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Novel cilostamide analogs, phosphodiesterase 3 inhibitors, produce positive inotropic but differential lusitropic and chronotropic effects on isolated rat atria

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ABSTRACT

Objective(s): Recently, we showed that some new synthetic compounds structurally related to cilostamide (4-(1,2-dihydro-2-oxoquinolin-6-hydroxy)- N-cyclohexyl-N-methylbutanamide), a selective phosphodiesterase 3 (PDE3) inhibitor, produce inotropic effect comparable to that of IBMX (3-isobutyl-1-methylxanthine), a non-selective PDE inhibitor, but with differential chronotropic effect. In this investigation, we compared the pharmacological effects of these compounds as potential cardiotonic agents using the spontaneously beating atria model.

Materials and Methods: In each experiment, rats were treated with reserpine. The atrium was isolated and mounted in an organ bath. We assessed chronotropic and inotropic effects using cumulative log concentration-response curves of isoprenaline alone or in combination of each test-compound.

Results: Majority of test compounds augment atria contraction force (ACF) significantly but with different potencies on atrium contraction rate. Cilostamide, MCPIP ([4-(4-methyl piperazin-1-yl)-4-oxobutoxy)-4-methylquinolin-2(1H)-one]), methyl carbostyril compounds- (mc1), mc2 and mc5 increased the isoprenaline effect on ACF synergistically. But, mc6 failed to potentiate the effect of isoprenalin; mc3 and mc4 did not increase ACF, which may be because of their higher hydrophilic nature. It was interesting that mc2, alone or in combination with isoprenaline, produced the highest inotropic effect while it did not affect the basal contraction rate and almost blocked the isoprenaline chronotropic effect.

Conclusion: Combination of mc2 with isoprenaline had synergistic effect on inotropic effect, but this combination reduced isoprenaline chronotropic effect; therefore, these effects cannot be related to reducing B-adrenergic receptors activity. These compounds showed different effects; probably all of them were not mediated via PDE3 inhibition and other mechanisms are involving.

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Introduction

Cyclic adenosine mono phosphate (cAMP) plays important roles in regulation of various cellular activities, such as metabolism, contractility, motility, and transcription (1). The concentration of cAMP is modulated via synthesis and degradation. Therefore, combination of adenylyl cyclase (AC) activators and cyclic nucleotide phosphodiesterase (PDEs) inhibitors may produce a synergistic effect via increasing of cAMP concentration.

PDEs are a superfamily of metallophosphohydrolases that specifically cleave the 3' phosphate-bound in cAMP and/or cyclic guanosine mono phosphate (cGMP) to produce their 5'-nucleotide (2). The human genome contains 21 distinct PDE genes that encode 11 PDE protein families in which PDE1, PDE3, PDE4, PDE6, PDE7 and PDE8 are multigene while PDE2, PDE5, PDE9, PDE10 and PDE11 are unigene families (3). In cardiac tissue and isolated cardiomyocytes, multiple variants of several PDE families (PDE2, PDE3A, PDE4 and PDE5) have been identified. In these tissues, PDE3A and PDE4 have functional role in regulating cardiac contractility (4) in which inhibitors of PDE3A produce higher efficacy (5). At the present time, some of the selective PDE3 inhibitors (e.g. amrinone, milrinone and enoximone) have been approved for treatment of chronic heart failure (CHF) (6).

PDE3 inhibitors possess positive inotropic and lusitropic properties in ventricular tissue. The potency of the PDE3 inhibitors as inotropic agents in this tissue is considered to correlate with their potency as inhibitors of the sarcoplasmic reticulum membrane-bound PDE3 activity, which might induce a local elevation of cAMP (7). Cilostamide, a selective PDE3 inhibitor, possesses both anti-thrombotic and anti-intimal hyperplasic actions. However, its clinical use is

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limited by tachycardia side-effects, which is assumed to inhibit cardiac PDE activity (8).

In previous work, we synthesized a new series of cilostamide analogues and studied their effects on heart contractile force and rate in which the PDE3A catalytic cavity was considered for modeling new PDE3 inhibitors (9). We showed that, among the tested compounds, the inotropic effect of [4-(4-methyl piperazin-1-yl)-4-oxobutoxy)-4-methylquinolin-2(1H)-one] (MCPIP) was more predominant. It increased the contractility in a dose-dependent manner with a maximum effect comparable with IBMX (3-isobutyl-1-methylxanthine), a non-selective PDE inhibitor (9). It was interesting that the 4-methyl analogue of cilostamide, in spite of having high inotropic effect, produced a lower chronotropic effect. This suggests that the test compounds may produce their inotropic and chronotropic effects via separated mode of action.

