SHORT THESIS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY (PH.D.)

Determination of susceptibility to thyroxine and of receptor reserve for the direct negative inotropic action concerning the A_1 adenosine receptor in the guinea pig atrium

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By Zsuzsanna Kiss Pharm.D.

Supervisor: Rudolf Gesztelyi M.D., Ph.D.

Doctoral School of Pharmaceutical Sciences (Pharmacology program), University of Debrecen

Head of the Examination Committee:	Lajos Gergely M.D., D.Sc.
Members of the Examination Committee:	Tamás Bíró M.D., D.Sc.
	Tamás Csont M.D., Ph.D.

The Examination takes place at Department of Pharmacology, Faculty of Pharmacy, Medical and Health Science Center, University of Debrecen
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Head of the Defense Committee:	Lajos Gergely M.D., D.Sc.
Reviewers:	Ilona Benkő M.D., Ph.D.
	Gábor Pethő M.D., Ph.D.
Members of the Defense Committee:	Tamás Bíró M.D., D.Sc.
	Tamás Csont M.D., Ph.D.

The PhD Defense takes place at the Lecture Hall of Bldg. A, Department of Internal Medicine, Medical and Health Science Center, University of Debrecen
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Contents

1. Introduction

The A_1 adenosine receptor (A_1 receptor) is expressed in almost every mammalian tissue, in which it mediates regulatory, retaliatory (energy consumption limiting), adaptive and regenerative functions. By virtue of their capacity to evoke these actions, several A_1 receptor agonists are in late stage clinical development or approved for clinical use at the time of this writing. Since an A_1 receptor agonist, administered for any reason, can bind to any A_1 receptor, differences in tissue responsiveness have great practical significance. In the traditional receptor theory, an important determinant of tissue responsiveness is the so-called receptor reserve.

The term receptor reserve refers to a phenomenon whereby stimulation of only a fraction of the whole receptor population apparently elicits the maximal effect achievable in a particular tissue. The existence (and magnitude) of receptor reserve depends on the agonist (efficacy), tissue (signal amplification ability) and measured effect (pathways activated to cause signal amplification). High-efficacy agonists usually act on most tissues expressing the given receptor as a full agonist. In turn, low-efficacy agonists exert significant effect only in tissues with large receptor reserve. Thus, use of low-efficacy agonists can ensure tissue selectivity in a sense that they will not evoke biologically significant effect in tissues with small (or no) receptor reserve.

Relative to the adipose and neuronal tissues, the heart has a small A_1 receptor reserve with respect to electrophysiological actions (such as the inwardly rectifying potassium current, L-type calcium current, action potential duration) (Dhalla et al., 2003). However, because receptor reserve depends on the particular effect (Brown and Goldstein, 1986; Srinivas et al., 1997), it is prudent to quantify the reserve for each important effect entity. To the best of our knowledge, the myocardial A_1 receptor reserve has not been yet quantified for

the negative inotropic effect, although this latter is an important possible adverse effect of the A_1 receptor agonists.

It is well-established that A_1 receptor agonists have limited direct effect (an effect without prior adenylyl cyclase activation) on the ventricle. On the other hand, A_1 receptor agonists exert a considerable direct negative inotropic effect on the atrium in most species, including the guinea pig and human. Starting from this, the aim of the present work was to determine the A_1 receptor reserve in the atrial muscle. Our investigations were conducted on isolated and paced guinea pig left atria, a model system where negative tropic effects mediated by the A_1 receptor manifest purely as a decrease of the contractile force. We focused on the direct negative inotropic effect of the atrial A_1 receptor, because this action can decrease the contractile force below the resting level.

To elicit the atrial A_1 receptor mediated direct negative inotropy, adenosine, the physiological ligand, furthermore NECA, CPA and CHA, three synthetic full agonists, were used. To determine receptor reserve, a fraction of the receptor population has to be permanently inactivated with the remaining fraction retaining its functional integrity. For this purpose, FSCPX, a selective, potent and irreversible A_1 receptor antagonist, was used for the present study. In order to compare our results with data of previous studies, we determined the receptor reserve with the operational model of agonism and Furchgott's method, two methods based on the traditional receptor theory. At the same time, irrespectively of the traditional receptor theory, the effect of FSCPX on the A_1 receptor mediated negative inotropy can itself characterize the susceptibility of atrial contractile force to A_1 receptor agonists that was the main goal of the present study.

