimental Design

Ventricles strips were driven electrically through parallel platinum electrodes by square-wave pulses of 3-ms duration and supramaximal voltage at a frequency of 0.5 Hz using a Grass S88 stimulator. Transient additional stimulations (TAS) were applied for 10 s with following parameters of train stimulation: 100 V, 5 ms duration and 2, 4, 8 and 16 Hz. The TAS has been used already for the investigation of presynaptic adrenergic receptors in the guinea-pig left atrium and in frog myocardium (6).

Isometric measurements

The concentration-response relationships of noradrenaline (NA) (10-7-10^{-4} M) were obtained on contractility of frog ventricular strips evoked by electrical stimulation. The concentration-response relationships of NA were repeated in the presence of agmatine ($3x10^{-4}$ M). The contraction responses of NA were presented as % of added contractions on TAS-induced contractions. The contractions induced by TAS were repeated in presences of agmatine ($3x10^{-4}$ M), idazoxan (10-5 M) +agmatine ($3x10^{-4}$ M) and yohimbine (10^{-5} M) +agmatine ($3x10^{-4}$ M). Agmatine, idazoxan and yohimbine were added into organ bath before 30 minutes from beginning of TAS.

Drugs

In this study, following drugs were used: noradrenaline (Sigma), agmatine (Sigma), yohimbine (Merck) and idazoxan (Sigma). All drugs were prepared daily, and were dissolved in distillated water.

Statistical Analysis

To evaluate the effects of an agonist, the maximum response (Emax), the concentration for a half-maximal response (EC_{50})

Table 1. Frog myocardium contractions during TAS (100 V, 5 ms)

Frequency	Contractions (g)
2 Hz	0.52 ± 0.09
4 Hz	0.64 ± 0.08
8 Hz	0.88 ± 0.08
16 Hz	0.94 ± 0.07

Values indicate mean ± SEM and number of experiments (n=8)
g- gram, TAS - transient additional stimulations

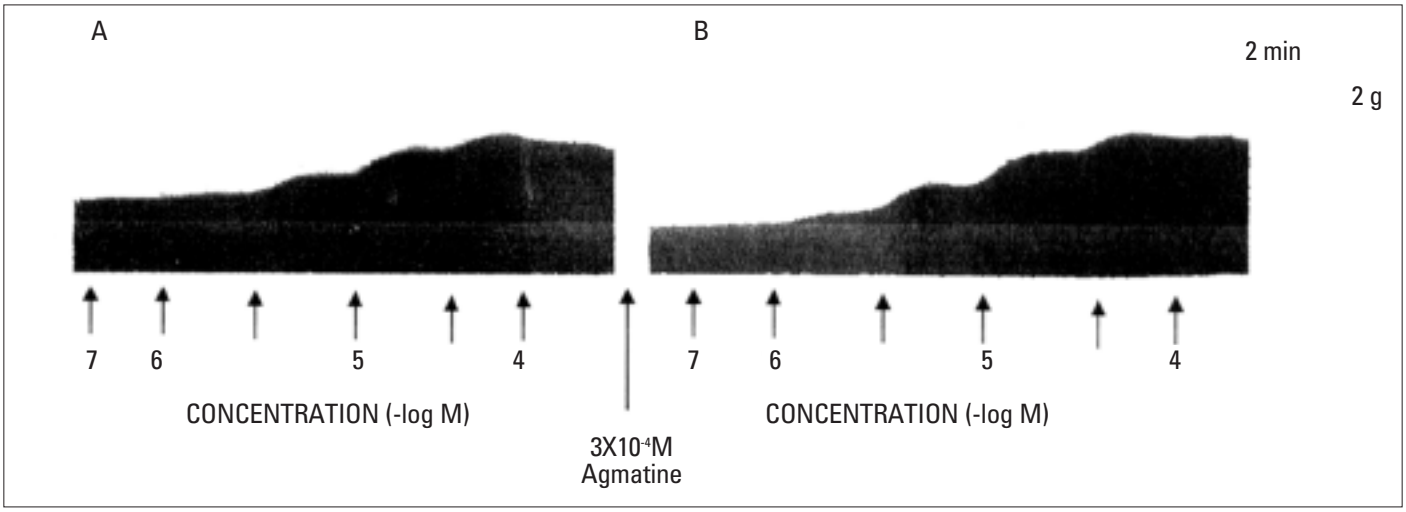

Figure 1. Contractility of frog ventricular strips evoked by electrical stimulation (n=8): effects of exogenous NA (10^{-7}-10^{-4} M) (A), and NA (10^{-7}-10^{-4} M) + Agmatine ($3x10^{-4}$ M) (B).