This research was aimed to design and evaluate new selective PDE3 inhibitor to potentiate the heart contractility but weakens chronotropic effect. The new compounds were designed and synthesized based on cilostamide structure. These chemical compounds are cilostamide analogues in which a methyl was added to the carbostyril moiety (mc, abbreviation for methyl-carbostiryl, Table1). The potencies of these compounds for PDE3A inhibition and their inotropic and chronotropic effects, alone and in the present of isoprenaline, were assessed using a spontaneously beating atria model.

Materials and Methods

Drugs and chemicals

The test compounds were synthesized by Sadeghian and co-workers which previously described in detail (10). Reserpine and isoprenaline hydrochloride were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Dimethyl sulfoxide (DMSO) was provided from Fluka Chemical Co. All other chemicals obtained from Merck (Germany). In each experiment, we dissolved isoprenaline in distilled water containing 0.1% vitamin C and diluted with Krebs-Henseleit media (mM, NaCl 118 , KCl 4.5 , $CaCl_2$ 1.36, $MgSO_4$ 1.2, $NaH_2PO_4$1.2, $NaHCO_3$ 25 and glucose 10) to final desired concentration. All the other test compounds were dissolved in DMSO and diluted with Krebs-Henseleit solution to the mentioned concentration (100 µM) (9).

Animals

The male Wistar rats (250-350 g) were purchased from animal house of Mashhad University of Medical Sciences, Medical School, Mashhad, Iran, where the environmental condition was programed to keep the temperature at about 23 ± 2°C in light-dark cycle from 7 am to 7 pm. Animals had free access to a standard laboratory diet and water. In order to obtain atria preparation, which depleted in endogenous catecho-lamine, 24 hr before euthanasia, each animal was injected intraperitoneally 5 mg/kg reserpine (11). Then animals were anaesthetized with injecting 80 mg/kg thiopental intraperitoneal. After midline thoracotomy, the heart was rapidly separated and transferred to a dissection dish filled with Krebs-Henseleit solution aired constantly with carbogen (95% O2 / 5% CO_2).

Methods

The atria was isolated from ventricles and suspended vertically in a 50 ml organ-bath filled with Krebs-Henseleit solution, which constantly gassed by 95% O_2 and 5% CO_2 at 37 ˚C, pH = 7.4 (12). The contractions were recorded using a high sensitive isometric transducer (model N^0, sensitivity 0-25 g), which was connected to a power lab system and recorded in a computer using Chart and Scope software. (Power lab 8/30, Chart and Scope are trademarks of AD Instruments Pty Ltd).

In each experiment, the basal resting tension was considered to be about 0.5 g (13), and the isolated atria was incubated for about 30 min (equilibration period). To evaluate the possible synergism between isoprenaline and test compounds, preparation were first treated with cilostamide or one of methyl carbostiryl compounds (mc1, mc2, mc3, mc4, mc5, mc6 or MCPIP). After stabilizing heart rate (about 20 min later), different concentrations of isoprenaline were added in a cumulatively manner.

For each treatment, the atria contraction force (ACF) was calculated using one minute of contraction curve in steady state. For lusitropic effect, the mean of minimum ACF (ACFmin), and for inotropic effect, the mean of maximum ACF (ACFmax) were calculated. For atria contraction rate (ACR), the number of contraction was counted in one minute. Each experiment was repeated for 5 to 6 times.

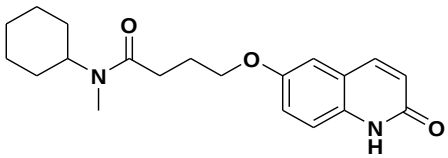
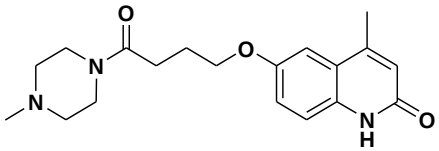
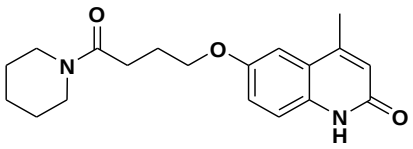
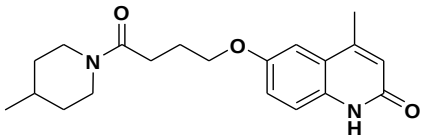
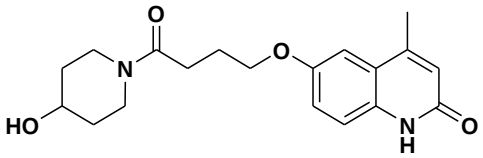
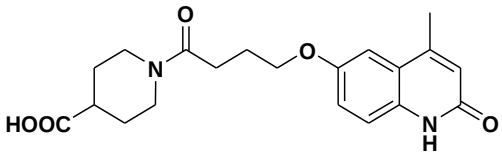
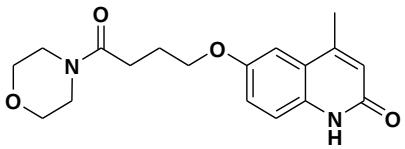
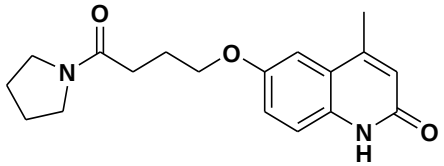
PDE Assay

