

Positive Inotropic Effect of the Inhibition of Cyclic GMP-Stimulated 3',5'-Cyclic Nucleotide Phosphodiesterase (PDE₂) on Guinea Pig Left Atria in Eu- and Hyperthyroidism

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Abstract. The significance of PDE₂ on the atrial inotropy was studied in eu- and hyperthyroidism. The contractile force was measured and negative inotropic capacity of N⁶-cyclopentyladenosine (CPA) was determined on left atria isolated from 8-day thyroxine- or solvent-treated guinea pigs, in the presence or absence of EHNA (adenosine deaminase and PDE₂ inhibitor) or NBTI (nucleoside transporter inhibitor). EHNA was administered to inhibit PDE₂, while NBTI was used to model the accumulation of endogenous adenosine.

The reduction of the contractile force caused by EHNA was smaller in the thyroxine-treated atria than in the solvent-treated samples. Contrary, NBTI induced a decrease in the contractile force without significant difference between the two groups. In addition, EHNA enhanced the efficiency of CPA in thyroxine-treated atria and did not affect it in solvent-treated samples, while the response to CPA was decreased by NBTI in all atria, especially in hyperthyroidism.

On the basis of greater retention of the contractile force and sustained/enhanced responsiveness to CPA in the presence of EHNA we conclude that PDE₂'s inhibition has a significant positive inotropic effect in guinea pig atria and this effect is proven to be augmented in hyperthyroidism.

Key words: Adenosine — Guinea pigs — Thyroxine — PDE₂ — Inotropic effect

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Introduction

By regulating the 3',5'-cyclic nucleotide phosphodiesterases (PDE), 3',5'-cyclic guanosine monophosphate (cGMP) intervenes in the cardiac function at the level of 3',5'-cyclic adenosine monophosphate (cAMP) (Fig. 1). In amphibian ventricular myocytes, the cGMP-stimulated PDE (PDE₂) significantly decreased the cAMP level and the L-type Ca²⁺ current (Méry et al. 1997). Similarly, the inhibition of

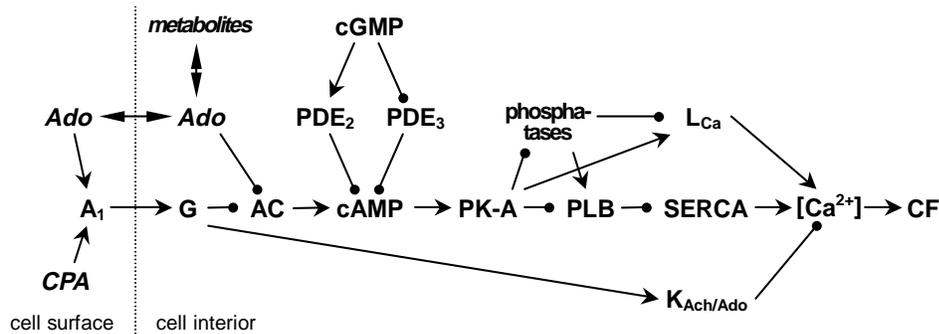


Figure 1. The A₁ adenosine receptor signaling and its relationship with cGMP in guinea pig left atrium designed by us based on the followings.

Among the several adenosine binding sites (e.g. P-site of adenylyl cyclase; Tesmer et al. 2000) the G protein coupled cell-surface A₁ receptors mediate most cardiac effects of adenosine (Belardinelli et al. 1995; Gardner and Broadley 1999). The actions of A₁ receptor agonists consist of an increase in inwardly rectifying K⁺ current and an inhibition of adenylyl cyclase (for review see Ralevic and Burnstock 1998), which results in the inhibition of the cAMP dependent protein kinase A, and in the activation of phosphoprotein phosphatases (Gupta et al. 1993). The decrease in phosphorylation by protein kinase A increases the inhibitory activity of phospholamban (Neumann et al. 1995), and inhibits the L_{Ca} (Jahnel et al. 1992), decreasing the contractile force (for review see Bers 2001). The A₁ receptors are coupled more efficiently to an inhibition of Ca²⁺ current than to an activation of K⁺ current (Srinivas et al. 1997), furthermore, the action of these K⁺ channels has little influence on the amplitude of the contractile force (Ford and Broadley 1999). cGMP regulates the level of cAMP *via* PDE₂ and PDE₃ (Shirayama and Pappano 1996; Bers 2001).