NA- Noradrenaline

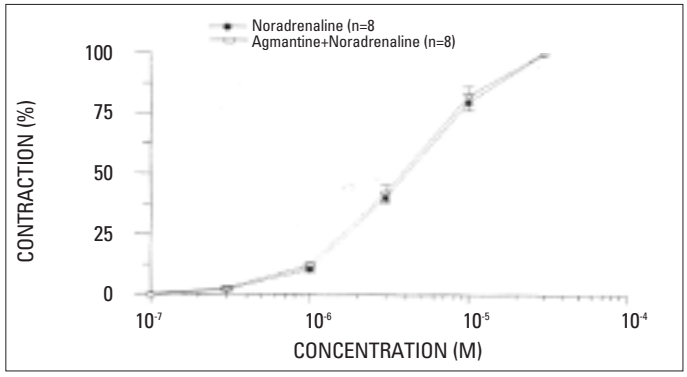

Figure 2. Concentration-response curves of NA and NA + Agmatine (3x10-4 M) in isolated of frog ventricular strips evoked by electrical stimulation. Values indicate mean ± SEM and number of experiments (n=8).

NA- Noradrenaline

and pD_2 values were calculated from the concentration-response curve obtained in each experiment, as predicted from the Scatchard equation for drug-receptor interaction, where: response/concentration=1/EC_{50}xresponse+Emax/EC_{50}. The pD_2 value was expressed as the negative logarithm of the EC_{50}. Groups were compared using general linear models of analysis, with independent paired t-test, and $P<0.05$ considered to indicate statistical significance.

Results

The effects of agmatine on ventricle strips driven electrically

The results of TAS-induced contractions (100 V, 5 ms duration and 2, 4, 8 and 16 Hz) on frog myocardium are presented in Table 1. Noradrenaline induced contractions with cumulative concentration on ventricular strips evoked by electrical stimulation (Fig. 1A). Agmatine did not change contractile responses of