The test compounds were assessed for PDE3A activity using PDE assay kit, produced by BPS Bioscience Company (BPS Bioscience Inc, San Diego, United States). The method is described in detailed previously () wherein the assay was performed at room temperature for 60 min in a 50 µM mixture containing IMAP (Immobilized Metal Ion Affinity Particle) reaction buffer, 100 nM FAM-cAMP (carboxyfluorescein or FAM, is a fluorescein derivative) 0.5 ng PDE3A and the test compound. The binding reactions were carried on using binding reagent. The absorbance of fluorescence was determined at an excitation of 485 nm and an emission of 528 nm using a BioTek Synergy ™ 2 microplate reader. Each assay was performed in duplicate.

Statistical analysis

Data were expressed as mean ± standard error of mean (SEM). In the case of examining more than two groups, one-way analysis of variance (ANOVA) and the Tukey, s *post hoc* test were used. Differences among means were considered significant if $P<0.05$. All data passed a normality test.

Table1. The structure of compounds

Name	Chemical Name	Structural Formula
Cilostamide		
MCPIP	4-(4-methyl piperazin-1-yl)-4-oxobutoxy)-4-methylquinolin-2(1H)-one	
mc1	6-(4-oxo-4-(piperidine-1-yl-)-4-methylquinolin-2(1H)-one	
mc2	6-(4-(4-methylpiperidin-1-yl)-4-oxobutoxy)-4-methylquinolin-2(1H)-one	
mc3	6-(4-(4-hydroxypiperidin-1-yl)-4-oxobutoxy)4-methylquinolin-2(1H)-one	
mc4	methyl 1-(4-(1, 2-dihydro-4-methyl-2- oxoquinolin -6-yloxy)butanoyl) piperidin -4-carboxylate	
mc5	6-(4-morpholino-4-oxobutoxy)-4-methy quinolin-2(1H)-one	
mc6	6-(4-oxo-4-(pyrrolidin-1-yl)butoxy)-4-methylquinolin-2(1H)-one	

Results

Effect of isoprenaline alone or in combination with cilostamide on ACF

In the presence of isoprenaline, ACF increased concentration dependently with threshold of 10^{-10} M. In concentration of 10^{-6}M, ACF was reached to maximum of 14±6% higher than systolic base line (HSBL) (Table2, Figure 1A). Adding cilostamide (selective PDE3 inhibitor, IC50=0.04 µM, Figur 3) to organ-bath increased ACF and potentiated the effect of isoprenaline (about 2.86 fold, Figur 1A).

Effect of isoprenaline on ACFmax & ACFmin

The analysis of ACF curve showed that ACFmax and ACFmin variations are not concomitant in presence of isoprenaline. The threshold of isoprenaline ACFmax curve was produced at concentration 10^{-10}M and reached to maximum in concentration of 3×10^{-8} M, which was 21% HSBL (Figure 4B). In higher concentration, ACFmax was decreased. While between 10^{-10}M - 10^{-8} M, ACFmin was not changed. At higher concentration of isoprenaline, ACFmin increased in a concentration dependently manner and in concentration of 10^{-6} M, it was 10.23±5% higher than diastolic base line (HDBL) (Figure 4A). Adding of cilostamide to organ bath has different effect on ACFmax and ACFmin. At the beginning of experiment, ACFmax was increased to 24±9% HSBL, which within 20 min decreased to 11±6% HSBL (Figure 4B). ACFmin at the beginning decreased to 9±4%HDBL, but subsequently it increased and reached to 16±6% HDBL within 20 min (fig 4A).

In the presence of cilostamide, isoprenaline increased both ACFmax and ACFmin concentration dependently with maximum effect in concentration of 3×10^{-6} and 10^{-6} M, respectively (ACFmax 25±5% HSBL and ACFmin 39 ±7% HDBL).

