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Modulation of the contractility of guinea pig papillary muscle by the activation of ATP-sensitive K^+ channels

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Abstract

The influence of activation of ATP-sensitive K^+ channels on the positive inotropic action of *l*-isoproterenol *d*-bitartrate (isoprenaline), 12b-hydroxydigitoxin (digoxin), 5-amino-[3,4′-bipyridin]-6[1*H*]-one (amrinone), 1,6-dihydro-2-methyl-6-oxo 3,4-bipyridine-5-carbonitrile (milrinone) and UD-CG 115 BS; 4,5-dihydro-6[2-(4-methoxyphenyl)-1*H*-benzimidazol-5-yl]-5-methyl-3(2*H*)pyridazinone (pimobendan) was investigated in guinea pig papillary muscle. The force of contraction (dF) and the rate of rise of force of contraction (dF/dt) were measured. After activation of ATP-sensitive K^+ channels by 1 μM of (3*S*,4*R*)-3-hydroxy-2,2-dimethyl-4-(oxo-1 pyrrolidinyl)-6-phenyl-sulfonylchroman hemihydrate (HOE 234) the dose-response curves for isoprenaline were shifted to the right (about 9-fold). The positive inotropic action of digoxin and milrinone was significantly enhanced (about 5-fold). The inotropic action of amrinone and pimobendan before and after pretreatment with HOE 234 was not significantly different. HOE 234 pretreatment decreased irreversibly the maximum effect (E_{max}) of isoprenaline only for the amplitude of force of contraction, but not for the rate of rise of force. Opposite to this, activation of ATP-sensitive K^+ channels evidently enhanced the positive inotropic effects of digoxin and milrinone. In the case of milrinone, the E_{max} for both parameters (dF and dF/dt) was greater after HOE 234 pretreatment. Only the E_{max} of digoxin for the amplitude of the force of contraction was significantly increased in the presence of HOE 234. The above mentioned results indicate that activation of ATP-sensitive K^+ channels by HOE 234 modulates the positive inotropic action of cardiotonic drugs. This change may be expressed as potentiation (digoxin, milrinone) or attenuation (isoprenaline) of the positive inotropic effects, depending on the mechanism of action.

Keywords: Inotropic drug; Heart failure; K^+ channel, ATP-sensitive; Papillary muscle; Digoxin; Milrinone

1. Introduction

Congestive heart failure is associated with marked dysfunction of heart contractility and requires treatment with positive inotropic drugs. Pharmacological agents that enhance the force of cardiac muscle contraction have a mechanism of action related to an increase of cAMP concentration (by stimulation of its synthesis or inhibition of degradation), inhibition of Na^+,K^+ ATP-ase or an increase in the sensitivity of the contractile apparatus to Ca^{2+} and prolongation of the activation of Na^+ channels. All these drugs reduce clinical signs of heart failure, increase exercise tolerance and improve left ventricular ejection fraction (DiBianco, 1991; Notterma, 1991; Pagel et al., 1994; Zierhut et al., 1994; Endoh, 1995).

However, regarding the safety of these drugs the situation is less clear. Recent long-term studies have shown an increasing mortality rate during treatment with positive inotropic drugs, specially in post-myocardial infarct patients (Komai et al., 1991; Packer et al., 1991; Nony et al., 1994; Kober et al., 1994).

Activation of ATP-sensitive K^+ channels, which occurs in ischemic heart muscle, could be a very important factor that modulates the action of the above-mentioned drugs in patients with congestive heart failure. HOE 234 is a new activator of ATP-sensitive K^+ channels. We have reported recently (Kocić, 1994) that HOE 234 activates ATP-sensitive K^+ channels in guinea pig papillary muscle, with high affinity ($ED_{50} = 0.5 \pm 0.1$ μM) as compared to cromacalim and pinacidil in the guinea pig atrium (Wai-Man, 1992).

The aim of the present study was to examine the influence of HOE 234 pretreatment on the positive inotropic effects of cardiotonic drugs with different mechanisms of action.

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2. Materials and methods

2.1. Animals and preparations

Locally bred guinea pigs (weighing 300–400 g) of either sex were killed by cervical dislocation. The hearts were immediately excised and washed free of blood with a modified Krebs-Henseleit solution. This solution contained (mM): NaCl 119.0; KCl 4.8; $MgSO_4$ 1.2; $CaCl_2$ 2.5; $NaHCO_3$ 24.8; KH_2PO_4 1.2 and glucose 10.0. In the case of isoprenaline, ascorbic acid was added to the solution, to prevent HOE 234 from influencing the rate of autooxidation of isoprenaline; the pH was 7.4. Papillary muscles were dissected from the right ventricle and mounted in a 2 ml bath (Steirt Organ bath, type 813 with DC temperature controller type 319, Hugo Sachs Elektronik, Germany). The muscles were maintained at 35 ± 0.1°C, with a constant perfusion rate (10 ml/min) of Krebs-Henseleit solution bubbled with 95% O_2-5% CO_2 (peristaltic pump, type 371, Unipan, Poland).