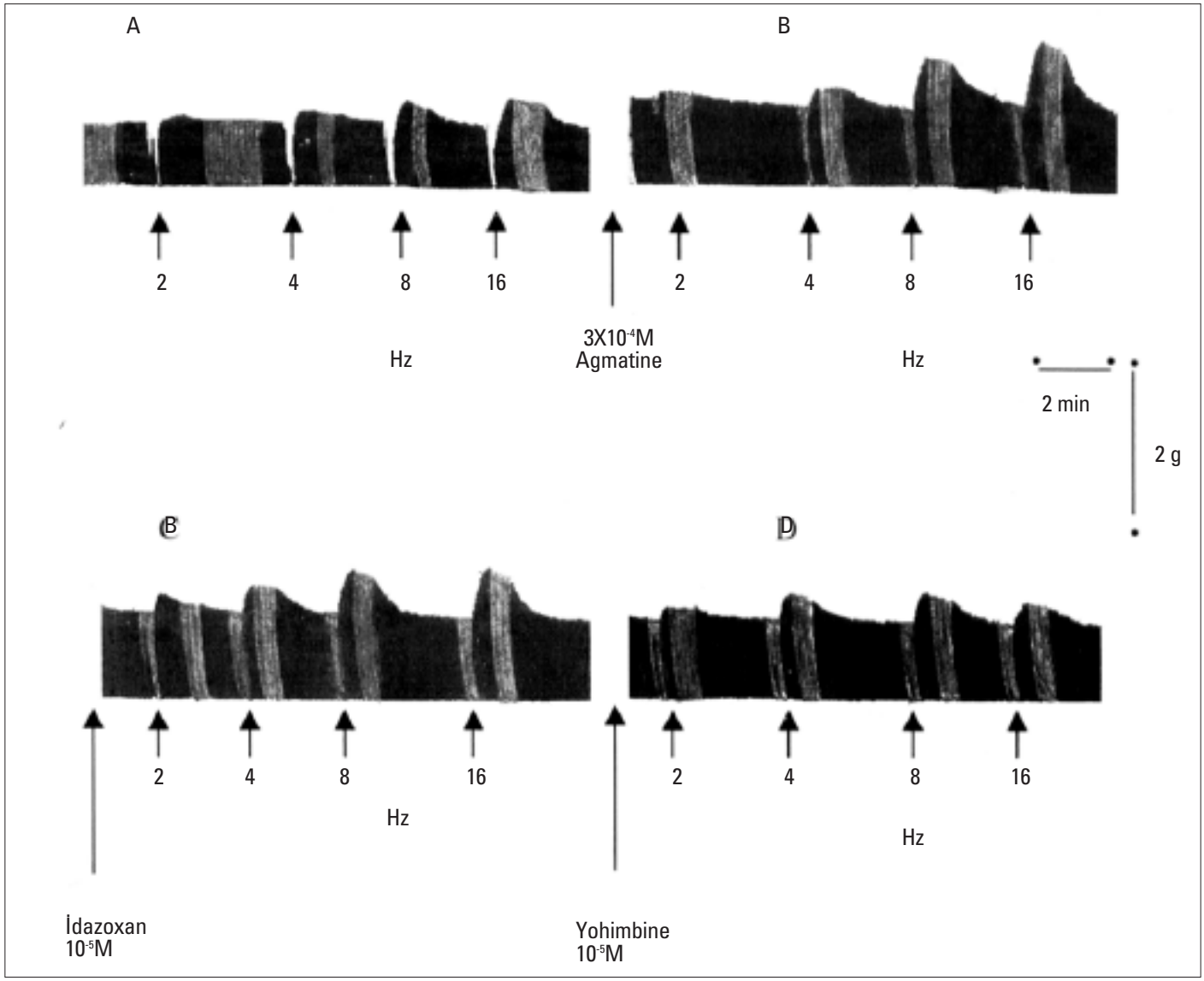

Figure 3. Contractions induced by TAS (n=8) (A), contractions induced by TAS in presence of Agmatine (3x10-4 M) (B), the effect of Idazoxan on contractions induced by TAS in presence of Agmatine (C) and the effect of Yohimbine on contractions induced by TAS in presence of Agmatine (D).

TAS - transient additional stimulations

NA (Fig. 1B). Agmatine did not change (p>0.05) concentration-response curves of NA (Fig. 2). The pD2 value of group given NA was5.22±0.08, the pD2 value of group given NA+ agmatine was 5.30±0.10.

The effects of drugs on the response to TAS of ventricle strips driven electrically

In isolated strips TAS produced contractions in a frequency-dependent way (Fig. 3A). Agmatine increased significantly (p<0.05) these contractions Fig. 3B). Idazoxan did not change (p>0.05) contractile responses of agmatine (Fig. 3C), but yohimbine diminished significantly (p<0.05) contractions of agmatine (Fig. 3D). Frequency-response curves of TAS with agmatine, idazoxan and yohimbine are showed in Figure 4.