Table 2. Comparison the effects of mc2 on isoprenaline-induced ACF and ACR augmentation

	Cont	10 µM	100 µM
ACR (% increase baseline)	68±12	50±10	6±7**
ACF (% increase baseline)	14±6	3±1	60±5**

The whole rat atrium was incubated in an organ bath, atria contraction force and atria contraction rate were recorded in the presence of solvent (cont) or a concentration of 10 µM or 100 µM methyl carbostiryl compounds-2 (mc2). After 20 min, isoprenaline was added to the organ bath. The percentage of increase of atria contraction force (ACF) or atria contraction rate (ACR) from baseline was calculated. Each number represents the effect of the test compound in combination with 1 µM isoprenaline. Results are means ± SEM from six experiments.
Difference between isoprenaline alone and its combination with mc2
**P<0.01

Similar to the protocol of cilostamide, the experiments were repeated with seven other methyl carbostiryl derivatives (mc1, mc2, mc3, mc4, mc5, mc6, MCPIP).

Effect of synthetic compounds on ACF

Adding mc1 and mc5 to organ bath significantly increased ACF with different efficacies (percentage over the base line, mc1=35±5%, mc5=19± 4%). Also MCPIP and mc6 significantly augmented ACF with relatively similar efficacies (percentage over the base line, MCPIP=15±3%, mc6=11±4%). However, mc3 and mc4 did not change ACF. On the other hand, mc2 changed ACF differently.

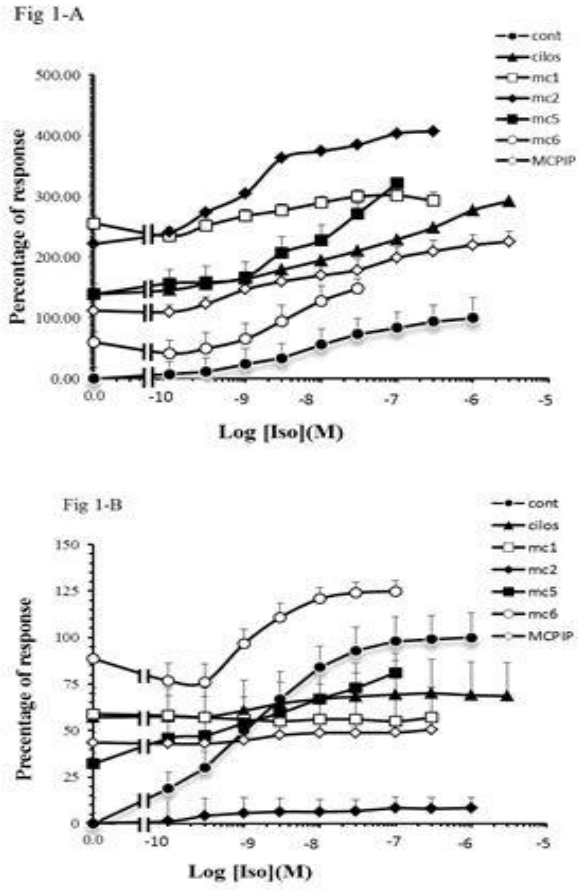


Figure 1. Comparing the efficacies of test compounds in combination with isoprenaline
The whole atrium was separated from rat and incubated in an organ bath. Average of tension (Atria Contraction Force, ACF) and heartbeat (Atria Contraction rate, ACR) were recorded using an isometric transducer. After stabilizing period (30 min), solvent (cont), cilostamide (cilos), or a methyl carbostiryl (MCPIP, mc1, mc2, mc5 or mc6) was added to incubation medium. After 20 min, isoprenaline (Iso) was added to organ bath with mentioned concentration in a cumulative manner. Each point is the average of 5 – 6 observations, which normalized according to the baseline and calculated with considering 100 percent for maximum effect of isoprenaline alone.
Figure 1-A; The efficacies of test compounds on atria contraction force (ACF)
Figure 1-B; The efficacies of test compounds on atria contraction rate (ACR)

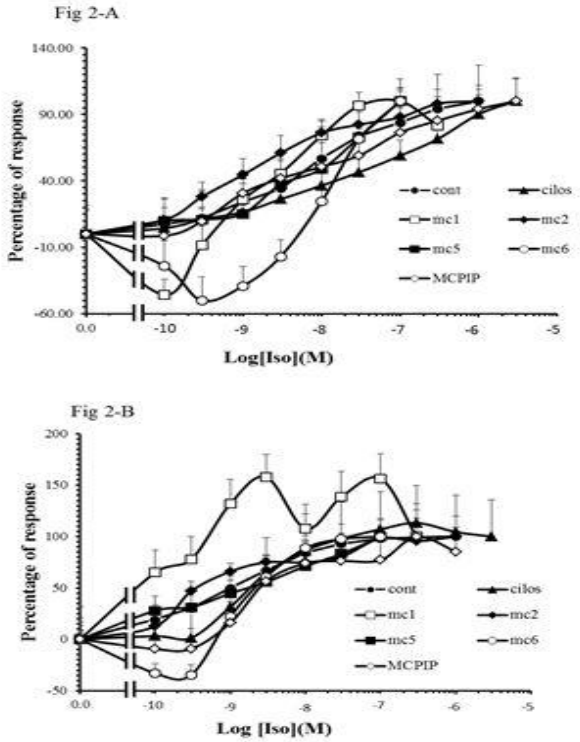


Figure 2. Comparing the potencies of test compounds in combination with isoprenaline