In addition, it is established that thyroid hormones (T_3, T_4) have a wide array of cardiovascular actions including well-known unfavorable effects and others offering potential advantages. Some of these actions affect the purinergic system of the heart, including the A_1 receptor and its signaling pathways.

Ability of a receptor to bind a ligand (affinity) plays an important role in receptor function, and beyond this, it can serve as an index of the structure of the binding site. One of the simplest and yet most reliable ways to explore prospective structural changes in a receptor is when the affinity of a selective and competitive antagonist for the given receptor is determined. Nevertheless, there are relatively scarce data available about ligand binding properties of the hyperthyroid A_1 receptor. In the present study, therefore, we investigated whether or not T_4 treatment affects the affinity of CPX, a selective and competitive orthosteric antagonist, for the guinea pig atrial A_1 receptor. For this purpose, concentration-response (E/c) curves were generated with adenosine and CPA, two A_1 receptor full agonists, in the absence and presence of CPX, a selective and reversible A_1 receptor antagonist, and then the E/c curves were evaluated by means of the Schild method.

2. Materials and methods

2.1. Chemicals

The following materials were used: salts for the modified Krebs-Henseleit buffer (Krebs buffer), L-thyroxin sodium salt pentahydrate (T_4), adenosine, NECA (5′-(N-ethylcarboxamido)adenosine), CPA (N^6-cyclopentyladenosine), CHA (N^6-cyclohexyladenosine), CPX (8-cyclopentyl-1,3-dipropylxanthine), FSCPX (8-cyclopentyl-N^3-[3-(4-(fluorosulfonyl)benzoyloxy)propyl]-N^1-propylxanthine), NBTI (S-(2-hydroxy-5-nitrobenzyl)-6-thioinosine) (Sigma). The bathing medium for the preparations was Krebs buffer (36 °C) in the entire course of the experiments.

2.2. Animals and preparations

Our experiments were performed on the isolated left atria of male guinea pigs weighing 500-900 g. The housing, pretreatment and processing of animals was according to the European Community guidelines and in agreement with the Ethical Codex of the Committee of Experimental Animal Research of the University of Debrecen (DE MÁB 35/2007.).

In vivo thyroxine treatment

The *in vivo* treated animals were randomly divided into two groups: the T_4 treated and the solvent (S) treated group. One group of the animals received 330 μg/kg T4 daily ip. for 8 days (in vivo T_4 treatment), while the vehicle of T4 (S) was administered daily ip. for 8 days to another group (in vivo solvent treatment). The animals were sacrificed on the ninth day *via* guillotine.

Tissue preparations and pre-incubation

After opening the thorax of the guillotined guinea pigs, left atria were removed and mounted in a 10 cm^3 organ chamber filled with Krebs solution (TSZ-04, Experimetria, Budapest). The Krebs solution was oxygenated with 95% O_2 and 5% CO_2 (pH 7.4). Atria were paced by platinum electrodes (3 Hz, 1 ms, twice the threshold voltage) with the use of a built-in programmable stimulator (Experimetria ST-02) and power amplifier (Experimetria PST-02). The contractile force was characterized by the amplitude of the isometric twitches, which were measured by a transducer (Experimetria SD-01) and strain gauge (Experimetria SG-01D), and recorded by a polygraph (Medicor R-61 6CH Recorder).

After starting the stimulation, every atrium was incubated in a Krebs solution for 50 minutes to get the contractility parameters stabilized. The bathing medium was changed every 15 to 20 minutes (washing).

2.3. Groups and protocols

Determination of affinity of A_1 adenosine receptor towards CPX

The solvent (n=33) and T_4 treated (n=32) atria underwent one of two protocols. Protocol 1 involved two groups: S1 (n=9) and T1 (n=9). Protocol 2 included six groups: S2 Control (n=10), S2 1 μM CPX (n=7), S2 10 μM CPX (n=7), T2 Control (n=9), T2 1 μM CPX (n=7), T2 10 μM CPX (n=7). S or T in the name of the groups denotes in vivo solvent or T_4 treatment, respectively; and the accompanying number reflects the protocol used.