Discussion

Agmatine is an endogenous amine synthesized from L-arginine and it has been identified in a number of mammalian tissues including gastrointestinal system (7,8). Imidazoline receptors were described in the pancreas and a variety of compounds containing an imidazoline ring have the ability to stimulate the insulin secretion (9). Some imidazoline derivatives modulate cholinergic activity of the guinea-pig ileum by interacting with presynaptic alpha 2-adrenoceptors (10) and play a role in gastric acid secretion (11). Some investigators have proposed that agmatine worsen gastric mucosal injury in rats (12), and depresses the contractility of guinea pig papillary muscle (13). Imidazoline-binding sites are non-adrenergic receptors and classified into I1/I2 subtypes (14). Agmatine binds to imidazoline receptors and alpha 2-adrenoceptors and stimulates the release of catecholamines from adrenal chromaffin cells in a dose-dependent manner (1). To investigate further the pharmacological mechanism involved in the positive inotropic effect of agmatine, in the present study two antagonists were used: 1-Idazoxan, an imidazoline that is known to bind to nonadrenergic imidazoline-preferring sites (15) and 2-Yohimbine, a classical α2 adrenoceptor antagonist (16). Yohimbine has become classified as a selective α2 adrenergic antagonist with low affinity for imidazoline-preferring receptors. It has recently been established that noradrenaline release in the human atrium and pulmonary artery, and release of noradrenaline is inhibited via presynaptic imidazoline receptors (17,18). These presynaptic imidazoline receptors appear to be related to those previously characterized in rabbit aorta and pulmonary artery (19). Interestingly agmatine has been also shown to have agonist activity at prejunctional α2 adrenoceptors in the rat tail artery (4) and multiple effects on sympathetic neurotransmission in rat vas deferens (20). In this study, the positive inotropic effect of agmatine was unaffected by idazoxan, indicating that imidazoline receptors have not functions in this response, however the positive inotropic effect of agmatine was abolished by yohimbine. Increases in exogenous NA-induced contraction powers were not change by agmatine, but TAS-dependent contractions were increased and these contractions were abolished by yohimbine, an alfa 2 antagonist. These results suggest that agmatine induces positive inotropic effect on frog myocardium increasing by sympathetic transmission. This effect can be due to a mechanism involving α2 adrenoceptor.

Imidazoline derivates exhibit antiarrhythmic properties and positive inotropic effect (21) that are due to blockade of postsynaptic α2 adrenoceptors in the heart and coronary arteries, and activating of α1 adrenoceptor, respectively (22). In our study, agmatine did not effect on contractions due to exogenous noradrenaline, indicating that there is not an agonist effect of agmatine on postjunctional adrenoceptors.

In conclusion, we can speculate that agmatine causes positive inotropic effects via presynaptic α2 adrenoceptors in frog myocardium.

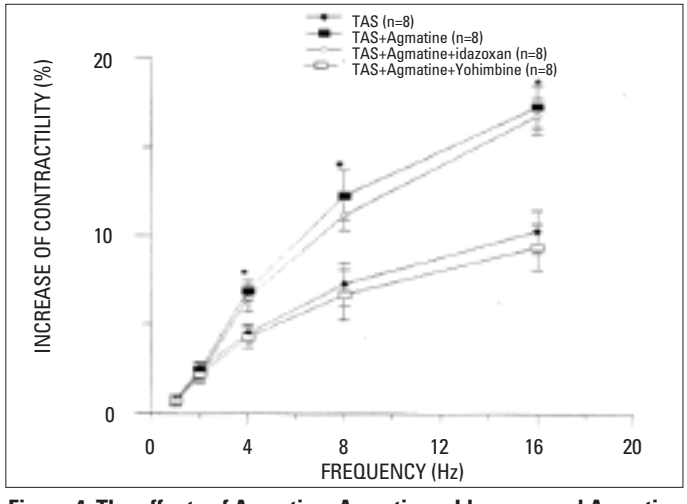

Figure 4. The effects of Agmatine, Agmatine + Idazoxan and Agmatine + Yohimbine on contractility of frog ventricular strips evoked by TAS. Values indicate mean ± SEM and number of experiments (n=8). p<0.05- significantly different from control pre drug values