The whole rat atrium was incubated in an organ bath. Average of tension and heartbeat were recorded using an isometric transducer, after stabilizing period (30 min). Solvent (cont) cilostamide, (cilos) or a methyl carbostiryl compound (MCPIP, mc1, mc2, mc5, or mc6) was added to incubation medium. After 20 min, isoprenaline (Iso) was added to organ bath with mentioned concentrations in a cumulative manner. Each point is the average of 5 – 6 observations, which normalized according to the baseline and calculated with considering 100 percent for its maximum effect.

Figure 2-A; The potencies of test compounds on atria contraction force (ACF)

Figure 2-B; The potencies of test compounds on atria contraction rate (ACR)

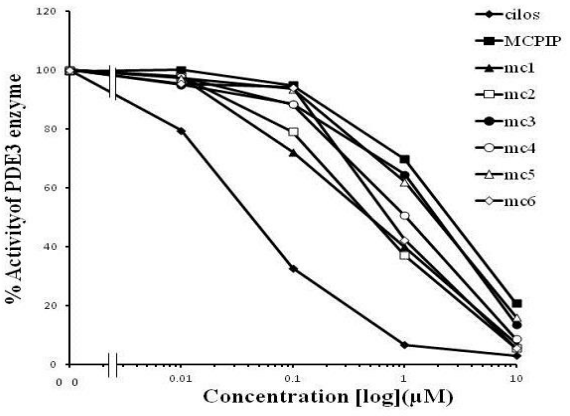


Figure 3. The inhibitory effects of test compounds on PDE3 activity

The effects of mentioned test compounds on phosphodiesterase 3A (PDE3A) activity, performed by BPS Bioscience Company (BPS Bioscience Inc, San Diego, United States). Each IC_{50} was calculated using GraphPad Prism software

When mc2 was added to medium, ACF decreased (-1.76 %), after a while, it began to increase gradually in 20 min and reached to 40±7% HSBL. Analysis of ACF showed that the primary ACF decline was because of initial decreasing the ACFmin (-6% lower than HDBL), which then increased within 20 min to maximum 36±6%HDBL (Figure 4A), while ACFmax was increased continuously from the beginning (Figure 4B).