Protocol 1: Atria in the groups S1 and T1 were allowed to equilibrate in Krebs solution for 30 min, and then 100 μM adenosine was administered for 2 min to exercise the preparations. Following a 15 min long wash-out period, a cumulative E/c curve was generated using adenosine. After a 15 min long wash-

out, atria were incubated in the presence of 0.1 µM CPX for 20 min, and then (without wash-out) another cumulative E/c curve was constructed with adenosine. Following a 15 min long wash-out, atria were incubated in the presence of 1 µM CPX for 20 min, and then a third cumulative E/c curve was generated with adenosine. After a 15 min long wash-out, atria were incubated in the presence of 10 µM CPX for 20 min, afterward a fourth cumulative E/c curve was generated with adenosine.

Protocol 2: Atria were allowed to equilibrate in Krebs solution for 45 min. Afterward, a cumulative E/c curve was constructed with adenosine to assess the responsiveness of A_1 receptors. After a 15 min long wash-out period, the following in vitro treatments were performed: in the groups S2 Control and T2 Control, atria were incubated in Krebs solution for 20 min; in the groups S2 1 µM CPX and T2 1 µM CPX, atria were subjected to 1 µM of CPX for 20 min; and in the groups S2 10 µM CPX and T2 10 µM CPX, atria received 10 µM of CPX for 20 min. Finally, a cumulative E/c curve was generated with CPA in all groups.

Determination of A_1 adenosine receptor reserve using NECA, CPA and CHA

Atria were allowed to equilibrate in Krebs solution for 40 min, then they were randomized into twelve groups: NECA N25 (n = 3), NECA X25 (n = 3), NECA N-W (n = 3), NECA X-W (n = 3), NECA N+W (n = 3), NECA X+W (n = 3), furthermore NECA N (n = 8), NECA X (n = 7), CPA N (n = 7), CPA X (n = 7), CHA N (n = 7), CHA X (n = 6). Afterward, a cumulative concentration-response (E/c) curve with adenosine was constructed (from 100 nM to 1 mM) to assess the responsiveness of atrial A_1 receptors.

FSCPX treatment: After washout, atria were subjected to one of four protocols receiving 10 µM FSCPX or 10 µL DMSO, the vehicle of FSCPX (as control). The protocols and groups receiving FSCPX are as follows: (1) 25 min incubation with FSCPX followed by a 75 min of washout with Krebs solution

(group NECA X25); (2) 10 min incubation with FSCPX without washout (group NECA X-W); (3) 45 min incubation with FSCPX followed by a 120 min of washout with Krebs solution (group NECA X+W); (4) 45 min incubation with FSCPX followed by a 75 min of washout with Krebs solution (groups NECA X, CPA X, CHA X). Every group had a counterpart marked N (instead of X) that was treated with 10 μL DMSO (instead of FSCPX) for an identical time period.

NECA, CPA and CHA E/c curves: After FSCPX (or vehicle) treatment, a cumulative E/c curve was generated with an A_1 receptor agonist indicated in the name of the group.

Determination of A_1 adenosine receptor reserve with adenosine

The atria were divided into six groups: P1 (in which Protocol 1 was carried out), P2 (a group for Protocol 2), P3-Control and P3-NBTI (two groups for Protocol 3), P4-Control and P4-FSCPX (two groups for Protocol 4).

Protocol 1 (demonstration of the effect of FSCPX on the adenosine E/c curve):

Control adenosine E/c curve: Atria (n = 8) were allowed to equilibrate in Krebs solution for 25 min, and then they were exposed to 100 μM of adenosine for 1 min followed by a 15 min long washout with Krebs solution. Afterward, a cumulative E/c curve with adenosine (from 1 nM to 3 mM) was constructed (P1-Control curve).

FSCPX treatment: After a 15 min washout, atria were subjected to 10 μM FSCPX for 45 min followed by a 75 min long washout with Krebs solution.

Second adenosine E/c curve: After the FSCPX-pretreatment, a cumulative E/c curve with adenosine (from 1 nM to 3 mM) was generated (P1-FSCPX curve).

Protocol 2 (attempt to determine the A_1 receptor reserve for adenosine):

Control adenosine E/c curve: Atria (n = 7) were allowed to equilibrate in Krebs solution for 25 min, and then they were exposed to 100 μM adenosine for 1 min followed by a 15 min long washout with Krebs solution. Subsequently, a cumulative E/c curve with adenosine (from 1 nM to 3 mM) was constructed (P2-Control curve).