Symbols: → stimulation or increase in quantity; —● inhibition or decrease in quantity; ↔ equilibrative process; Ado, adenosine; CPA, N⁶-cyclopentyladenosine; A₁, A₁ adenosine receptor; metabolites, products of adenosine from catabolic and anabolic processes; G, GTP-binding protein; AC, adenylyl cyclase; cAMP, 3',5'-cyclic adenosine monophosphate; cGMP, 3',5'-cyclic guanosine monophosphate; PDE₂, cGMP-stimulated 3',5'-cyclic nucleotide phosphodiesterase; PDE₃, cGMP-inhibited 3',5'-cyclic nucleotide phosphodiesterase; PK-A, cAMP-dependent protein kinase A; PLB, phospholamban; SERCA, SR Ca²⁺-ATP-ase (Ca²⁺ pump); phosphatases, intracellular phosphoprotein phosphatases; L_{Ca}, L-type Ca²⁺ channel (slow Ca²⁺ channel); K_{Ach/Ado}, atrial muscarinic-activated K⁺ channel; [Ca²⁺], concentration of the cytosolic Ca²⁺ during the plateau of the action potential; CF, contractile force.

PDE₂ by erythro-9-(2-hydroxy-3-nonyl)adenine (EHNA) was found to significantly increase the L-type Ca²⁺ current in human atrial myocytes (Rivet-Bastide et al. 1997). This effect was not reproduced with 2'-deoxycoformycin, a pure inhibitor of adenosine deaminase (ADA), therefore this effect cannot be attributed to increasing the adenosine (Ado) level by EHNA. The importance of PDE₂ was further emphasized, as EHNA enhanced the increase in the L-type Ca²⁺ current in response to cGMP on human atria (Vandecasteele et al. 2001). EHNA was also found to produce positive chronotropic effect in guinea pig sinoatrial node (Herring et al. 2001). On the other hand, it was also reported that EHNA had no effect on the L-type Ca²⁺ current in rat atrial and ventricular myocytes (Rivet-Bastide et al. 1997). In these and other studies involving human atrial, rat and guinea pig ventricular myocytes, the role of the cGMP-inhibited PDE (PDE₃) was found dominant in decreasing the L-type Ca²⁺ current (Shirayama and Pappano 1996; Verde et al. 1999; Vandecasteele et al. 2001).

Summarizing, the myocardial action of PDE₂ was investigated with electrophysiological and biochemical methods. Although cAMP, the target of PDE₂ is the major second messenger determining the contractile force (Fig. 1), to our best knowledge, no report has appeared on the influence of PDE₂ on the cardiac inotropic function. The execution is impeded by the fact that EHNA, the only known specific inhibitor of PDE₂, is also an inhibitor of ADA. Therefore, the inotropic manifestation of PDE₂'s inhibition cannot be measured without simultaneous negative inotropic action of the elevated endogenous Ado level.

In the present work, our primary aim was to explore the significance of PDE₂ on the cardiac inotropic function. We hypothesized that the inhibition of PDE₂ had significant positive inotropic effect in guinea pig. To consider the distortion caused by Ado accumulation, we implemented a model protocol with S-(2-hydroxy-5-nitrobenzyl)-6-thioinosine (NBTI). NBTI is one of the most potent agents to increase the Ado level, because it protects the endogenous Ado from intracellular degradation *via* specific inhibition of the *es* subtype (equilibrative and sensitive to NBTI) nucleoside transporter (ENT1) (Van Belle 1993). Beyond the development of the contractile force caused by PDE₂'s inhibition, we also studied the alteration of a receptor function with negative inotropic effect. We assessed the A₁ Ado receptor (A₁ receptor) function, because it involves cAMP, the target of PDE₂, but it does not interfere with cGMP, the regulator of PDE₂ (Fig. 1).