TAS - transient additional stimulations

References

1. Li G, Regunathan S, Barrow CJ, Eshraghi J, Cooper R, Reis DJ. Agmatine: an endogenous clonidine-displacing substance in the brain. Science 1994; 263: 966-9.
2. Raasch W, Regunathan S, Li G, Reis DJ. Agmatine is widely and unequally distributed in rat organs. Ann NY Acad Sci 1995; 763: 330-4.
3. Reis DJ, Regunathan S. Agmatine: an endogenous ligand at imidazoline receptors is a novel neurotransmitter. Ann NY Acad Sci 1999; 881: 65-80.
4. Gonzalez C, Regunathan S, Reis DJ, Estrada C. Agmatine, an endogenous modulator of noradrenergic neurotransmission in the rat tail artery. Br J Pharmacol 1996; 119: 677-84.
5. Li J, Li X, Pei G, Qin BY. Agmatine inhibited tolerance to and dependence on morphine in guinea pig ileum in vitro. Zhongguo Yao Li Xue Bao 1998; 19: 564-8.
6. Sarioglu Y, Ilhan M, Kayaalp SO. An evidence for presynaptic excitatory alpha 2-adrenergic receptors in frog myocardium. Arch Int Physiol Biochim 1988; 96: 35-40.
7. Raasch W, Regunathan S, Li G, Reis DJ. Agmatine, the bacterial amine, is widely distributed in mammalian tissues. Life Sci 1995; 56: 2319-30.
8. Reis DJ, Li G, Regunathan S. Endogenous ligands of imidazoline receptors: classic and immunoreactive clonidine-displacing substance and agmatine. Ann NY Acad Sci 1995; 763: 295-313.
9. Morgan NG, Chan SL, Mourtada M, Monks LK, Ramsden CA. Imidazolines and pancreatic hormone secretion. Ann NY Acad Sci 1999; 881: 217-28.
10. Colucci R, Blandizzi C, Carignani D, Placanica G, Lazeri G, Del Tac-

ca M. Effects of imidazoline derivatives on cholinergic motility in guinea-pig ileum: involvement of presynaptic alpha 2-adrenoceptors or imidazoline receptors? Naunyn Schmiedebergs Arch Pharmacol 1998; 357: 682-91.
11. Molderings GJ, Donecker K, Burian M, Simon WA, Schroder DW, Göthert M. Characterization of I2 imidazoline and sigma binding sites in the rat and human stomach. J Pharmacol Exp Ther 1998; 285: 170-7.
12. Utkan T, Ulak G, Yildiran HG, Yardimoglu M, Gacar MN. Investigation on the mechanism involved in the effects of agmatine on ethanol-induced gastric mucosal injury in rats. Life Sci 2000; 66: 1705-11.
13. Li XT, Fan ZZ, He RR. Electrophysiologic effects of agmatine on pacemaker cells in sinoatrial node of rabbits. Zhongguo Yao Li Xue Bao 1999; 20: 897-901.
14. Raasch W, Chun KRJ, Dendorfer A, Dominiak P. Positive inotropic effects of imidazoline derivatives are not mediated via imidazoline binding sites but alpha1-adrenergic receptors. Jpn J Pharmacol 2000; 84: 1-6.
15. Michel MC, Regan JW, Gerhardt MA, Neubg RR, Insel PA, Motulsky HJ. Nonadrenergic (3H) idazoxan binding sites are physically district from alpha 2-adrenergic receptors. Mol Pharmacol 1990; 122: 43-50.
16. Goldberg MR, Robertson D. Yohimbine a pharmacological probe for study of the alpha 2-adrenoceptors. Pharma Rev 1983; 35: 143-70.
17. Likungu J, Molderings GJ, Göthert M. Presynaptic imidazoline receptors and _2 adrenoceptors in the human heart discrimination by clonidine and noxonidine. Naunyn Schmiedebergs Arch Pharmacol 1996; 354: 689-92.
18. Molderings GJ, Likungu J, Jakschik J, Göthert M. Presynaptic imidazoline receptors and non-adrenoceptor (3H) idazoxan binding sites in human cardiovascular tissues. Br J Pharmacol 1997; 122: 43-50.
19. Docherty JR, Göthert M, Dieckhöfere C, Starke K. Effects of 4-chloro-2 (2-imidazoline-2-ylamino) isoindoline hydrocloride (BE 6143) at pre- and postsynaptic alpha-adrenoceptors in rabbit aorta and pulmonary artery. Arzneim-Forsch 1982; 32: 1534-40.
20. Jurkiewicz NH, Carme LG, Hirata H, Santos WC, Jurkiewicz A. Functional properties of agmatine in rat vas deferens. Eur J Pharmacol 1996; 307: 299-304.
21. Molderings GJ. Imidazoline receptors: basic knowledge, recent advances and future prospects for therapy and diagnosis. Drugs Future 1997; 22: 757-72.
22. Molderings GJ, Göthert M. Imidazoline binding sites and receptors in cardiovascular tissue. Gen Pharmacol 1999; 3281: 17-22.