NBTI treatment: After washout with Krebs solution (15 min), atria were incubated in 10 μM NBTI for 15 min.

Second adenosine E/c curve: A cumulative E/c curve with adenosine (from 1 nM to 3 mM) was generated in the presence of 10 μM NBTI (P2-NBTI curve).

FSCPX+NBTI treatment: After washout with Krebs solution (20 min), atria were subjected to 10 μM FSCPX for 45 min followed by a 60 min long washout with Krebs solution. Then, preparations received 10 μM NBTI and were incubated for 15 min.

Third adenosine E/c curve: A cumulative E/c curve with adenosine (from 1 nM to 3 mM) was generated in the presence of 10 μM NBTI (P2-FSCPX+NBTI curve).

Protocol 3 (data collection to determine c_x, the CPA concentration that is equieffective with the surplus endogenous adenosine accumulated interstitially in the presence of NBTI)

Adenosine E/c curve: Atria were allowed to equilibrate in Krebs solution for 40 min. Then, a cumulative E/c curve was constructed with adenosine (from 10 nM to 1 mM) to assess the responsiveness of atrial A_1 receptors.

Control or NBTI treatment: After washout with Krebs solution (15 min), atria were randomized into two groups. Atria in the P3-Control group (n = 8) received 10 μl DMSO, the vehicle of NBTI, then they underwent a 15 min long incubation period. Atria in the P3-NBTI group (n = 8) were incubated in the presence of 10 μM NBTI for 15 min.

CPA E/c curve: A cumulative E/c curve was generated with CPA (from 0.1 nM to 100 µM) in the presence of 10 µl DMSO (P3-Control group) or 10 µM NBTI (P3-NBTI group).

Protocol 4 (data collection to compute the negative inotropic effect of c_x on the FSCPX-pretreated atria)

Adenosine E/c curve: Atria were allowed to equilibrate in Krebs solution for 40 min. Afterward, a cumulative E/c curve was constructed with adenosine (from 10 nM to 1 mM) to determine the responsiveness of atrial A_1 receptors.

Control or FSCPX treatment: After washout with Krebs solution (15 min), atria were randomly divided into two groups. Atria in the P4-Control group (n = 11) received 10 µl DMSO, the solvent of FSCPX, for 45 min followed by a 75 min long washout with Krebs solution. Atria in the P4-FSCPX group (n = 12) were subjected to 10 µM FSCPX for 45 min, succeeded by a 75 min long washout with Krebs solution.

CPA E/c curve: A cumulative E/c curve was generated with CPA (from 0.1 nM to 100 µM).

2.4. Determination of A_2 or K_B

To obtain A_2 (the antagonist concentration causing two-fold rightward shift of the E/c curve) or K_B (the equilibrium dissociation constant of the antagonist-receptor complex) values, the corresponding E/c curves of protocols 1 and 2 for the determination of A_1 receptor affinity were globally fitted to the Schild equation with both variable and fixed (at unity) Schild coefficient. If the Schild equation with S=1 showed a better fit, A_2 was considered to equal K_B, if not, then A_2 did not give K_B.

2.5. Determination of K_A

To estimate K_A (the equilibrium dissociation constant of the agonist-receptor complex), the following data sets were used: data of E/c curves generated with a synthetic agonist in groups NECA N, NECA X, CPA N, CPA X, CHA N, CHA X (receiving the FSCPX protocol (4) and its control). The data sets were processed using the operational model of agonism and (alternatively) Furchgott's method.

2.6. Quantification of receptor reserve

The receptor reserve was quantified with the pharmacological shift ratio ($PSR = K_A/EC_{50}$), and with the following percentage form ($RR_{\%}$) as well ($RR_{\%} = E_{\%}-\rho_{\%}$). $E_{\%}$ is the percentage effect, while $\rho_{\%}$ is the percentage receptor occupancy that can be computed from the K_A. Then, $RR_{\%}$ was plotted against the $E_{\%}$.