In mammalian ventricles, Ado fails to exert the negative inotropic effect without prior cAMP stimulation (Endoh 1987). In atria, however, Ado efficiently decreases the contractile force without previous pharmacological intervention (Kurachi et al. 1986). To minimize the number of the applied agents and their possible interactions, we focused our experiments on atria.

It has been well established that thyroid hormones augment the quantity of the sarcoplasmic reticulum (SR) Ca²⁺-ATP-ase furthermore decrease the amount and efficiency of phospholamban (PLB) both in atria and ventricles (Kaasik et al. 1997; Ojamaa et al. 2000; Shenoy et al. 2001). Some authors found an increase in the amount of L-type Ca²⁺ channel (L_{Ca}) (Kim et al. 1987; Kreuzberg et al. 2000),

others reported a decreased quantity of the L_{Ca} (Seppet et al. 1993; Gøtzsche 1994) in the background of the elevated transsarcolemmal slow Ca^{2+} current in hyperthyroidism (Rubinstein and Binah 1989; Mager et al. 1992; Kreuzberg et al. 2000). In any case, in rat heart atria show a greater responsiveness to thyroid hormones than ventricles (Shenoy et al. 2001). This chamber specific phenomenon causes that the contractile force of rat atrium is unable to increase in hyperthyroidism (it reaches the maximum even in the euthyroid state), in contrast to ventricle (Kaasik et al. 1997). Since the alteration of the Ca^{2+} -handling proteins plays considerable role in determining inotropy, we extended our study to the hyperthyroid state. Therefore, our secondary aim was to investigate, how thyroid hormones alter the contractile force in guinea pig atria and how PDE_2 's inhibition affects this contractile force and the negative inotropic capacity of A_1 receptor function in hyperthyroid atria.

Materials and Methods

Animals

These experiments have been performed according to the European Community guidelines for the use of experimental animals. The protocol has been approved by the institutional ethics committee (Committee of Animal Research, University of Debrecen, DE MÁB 45/2001).

Male guinea pigs weighing 500–700 g were used. One group of the animals received 330 $\mu\text{g}/\text{kg}$ L-thyroxine sodium salt pentahydrate (T_4) daily i.p. for 8 days (*in vivo* T_4 treatment), the vehicle of T_4 was administered to the other group (*in vivo* solvent treatment). The animals were sacrificed on the ninth day.

Atria

The animals were killed by one firm blow on the head. The left atria were removed immediately and set up under a resting tension of 10 mN in 10 ml vertical organ baths (TSZ-04, Experimetria, Budapest) containing Krebs solution (36°C; pH 7.4; gassed with 95% O_2 and 5% CO_2). The composition of the Krebs solution was (in mmol/l): NaCl, 118; KCl, 4.7; $CaCl_2$, 2.5; NaH_2PO_4 , 1; $MgCl_2$, 1.2; $NaHCO_3$, 24.9; glucose, 11.5; ascorbic acid, 0.1 (dissolved in redistilled water). This solution was used during the course of the experiments, and the appropriate drugs were added to this solution. Atria were electrically paced by platinum electrodes (3 Hz, 1 ms, twice the threshold voltage) by a programmable stimulator (PST-02, Experimetria, Budapest). Isometric contractions were measured with a transducer (SD-01, Experimetria, Budapest), and recorded by a polygraph (BR-61, Medicor, Budapest).