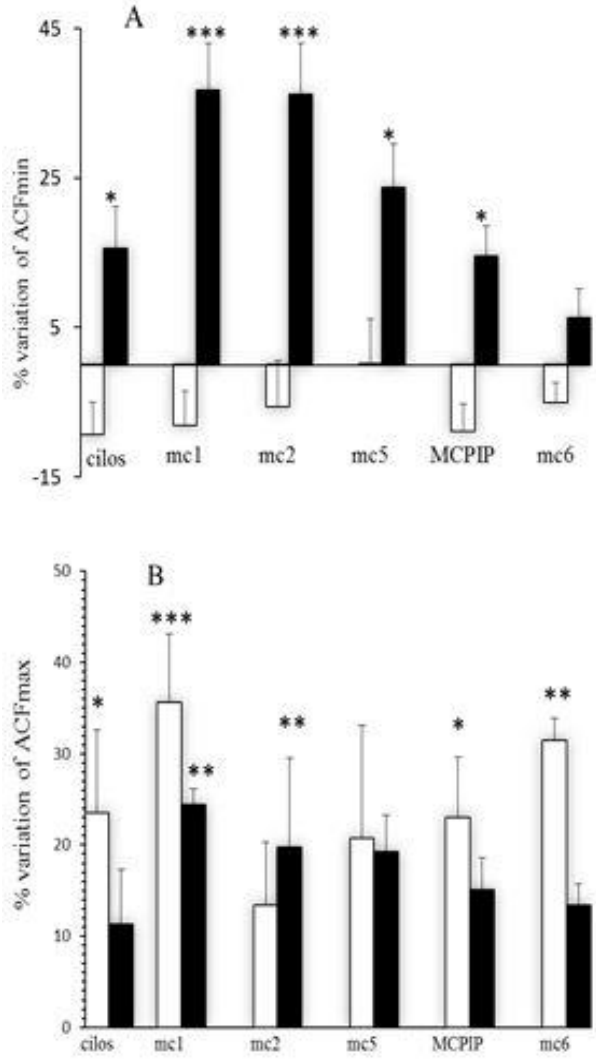


Figure 4. The effect of test compounds on ACFmin and ACFmax

The whole rat atrium was incubated in an organ bath. After equilibration of atria contraction force, 100 µM of a test compounds (cilostamide, MCPIP, mc1, mc2, mc5 or mc6) was added to medium. ACFmin (minimum atria contraction force, Figure A) and ACFmax (maximum atria contraction force, Figure B) were recorded at the beginning of adding the test compounds (white column) and 20 min latter (black column). For each compound, results are means ± SEM from six experiments and presented as percentage of increase of basal (basal ACFmin; 430±4.5 mg and basal ACFmax; 740±12 mg).

*) Difference between baseline and test compounds *P<0.05, **P<0.01 and ***P<0.001

Effect of isoprenaline on ACF in presence synthetic compounds

In the presence of each test compound, the effects of different concentrations of isoprenaline on ACF were assessed. In the presence of mc1 and mc6, low concentrations of isoprenaline decreased ACF, while higher concentrations augmented ACF in a concentration-dependent manner (Figure 1A). The decreasing ACF in low concentrations was because of decreasing both ACFmin and ACFmax (Figure 4A, 4B). However, in the presence of mc2, mc5 and MCPIP, low concentrations of isoprenaline did not produce diminishing effect and increased ACF concentration dependently. Increasing ACF was correlated to increasing both ACFmin and ACFmax, which were concomitant with increasing isoprenaline concentration. The presence of mc3 or mc4 did not change isoprenaline concentration-response curve. It was also shown that 100 µM mc2 augmented the effect of isoprenaline on ACF (Figure 1A). Comparing with the effect of other test compounds, mc2 produced the highest effect on ACF (Figure 1A). However, this effect was not concentration dependently. Actually, 10 µM mc2 decreased isoprenaline-augmented ACF signify-cantly (isoprenaline 10^{-6}M, ACFmax 14±6% HSBL, isoprenaline +mc2 10^{-5}M, ACF 3±1% HSBL) (Table2). However, the screening assay for potencies of test compounds on ACF demonstrated that they have a similar potency and for concentration-dependent curve of isoprenaline, neither of them produced a significant shift to the left (Figure 2A).

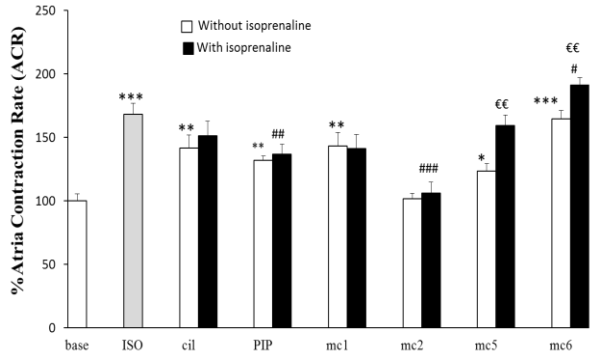


Figure 5. Chronotropic effects test compounds and their combination with isoprenaline

The whole rat atrium was incubated in an organ bath and atria contraction rate (ACR) was recorded in the presence of solvent (cont, 210±25 contraction/min) or a concentration of 100 µM cilostamide (cil), MCPIP (PIP), mc1, mc2, mc5 or mc6. After 20 min, isoprenaline was added to the organ bath. Each column represents the means of ACR for each test compound alone or its combination with 1 µM isoprenaline (+ISO) ±SEM from six experiments.

*) Difference between baseline and test compounds $^{*}P<0.05$, $^{**}P<0.01$ and $^{***}P<0.001$

#) Difference between isoprenaline alone and its combination with test compounds $^{\#}P<0.05$, $^{\#\#}P<0.01$, and $^{\#\#\#}P<0.001$

€) Difference between test compounds alone and its combination with isoprenaline $^{€€}P<0.01$

Atria contraction rate (ACR)

ACR was concentration dependently increased by isoprenaline with threshold concentration of 10^{-10}M. In 10^{-6}M, ACR was reached to maximum value, 68±9% HSBL (Figure 5, Figure 1B). Cilostamide significantly increased ACR (42±10% HSBL), while in combination with isoprenaline, it did not increase isoprenaline-induced ACR (isoprenaline 10^{-6}M, ACR 68±9% HSBL, + cilostamide 10^{-4} M 51±11% HSBL) (Figure 5, Figure 1B).