2.7. Quantification of the bias in adenosine E/c curves generated in the presence of NBTI

Effect values of E/c curves generated in the presence of NBTI were considered to be biased by an increase in interstitial concentration of endogenous adenosine that was produced by NBTI. This surplus interstitial adenosine concentration (above the resting level) biased the E/c curves because it was unknown and was not taken into account (it was "neglected"). The biased adenosine E/c curves were not suitable for the assessment of A_1 adenosine receptor reserve, the goal of the present study. Therefore, effects of adenosine E/c curves biased by NBTI were corrected by means of RRM, our method

validated for quantifying changes in agonist concentrations in the microenvironment of the receptors. To characterize the surplus interstitial adenosine concentration by RRM, the averaged CPA E/c curve of the P3 NBTI group was fitted to the equation of RRM. Since RRM compares the biased curve to a control one (generated under identical conditions excepting the cause of bias), its equation contained the empirical parameters of the averaged CPA E/c curve of the P3 Control group. RRM provided a parameter, c_x, which is the CPA concentration that is equieffective with the surplus interstitial concentration of endogenous adenosine produced by NBTI. So, c_x was a "surrogate parameter" for the present investigation.

2.8. Correction of effect values of adenosine E/c curves generated in the presence of NBTI

The effect belonging to c_x (E_x) could be determined by means of the Hill equation. When E_x was computed for correcting the averaged P2 NBTI curve, empirical parameters of the averaged CPA E/c curve of the P3 Control group were used. In turn, when E_x was calculated for correcting the averaged P2 FSCPX+NBTI curve, empirical parameters of the averaged CPA E/c curve of the P4 FSCPX group were applied. The corrected effect values reflected the action of NBTI on the adenosine E/c curve without the bias caused by the extracellularly accumulated endogenous adenosine.

2.9. Statistical analysis and curve fitting

Two data sets passing the normality test as well as the equal variance test were compared by paired or unpaired t-test. If only the normality test was passed, Welch's test was used. More than two data sets were compared with one-

way ANOVA followed by Tukey post-testing (because all data sets passed both the normality and equal variance tests). Values of $P<0.05$ were considered to be significant. Data are presented as mean ± S.E.M..

GraphPad Prism version 4.03 for Windows was used for the statistical analysis and curve fitting. Calculations were done with the help of Microsoft Office Excel 2003.

3. Results

3.1. Change in affinity of A_1 adenosine receptor towards CPX after a 8-day long thyroxine treatment

When fitting the corresponding adenosine E/c curves, fit of the model was significantly better if the Schild slope was variable rather than unity, in the case of both solvent and T_4 treated atria. Consequently, A_2 (and not K_B) values could only be determined for CPX.

In contrast, when fit of the Schild equation to the CPA E/c curve families were evaluated, the model with a Schild slope fixed at unity was significantly superior to that with variable Schild slope in both the solvent and T_4 treated atria. Thus, the use of CPA enabled to determine K_B values. The K_B related to the solvent treated atria was greater than that belonging to the T_4 treated ones, indicating that CPX had lower affinity for the hyperthyroid guinea pig atrial A_1 receptor than for the euthyroid one. As K_B values characterize purely the interaction between CPX and the atrial A_1 receptors, the observed difference between those strongly suggest a difference in the orthosteric binding sites of the eu- and hyperthyroid A_1 receptors.

3.2. A_1 adenosine receptor reserve for the direct negative inotropic effect

A_1 receptor reserve for atrial negative inotropy evoked by NECA, CPA and CHA

The PSR values for all three agonists were considerably greater than unity. Using K_A values provided by both methods, the rank order of receptor reserve was: CPA > CHA > NECA.

The $RR_{\%}$ values corroborated with the PSR values. When $RR_{\%}$ was plotted *vs* $E_{\%}$, the functions tightly approximated the asymptotic upper limit of RR% (which is the value of the corresponding $E_{\%}$) up to about 70% (NECA) or 90% (CPA, CHA) of $E_{\%}$. Then, after reaching a maximum, they steeply dropped to zero value at the $100_{\%}$ of $E_{\%}$.