Experimental protocols

Following the 50-min equilibrative period, an Ado concentration-response ($E/[A]$) curve was constructed on all atria according to the cumulative method of Van Rossum (1963). Ado was used to compare atria before *in vitro* treatment and to

exclude anomalous atria, since Ado does not cause A₁ receptor desensitization due to its rapid metabolism. After a washout period, one of three protocols was implemented. The first group of atria was incubated in Krebs solution (control). The second group of atria was incubated in the presence of 10 μmol/l EHNA for 50 min (*in vitro* EHNA treatment). The third group of atria was incubated in the presence of 10 μmol/l NBTI for 50 min (*in vitro* NBTI treatment). Following this, another cumulative E/[A] curve was determined with N⁶-cyclopentyladenosine (CPA), a selective full agonist of A₁ receptor. CPA was employed to examine the effect of the different *in vitro* treatments on the A₁ receptor function, because CPA is a poor substrate of the Ado-handling enzymes/carriers and its fate is practically unaffected by EHNA and NBTI in our experimental preparations. The combination of two *in vivo* and three *in vitro* treatments produced 6 different subgroups: solvent-treated control, *n* = 11; solvent-treated with EHNA, *n* = 6; solvent-treated with NBTI, *n* = 12; T₄-treated control, *n* = 10; T₄-treated with EHNA, *n* = 7; T₄-treated with NBTI, *n* = 8.

The following inclusion criteria were met for the statistical analysis:

1. The resting contractile force before the first E/[A] curve reached 1 mN (excluded atria, *n* = 2).
2. The mechanical activity of the paced atrium was regular (excluded atria, *n* = 4).
3. The response to the dose closest to the EC₅₀ (see in Statistical analysis) of the E/[A] curve for Ado was within the mean ±2 standard deviations (SD) interval (excluded atria, *n* = 5). The doses closest to the EC₅₀ were 10⁻⁵ mol/l for the solvent-treated, 10⁻⁴ mol/l for the T₄-treated atria. The SD was defined within the two *in vivo* treated groups for every atrium that met the first two criteria.

All data meeting the above-mentioned 3 criteria were processed.

Statistical analysis

The negative inotropic responses to Ado and CPA were expressed as the percentage decrease of contractile force

$$E = \frac{CF_R - CF_{[A]}}{CF_R} \cdot 100\%$$

where: *E*, the response (effect); *CF_R*, the resting contractile force (the contractile force in equilibrium before the administration of the first agonist dose); *CF_[A]*, the contractile force produced by the given [A] agonist concentration.

The individual E/[A] curve data were characterized by fitting the Langmuir-Hill equation (based on the law of mass action)

$$E = E_{\max} \cdot \frac{[A]^{n_H}}{[A]^{n_H} + EC_{50}^{n_H}}$$

where: $[A]$, the concentration of the agonist; E , the effect at $[A]$; E_{\max} , the maximal effect (maximum efficacy, asymptote); EC_{50} , the agonist concentration producing half maximal effect (midpoint location); n_H , the Hill coefficient (midpoint slope). The data are expressed as mean \pm standard error of the mean (SEM).

For the comparison of two data sets an unpaired Student's t -test was employed if the data passed the normality test and the equal variance test. If only the normality test was passed, the Welch's test was used; when even the normality test was not passed, the Mann-Whitney U-test was performed. For the comparison of three or more data sets a one-way analysis of variance (ANOVA) with Newman-Keuls post-test was used if all data sets passed the normality test and the equal variance test. If any data set failed than the Kruskal-Wallis test with Dunn's post-test was used. For the assessment of the interaction between the *in vivo* and *in vitro* treatment, two-way ANOVA was performed, where appropriate.

Values of $p < 0.05$ were considered to be significant. For the statistical analysis, GraphPad Prism 3.02 software was used.

Materials

The following drugs were used: adenosine (Ado); N⁶-cyclopentyladenosine (CPA); erythro-9-(2-hydroxy-3-nonyl)adenine (EHNA); S-(2-hydroxy-5-nitrobenzyl)-6-thioinosine (NBTI); L-thyroxine sodium salt pentahydrate (T₄) from Sigma (St. Luis, MO, USA).