Adding mc1, mc5, MCPIP and mc6 to organ bath significantly increased ACR with different efficacies (percentage over the base line, 43±11%, 23±6%, 32±4% and 65±6%, respectively) (Figure 5, Figure 1B), while mc2, mc3 and mc4 did not change ACR.

In the presence of each test compounds, the effects of different concentrations of isoprenaline on ACR were assessed. Findings showed that mc3 and mc4 did not make any significant changes in the isoprenaline concentration-response curve. In the presence of mc6, low concentrations of isoprenaline (10^{-10}-10^{-9}) decreased ACR, while from the concentration of 10^{-9}M, ACR was concentration dependently increased with a maximum effect at 10^{-6}M isoprenaline (91±6% HSBL, Figure 5, Figure 1B). Isoprenaline-augmented ACR was not changed significantly in the presence of mc5 (isoprenaline 10^{-6}M, ACR 68±12% HSBL, Iso+ mc5 10^{-4}M 59±8% HSBL). Mc1 and MCPIP significantly decreased isoprenaline-augmented ACR (isoprenaline 10^{-6}M, Iso+MCPIP 10^{-4}M, 37±8% HSBL $P<0.05$, Figure 5, Figure 1B). Mc2 in concentration of 100 µM did not change ACR, but concentration dependently inhibited isoprenaline-augmented ACR. It was interesting that in the presence of 100 µM mc2, the maximum isoprenaline-augmented ACR was inhibited and reached to the baseline limitations (Table 2, Figure 1B).

However, the screening assay for potencies of test compounds on ACR demonstrated that they have a similar potency except mc1, and for concentration-dependent curve of isoprenaline, only mc1 produces a significant shift to the left (Figure 2B).

PDE3 inhibitory effect of synthetic compounds

Figure 3 shows inhibitory effect of synthetic compounds on PDE3A. IC50 for inhibition of PDE3A for mc1, mc2, mc3, mc4, mc5 and MCPIP are 0.5 µM, 2 µM, 1 µM, 2 µM, 0.7 µM and 3 µM, respectively.

Discussion

The test compounds (MCPIP and mc1-mc6) are potent PDE3 inhibitors and most of them produced cardiotonic effect with different efficacy. However, mc3 and mc4 failed to modify basal atria activity, which seems that adding a polar group on piperidin (OH and COOH for mc3 and mc4, respectively) decreases penetration of these compounds to the cell. Cilostamide and other test compounds (mc1, mc2, mc5, mc6 and MCPIP) produced different lusitropic and chronotropic effects.

It has been shown that in heart and isolated cardiomyocytes, cAMP-dependent signaling is conducted by activity of different PDE isoenzymes (PDE1, PDE2, PDE3A, PDE4 and PDE5) (4). Selective PDE3A inhibitors (e.g. cilostamide) increase both rate and force of cardiac contraction. In our previous work, we showed that, a methyl carbostiryl derivative, [4-(4-methyl piperazin-1-yl)-4-oxobutoxy)-4-methylquinolin -2 (1H)-one) (MCPIP), increased the contractility in a concentration-dependent manner with a maximum effect comparable with IBMX, a non-selective PDE inhibitor (9). However, in spite of having the highest positive inotropic effect, it reduced the isoprenaline-induced tachycardia. So, we synthesized a new series of methyl carbostiryl compounds (mc is abbreviated for methyl carbostiryl derivative, Table 1) and assessed their effects on atria contractility. Based on the structure-activity relationship studies and observation of cardiotonic effect, we used the compounds as possible selective PDE3 inhibitors. PDE assay confirmed that all of these compounds inhibited PDE3A with a high potency. Possible inotropic and chrono- tropic effects were evaluated for the test compounds alone and in combination with isoprenaline. Adrenergic receptor agonists (e.g. isoprenaline) activate adenylyl cyclase via G-protein dependent pathway, which increases cAMP levels in the cells. It increases contractile force (CF) and heart rate through activation of adenylyl cyclase (14).

In this experiment, isoprenaline simultaneously increased ACR and ACF from 10^{-10} M concentration dependently, which shows concomitant increasing of cAMP in sinoatrial node (SAN) and atria myocytes. Isoprenaline increased ACFmin concentration dependently with a threshold effect in concentration of 10^{-8}M, while ACFmax was increased between 10^{-10}-10^{-8}M. This may implicate the separation of two compartments in which cAMP accelerates the processes of contraction and relaxation. The compartment beneath membrane, where cAMP is produced, accelerates Ca^{+2} channels activity; the second compartment, near sarcoplasmic reticulum, increases Ca^{+2} pumps activity. Both compartments are separated by soluble PDEs in cardiomyocytes and restrict cAMP diffusion (15).