A_1 receptor reserve for atrial negative inotropy elicited by adenosine

After correcting for the bias caused by the surplus interstitial adenosine accumulated by NBTI, effect values of the P2 NBTI and P2 FSCPX+NBTI curves changed in their relationship to each other. As the total (endogenous + exogenous) interstitial adenosine concentration in the microenvironment of A_1 receptors was unknown, the corrected effect values of the P2 NBTI and P2 FSCPX+NBTI curves could only be plotted against the concentration of exogenous adenosine in the bathing medium. Consequently, the corrected E/c curves were not suitable for assessing exact receptor reserve values. Nevertheless, the inherently correct and corrected effects in the P2 group that belonged to the same x value could be compared to one another. The maximal effects related to 3 mM adenosine were as follows: 93.06%, 93.36% and 91.33% for the P2 Control curve, P2-NBTI curve and P2-FSCPX+NBTI curve, respectively. Thus, the corrected P2-NBTI and P2-FSCPX+NBTI curves changed places with each other as compared to the original curves, according to the expectations concerning the action of FSCPX, an irreversible A_1 receptor antagonist. On the other hand, the final (saturated) parts of the corrected P2 NBTI and P2 FSCPX+NBTI curves got very close to each other, indicating great A_1 receptor reserve for the direct negative inotropic effect of adenosine in the guinea pig atrium.

4. Discussion

4.1. Affinity of A_1 adenosine receptor to CPX after thyroxine treatment

Our results indicate that affinity of the hyperthyroid guinea pig atrial A_1 receptor towards CPX, a selective and competitive A_1 receptor antagonist, is moderately lower than that of the euthyroid A_1 receptor. This decrease in affinity suggests a modification in the orthosteric binding site of the hyperthyroid A_1 receptor, and can contribute to the diminution of the A_1 receptor mediated responses in hyperthyroidism. With regard to the intense reduction in the negative inotropic effect of adenosine and CPA in the hyperthyroid atria, it is reasonable to assume that the moderate decrease in affinity of the guinea pig atrial A_1 receptor is only in part responsible for the diminished negative inotropic effect of A_1 receptor agonists under hyperthyroid conditions. On the other hand, the reduction in affinity of the A_1 receptor to its orthosteric ligands may theoretically affect every A_1 receptor mediated biological process in hyperthyroidism. In addition, CPX proved to be a competitive antagonist not only for the euthyroid but hyperthyroid guinea pig atrial A_1 receptor.

4.2. A_1 adenosine receptor reserve for the atrial direct negative inotropic action

Our results show that FSCPX, an irreversible A_1 receptor antagonist (used in 10 μM for 45 min), failed to decrease the number of operable guinea pig atrial A_1 receptors in an extent that would had been sufficient to visibly reduce the maximum of the direct negative inotropic effect of NECA, CPA, CHA and adenosine, four A_1 receptor full agonists, in guinea pig left atria. In other words, to reach the (apparently) maximal direct negative inotropy, a smaller fraction of

the A_1 receptor population is needed than the intact receptor fraction after a treatment with FSCPX (10 μM, 45 min). Accordingly, the atrial A_1 receptor reserve have been found considerably great, ranging between 80-92% for the (near maximal) direct negative inotropic effect evoked by NECA, CPA and CHA, three synthetic A_1 receptor full agonists. These receptor reserve values are higher than historical values determined for any other A_1 receptor mediated effect in the guinea pig atrium, indicating that atrial contractility is very sensitive to the stimulation of A_1 receptors. The importance of this finding is further emphasized by the fact that this investigation is the first to assess the A_1 receptor reserve for atrial contractility.

In addition, a further result is that A_1 receptor reserve in the guinea pig supraventricular myocardium appertaining to the direct negative inotropic effect of the physiological ligand adenosine is considerably great, similarly to A_1 receptor reserve related to the direct negative inotropic action of NECA, CPA and CHA, synthetic A_1 receptor agonists. A limitation of this finding is that the exact concentrations of interstitial adenosine (the resting level and its changes) remained unknown; therefore the corrected effects could only be plotted *versus* the concentrations of administered (exogenous) adenosine evolved in the organ bath. This fact, unfortunately, impeded the exact quantification of A_1 receptor reserve by methods used for functional data (e.g. the operational model of agonism or Furchgott's method). However, the quasi-E/c curves created from the mathematically corrected effects are unique in that they are free of the bias caused by the interstitial accumulation of endogenous adenosine, despite the repressed adenosine elimination. These quasi-E/c curves are fully saturated, so maximal responses to adenosine can be well demonstrated.