Ado was dissolved in Krebs solution of 36°C. A 10⁻² mol/l solution of CPA was prepared with 20% ethanol, which was further diluted with Krebs solution. EHNA was dissolved in 96% ethanol, NBTI in dimethyl sulfoxide (DMSO). The concentration of ethanol and DMSO did not exceed 0.14% and 0.1% by volume, respectively, in the organ baths at any time. T₄ was dissolved in physiological salt solution containing 0.01% NaOH.

Results

1. Verification of the thyroid state

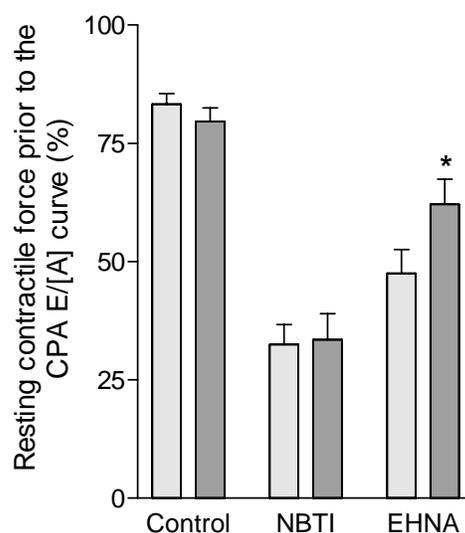
By the ninth day the average body weight of the solvent-treated animals (mean \pm SEM) varied from 607.3 \pm 23 g to 596.6 \pm 26 g (not significant), the rectal temperature changed from 36.8 \pm 0.1°C to 36.9 \pm 0.2°C (not significant). The body weight of the T₄-treated animals decreased from 621.3 \pm 31 g to 512.7 \pm 29 g ($p < 0.0001$, significant), while the rectal temperature rose from 36.7 \pm 0.2°C to 37.6 \pm 0.2°C ($p < 0.0001$, significant).

2. Contractile forces

The resting contractile forces before the construction of the first E/[A] curve did not differ significantly neither in the six subgroups nor in the pooled solvent- (6.5 \pm 0.4 mN) and T₄-treated (5.9 \pm 0.4 mN) groups.

The resting contractile forces before the second E/[A] curve were decreased as compared to the resting contractile forces prior to the first E/[A] curve. By defining

Figure 2. The light columns represent the solvent-treated atria, the dark columns the thyroxine-treated atria. The *in vitro* treatment is indicated below the columns. The height of the columns represents the resting contractile forces (%) prior to the construction of the second E/[A] curves (+SEM). 100% is defined as the resting contractile force before the first E/[A] curve in the same subgroup. Solvent-treated control, $n = 11$; solvent-treated with EHNA, $n = 6$; solvent-treated with NBTI, $n = 12$; thyroxine-treated control, $n = 10$; thyroxine-treated with EHNA, $n = 7$; thyroxine-treated with NBTI, $n = 8$; for specific data see Results. * statistically significant difference between the eu- and hyperthyroid subgroups at $p < 0.05$.



the resting forces before the first E/[A] curve as 100%, the resting forces before the second E/[A] curve were as follows (mean ± SEM): solvent- and T₄-treated control subgroups, 83.3 ± 2.2% and 79.6 ± 2.9%; solvent- and T₄-treated subgroups with NBTI, 32.5 ± 4.2% and 33.5 ± 5.5%; solvent- and T₄-treated subgroups with EHNA, 47.5 ± 5.1% and 62.1 ± 5.3%; respectively (Fig. 2). No significant difference was found in the force development between the two control subgroups and between the two NBTI-treated subgroups, however, the force development was significantly higher in the T₄-treated atria as compared to that of the solvent-treated samples following EHNA treatment ($p < 0.05$). The force developments were significantly different between the distinct *in vitro* treated subgroups. The interaction between the *in vivo* and *in vitro* treatments was found to be significant by two-way ANOVA for control and EHNA-treated subgroups ($p = 0.04$).

3. Response to Ado

Ado evoked a concentration-dependent decrease of the contractile force in left atria obtained from both solvent- and T₄-treated guinea pigs. No significant changes were detected within the three solvent-treated subgroups and within the three T₄-treated subgroups, the same *in vivo* treated groups proved to be homogenous.