The test compounds, in spite of having similar PDE3 inhibitory activity, produced different effects on ACF and ACR. In combination with isoprenaline, they increased ACF with different efficacies and produced completely different effects on ACR, which are not correlated with their PDE inhibition effects. The isoprenaline-induced ACR was potentiated by mc5 and mc6, while it was interesting that mc2 and MCPIP significantly reduced isoprenaline-induced ACR. This observation suggests that the mechanism of action of these compounds is complex. Probably, other mechanism may play a role in reduction of ACR. In addition to PDE inhibitory effect, these compounds may act via other mechanisms such as effect on ion channel activity. For instance, OPC-8212 (16) and OPC-8490 (17, 18) as quinolinone derivatives have reported to have inhibitory effect on K^+ current in guinea pig ventricular myocytes. Also, the involvement of voltage-dependent Ca^{2+} channels is suggested for the effect of these compounds (17).

Cyclic AMP plays an important role in SAN automaticity (19) and modulation of cardiac excitation-contraction coupling (20). PDE3 and/or PDE4 reduce basal sinoatrial beating rate via hydrolysis of cAMP. In rodents, the tachycardia elicited by catecholamines, via production of cAMP by β-adrenoceptor activation, is not controlled by PDE3 and PDE4, despite a blunting effect of PDE3 or/and PDE4 on basal sinoatrial beating (21, 22). It has been proved that cardiac contractility of isoprenaline is mediated by increasing adenylyl cyclase activity through a β-adrenoceptor-dependent mechanism. This effect is potentiated in the presence of a PDE inhibitor (23). Among the test compounds, mc2 produced the highest inotropic effect and potentiated the effect of isoprenaline. This suggests that the inhibitory effect of mc2 on isoprenaline-induced chronotropic effect is produced via a mechanism separated form β-adrenergic.

Also, increasing the activity of cAMP-dependent protein kinase promote Ca^{+2} overload and several proteins involved in Ca^{2+} signal transduction, which in combination with stimulated hyperpolarization-activated cyclic nucleotide–gated (HCN) channels increase SAN electrical activity. In myocardial cells, activation of protein kinase A (PKA) leads to phosphorylation of voltage-gated L-type calcium channels (or dihydropyridine receptor), and sarcoplasmic reticulum Ca^{2+} release channels (Ryanodine receptor 2). Phosphorylation of these channels elevates cytosolic calcium, which augments CF. At the same time, PKA phosphorylates the sarcomeric proteins troponin I, and myosin binding protein C, which reduces the sensitivity of the sarcomeric myofilaments to Ca^{2+} and accelerates the Ca^{2+} dissociation from the myofilaments. This effect in combination with phosphorylation of phospholamban by PKA, which reduces its inhibitory effect on sarcoplasmic Ca^{+2} ATPase pump, accelerates the relaxation process or lusitropy (15, 20). Also PKA phosphorylates PDEs, which increases cAMP-hydrolyzing activity of myocardial PDEs by 2-3 folds. It leads to decrease in cAMP concentration (24). Activating cAMP-dependent protein kinase by cilostamide resulting in augmenting Ca^{2+} signaling has been shown in rat ventricular myocytes. Attenuation of ACFmax, following of its initial augmentation, implicates the activation of a desensitization process, which has been shown previously (15, 20 and 25). Also, the initial decreasing ACFmin, which was followed by its increasing, may represent a desensitization processes in the relaxation compartment. However, the

exact mechanisms of these phenomena are a matter of further studies.

Conclusion

Cilostamide, MCPIP, mc1, mc2, mc5 and mc6, all produced inotropic effects accompanied by deferential chronotropic activities. Mc3 and mc4 did not change basal contraction and heart rate and referred to their increased polarity, which reduces penetration of the compounds in the atria cells. The test compounds augmented the effect of isoprenaline on ACF with different efficacies (mc2 > mc1 > mc5 > cilostamide > MCPIP > mc6), while these compounds inhibit PDE activity with similar potencies.

It was interesting that among test compounds, mc2 produced the highest efficacy for arterial contraction effect, but did not affect the basal contraction rate. Furthermore, it decreased the stimulating effect of isoprenaline on heart rate. However, mc6 potentiated the chronotropic effect of isoprenaline. Considering the synergism of mc2 with isoprenaline on ACF, reducing the isoprenaline chronotropic effect cannot be mediated via interfering with β-adrenergic receptors mediated processes. The differential effects of the test compounds on ACF and ACR are not correlated with their PDE3 inhibitory effect.

Thus, these compounds should be considered as mixed action agents with the positive inotropic action mediated by PDE3 inhibition and the negative chronotropic action mediated by electrophysiological effects.

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