The great A_1 receptor reserve found in the present study indicates that even low-efficacy A_1 receptor agonists will decrease atrial contractility. Starting from the great amino acid homology of A_1 receptors and from the similarity in atrial postreceptorial signaling pathways between the human and guinea pig, the

great porcelline A_1 receptor reserve for the direct negative inotropy suggests that partial A_1 receptor agonists may decrease the atrial contractile force in human subjects as well. Thus, it is worthwhile considering the possibility of a weakening in atrial mechanical activity with the long term use of compounds possessing A_1 receptor agonist property.

5. Summary of the findings

A main finding of the present thesis is that affinity of the guinea pig atrial A_1 receptor towards CPX, a selective, orthosteric A_1 receptor antagonist, moderately decreases in hyperthyroidism as compared to the euthyroid state. The reduced affinity of the binding site may contribute to the well-known decrease of A_1 receptor mediated actions in hyperthyroidism (although it is probable that this mechanism is only in part responsible for the phenomenon). In addition, we found that CPX is a pure competitive antagonist for the hyperthyroid and not only for the euthyroid atrial A_1 receptor.

Furthermore, results of the present thesis show that FSCPX, an irreversible A_1 receptor antagonist (used in 10 μM for 45 min, followed by 75 min wash-out), failed to decrease the number of operable A_1 receptors in an extent that would be sufficient to significantly reduce the maximum of the direct negative inotropic effect of NECA, CPA, CHA and adenosine, four A_1 receptor full agonists, in guinea pig left atria. Accordingly, A_1 receptor reserve has been found to be considerably great, ranging between 80-92% for the near maximal direct negative inotropic effect evoked by NECA, CPA and CHA, three stable synthetic A_1 receptor full agonists. These receptor reserve values are higher than all historical values determined for any other A_1 receptor mediated effect in the guinea pig atrium. Quantification of A_1 receptor reserve failed for adenosine, a compound that is eliminated and compartmentalizes rapidly in the living tissues. Moreover, adenosine E/c curves generated in the presence of NBTI, a nucleoside transport inhibitor preventing the intracellular elimination of adenosine, seemed to behave paradoxically. However, after the mathematical correction of adenosine E/c curves biased by NBTI, it turned out that A_1 receptor reserve for the direct negative inotropic effect of adenosine is similarly great as that of the synthetic agonists. These results indicate that atrial contractility is very sensitive to the stimulation of A_1 receptors. Thus, a decrease in the contractile force of atria, as a possible side effect, should be considered even in the case of partial A_1 receptor agonists and A_1 receptor enhancers.

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List of publications related to the dissertation

1. Gesztelyi, R., **Kiss, Z.**, Wachal, Z., Juhász, B., Bombicz, M., Csépányi, E., Pak, K., Zsuga, J., Papp, C., Galajda, Z., Branzaniuc, K., Pórszász, R., Szentmiklósi, J.A., Tósaki, Á.: The surmountable effect of FSCPX, an irreversible A1 adenosine receptor antagonist, on the negative inotropic action of A1 adenosine receptor full agonists in isolated guinea pig left atria.
   Arch. Pharm. Res. Epub ahead of print (2013)
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2. **Kiss, Z.**, Pak, K., Zsuga, J., Juhász, B., Varga, B., Szentmiklósi, J.A., Haines, D.D., Tósaki, Á., Gesztelyi, R.: The guinea pig atrial A1 adenosine receptor reserve for the direct negative inotropic effect of adenosine.
   Gen. Physiol. Biophys. "accepted by publisher", 2013.
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3. Gesztelyi, R., **Kiss, Z.**, Zsuga, J., Pak, K., Papp, C., Galajda, Z., Branzaniuc, K., Szentmiklósi, J.A., Tósaki, Á.: Thyroid hormones decrease the affinity of 8-cyclopentyl-1,3-dipropylxanthine (CPX), a competitive antagonist, for the guinea pig atrial A(1) adenosine receptor.
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7. Abstracts

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Pák K, **Kiss Z**, Tósaki Á, Gesztelyi R. A tengerimalac pitvari A_1 adenozin receptor rezerv megbecslése az adenozin által kiváltott direkt negatív inotróp hatásra nézve. MÉT LXXVII. Vándorgyűlése, 2013., Budapest (oral presentation)

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