The effect of the T₄ treatment: The E_{\max} values of the pooled solvent- and T₄-treated groups showed no significant difference, the EC_{50} of the T₄-treated group increased 10.6 fold as compared to that of the solvent-treated group ($p < 0.0001$, significant rightward shift) (Fig. 3). The slope of T₄-treated atria was found to be lower by 0.13 than the slope of solvent-treated atria ($p < 0.0001$, significant).

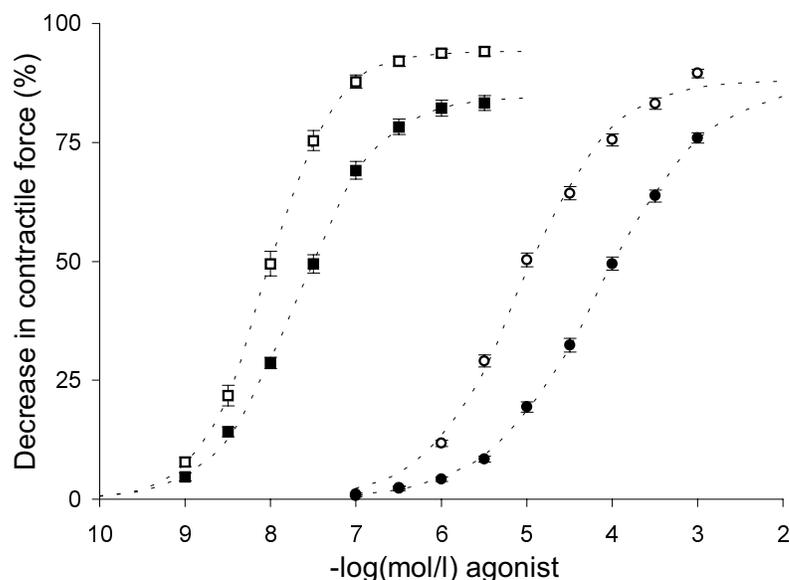


Figure 3. The x axis demonstrates the negative common logarithm of the administered adenosine receptor agonist (adenosine or CPA) concentrations, the y axis shows the percentage decrease in contractile force. The symbols represent the means of the individual responses (\pm SEM) of atria without EHNA or NBTI treatment. The broken lines represent the fitted curves with the Langmuir-Hill function.

Symbols: empty square (\square), solvent-treated control atria ($n = 11$) with CPA as agonist; solid square (\blacksquare), thyroxine-treated control atria ($n = 10$) with CPA as agonist; empty circle (\circ), all solvent-treated atria ($n = 29$) with adenosine as agonist; solid circle (\bullet), all thyroxine-treated atria ($n = 25$) with adenosine as agonist.

4. Response to CPA

CPA also induced a concentration-dependent decrease of the contractile force in left atria isolated from solvent- and T_4 -treated guinea pigs.

The effect of the T_4 treatment: In the T_4 -treated control subgroup the E_{\max} decreased to 90% ($p < 0.0001$, significant), the EC_{50} increased 2.2 fold ($p < 0.0001$, significant rightward shift) and the slope decreased by 0.23 ($p < 0.0001$, significant) in comparison to the solvent-treated control subgroup (Fig. 3).

The effects of the in vitro treatments within the solvent-treated group: The E_{\max} of the EHNA-treated subgroup decreased to 98% (not significant), the EC_{50} increased 1.1 fold (not significant rightward shift) as compared to the control subgroup. The E_{\max} of the NBTI-treated subgroup decreased to 85% ($p < 0.01$, significant), the EC_{50} increased 2.2 fold when compared with the control subgroup (not significant rightward shift) (Fig. 4A). No significant difference was detected between the slopes in the different subgroups.

The effects of the in vitro treatments within the T_4 -treated group: The E_{\max}

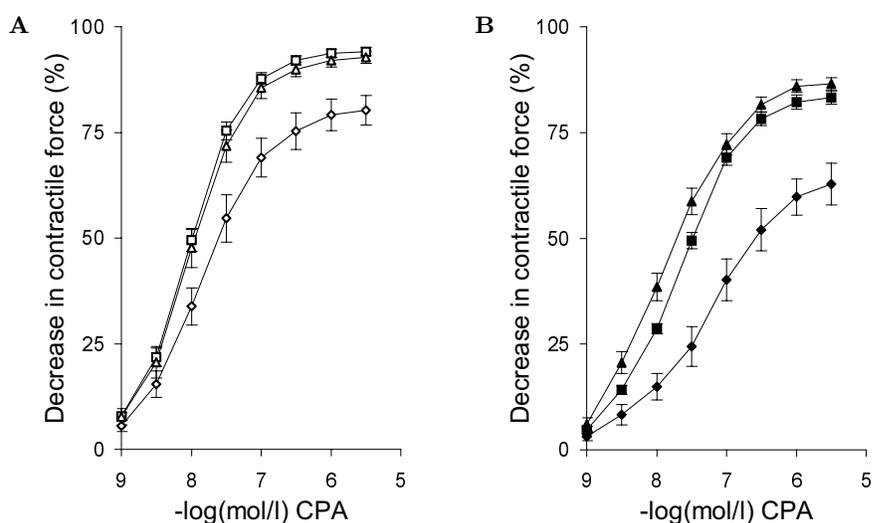


Figure 4. On the x axis the negative common logarithm of the administered CPA concentrations is exposed, the y axis shows the percentage decrease in contractile force. The symbols represent the means of the individual values (\pm SEM). The effect of the *in vitro* treatments on the response to CPA is shown in eu- (part A) and hyperthyroid (part B) atria.

Symbols: empty square (\square), solvent-treated control atria ($n = 11$); empty rhomboid (\diamond), solvent-treated atria in the presence of NBTI ($n = 12$); empty triangle (Δ), solvent-treated atria in the presence of EHNA ($n = 6$); solid square (\blacksquare), thyroxine-treated control atria ($n = 10$); solid rhomboid (\blacklozenge), thyroxine-treated atria in the presence of NBTI ($n = 8$); solid triangle (\blacktriangle), thyroxine-treated atria in the presence of EHNA ($n = 7$).

of the EHNA-treated subgroup increased to 103% (not significant), the EC_{50} decreased 0.75 fold ($p < 0.01$, significant shift to the left) in comparison to the control subgroup. In the NBTI-treated subgroup the E_{max} decreased to 78% ($p < 0.05$, significant), the EC_{50} increased 3.4 fold ($p < 0.01$, significant shift to the right) as compared to the control subgroup (Fig. 4B). There was no significant difference between the slopes of the different subgroups.

The two-way ANOVA for the control and the EHNA-treated subgroups showed a significant ($p = 0.013$) interaction between the *in vivo* and *in vitro* treatment considering their effect on the EC_{50} .

Discussion

This study is the first to explore the importance of PDE₂ in the atrial inotropy. The inhibition of PDE₂ has detectable positive inotropic effect in guinea pig and this is more extensive in hyperthyroidism – these are our major original observations. In addition, our experiments demonstrate that the A₁ receptor action

is a more sensitive indicator of PDE₂'s inhibition, than the resting contractile force.

Characteristics of experimental setup: It was found in the present study that T₄ treatment does not cause a significant alteration in the amplitude of the contractile force in guinea pig left atria. This observation corroborates with finding of Kaasik et al. (1997) in rat left atria, i.e., only the rate of contraction (+dT/dt) and the rate of relaxation (-dT/dt) are enhanced, the maximum force of the isometric twitches is unaltered in hyperthyroidism.

We confirmed the earlier observation that the response to Ado and CPA is decreased in hyperthyroidism, indicated by decreased E_{\max} and/or increased EC_{50} values (Fig. 3). This is in accordance with the previous findings from our laboratory on guinea pig left atrium (Szentmiklosi et al. 1992) and with the similar results of Kaasik et al. (1994) in rat left atrium. To circumvent this phenomenon, we normalized the A₁ receptor responses in the presence of EHNA or NBTI to the identical (same *in vivo* treatment) control before comparing different *in vivo* treated subgroups.

Responses to EHNA and NBTI – the role of PDE₂: The decrease in contractile forces of both the eu- and hyperthyroid atria in the presence of EHNA or NBTI (Fig. 2) is due to the accumulated endogenous Ado, which reduces the contractile force *via* stimulation of A₁ receptors (Fig. 1). This receptor-stimulation leads to the reduction of negative inotropic capacity of A₁ receptor function as well, which results in diminished susceptibility to CPA in all atria incubated with NBTI. However, in the presence of EHNA the response to CPA was not found to be diminished (Fig. 4A,B). This phenomenon can be explained by the earlier findings that the elevated cAMP level augments the efficiency of the A₁ receptor agonists *via* previous phosphorylation of L_{Ca} (Jahnel et al. 1992) and PLB (Neumann et al. 1995). By reason of this, the sustained/increased responsiveness to CPA in the presence of EHNA provides evidence about the significant effect of PDE₂ on the inotropy of guinea pig left atria. Furthermore, the sustained/increased susceptibility to CPA reflects that the A₁ receptor action characterizes the inhibition of PDE₂ more markedly than the resting contractile force.

The resting contractile force following incubation with EHNA was significantly greater in case of the T₄-treated atria than in case of the solvent treated-samples. On the contrary, the elevated endogenous Ado level in the presence of NBTI did not cause a significant difference (Fig. 2). This denotes that the inhibition of PDE₂ causes a greater positive inotropic effect in hyperthyroidism. In accordance with this, EHNA enhanced the response to CPA more extensively in hyperthyroid atria, than in euthyroid samples, while NBTI reduced the response to CPA greater in hyperthyroidism (Fig. 4A,B).

Summarizing, our experiments show a synergism between the PDE₂'s inhibition and the T₄ treatment in enhancing the contractile force and the negative inotropic capacity of A₁ receptor function. We suggest two possibilities to explain this phenomenon. It may be speculated that the function of PDE₂ is more significant in hyperthyroidism. Moreover, the alterations of L_{Ca} can also be responsible

for the increased positive inotropy of PDE₂'s inhibition *via* enhancement of their activity (T₄ and EHNA effect) and probably *via* increase in their number (T₄ effect). This concept is supported by the finding that the L-type Ca²⁺ current was measured to be significantly greater in hyperthyroid guinea pig ventricular myocytes, than in naives (Rubinstein and Binah 1989). In addition, acetylcholine decreased the L-type Ca²⁺ current more extensively in guinea pig ventricular myocytes from hyperthyroid animals than from naives (Mager et al. 1992). In human right atria, increased amount and activity of the L_{Ca} is shown in latent hyperthyroidism (Kreuzberg et al. 2000). However, since the amount of PLB is small in atrium (Shenoy et al. 2001) and shows further decrease in hyperthyroidism, the role of PLB, the other common target of thyroid hormones and PDE₂, appears to be less significant in the increased negative inotropic capacity. Whatever the precise mechanism is, as the PDE₂ is included in the nitric oxide (NO)-cGMP pathway (an important determinant of the cardiac function), an interesting possibility for intervention is revealed. Further inspiring investigations would be possible by the development of a pure PDE₂ inhibitor *sine* ADA inhibition.

In conclusion, our experiments demonstrate the significant positive inotropic effect of PDE₂'s inhibition in guinea pig atria. Furthermore, we found that T₄ treatment does not alter the amplitude of the atrial contractile force, but enhances the positive inotropic effect of PDE₂'s inhibition in comparison to the euthyroid state.

Acknowledgements. This study was supported by a grant from Hungarian Ministry of Health (ETT 04/473/2000).

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Final version accepted: July 7, 2003