Tissues were maintained at 0.4 g of resting tension and electrically paced by two silver electrodes in contact with the muscles, with square waves (1 Hz, 1–3 ms duration, threshold voltage +20%) generated by an electronic stimulator (SC-04, COTM, Poland). The developed tension (dF) and rate of rise of force of contraction (dF/dt) were measured by a force-displacement transducer F-30 and bridge amplifier with a differentiator (type 336, Hugo Sachs Elektronik, Germany). The signals were displayed on a line recorder (TZ-4200, Laboratorni Pristroje Praha, Czech Republic). A stabilization period of 60 min was used before measurements.

2.2. Experimental protocol and statistical evaluation

The concentration-response curves for isoprenaline, digoxin, amrinone, milrinone or pimobendan were made 60 min after the preparation procedure. Baseline measurements were taken before drug administration. The maximal effect of each concentration of the investigated inotropic drug was recorded. The papillary muscles were incubated 1–2 min with isoprenaline, 8–10 min with digoxin, amrinone and milrinone and about 15 min with pimobendan before registration of the effects of each concentration. The tissue was then allowed to recover (about 120 min) and the same protocol was repeated 7 min after the start of the perfusion with HOE 234 alone or after glibenclamide for 15 min, then HOE 234 in the presence of glibenclamide. The increase in force of contraction (dF) and in the rate of rise of force is expressed as % of control values obtained before administration of any drug (average control values were: $F = 0.47 \pm 0.078$ mN/mm^2; $dF/dt = 4.64 \pm 0.64$ mN/s; $n = 20$). Dose-response curves were constructed using a computer program according to Tallarida and Murray (1987). A coefficient of correlation (r) of linear regression was used to determine the existence of a dose-response relationship. Experimental values are presented as means ± S.E.M.. The statistical significance of the difference between two means was calculated with Student's t-test for paired samples. Only P values < 0.05 were considered to be statistically significant.

2.3. Drugs

All chemicals used in these experiments were of analytical grade.

Isoprenaline (*l*-isoproterenol *d*-bitartrate) and digoxin (12b-hydroxydigitoxin) were supplied by Sigma Chemical, St. Louis, MO, USA. Amrinone (5-amino-[3,4′-bipyridin]-6[1*H*]-one) and milrinone (1,6-dihydro-2-methyl-6-oxo-3,4-bipyridine-5-carbonitrile) were kindly donated by Sterling-Wintrop Research Institute and glibenclamide (*N*-*p*[2-(5-chloro-2-methoxybenzamido)ethyl] benzenesulfonyl *N*′-cyclohexylurea) by Polfa, Starogard, Poland. Pimobendan (UD CG 115 BS; 4,5-dihydro-6-[2-(4-methoxyphenyl)-1*H*-benzimidazol-5-yl]-5-methyl-3-(2*H*)-pyridazinone) and HOE 234 ((3*S*,4*R*)-3-hydroxy-2,2-dimethyl-4-(2-oxo-1 pyrrolidinyl)-6-phenylsulfonylchroman hemihydrate) were kindly donated by Boehringer Ingelheim, Ingelheim am Rhein, Germany, and Hoechst AG, Frankfurt, Germany, respectively.

Isoprenaline and HOE 234 were dissolved in distilled water, digoxin in 70% ethanol, amrinone and milrinone in 1% lactic acid and pimobendan and glibenclamide in DMSO (dimethylsulfoxide). All of these solvents were added to the control solution in the same concentrations and had no influence on the contractility of the muscle.

3. Results

Fig. 1 illustrates concentration-response data for isoprenaline obtained before and after incubation with HOE 234.

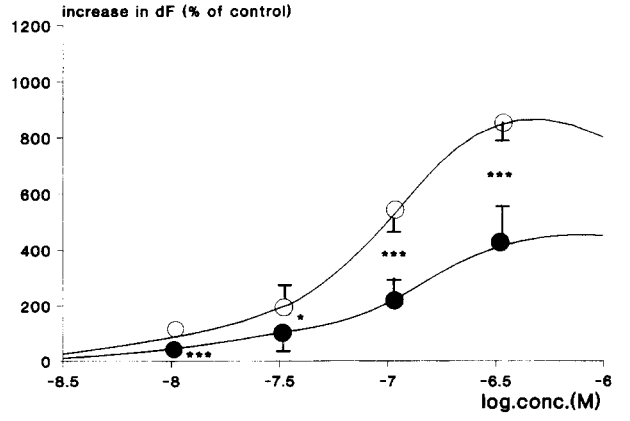

Fig. 1. Effect of HOE 234 pretreatment (1 μM) on the increase in the force of contraction induced by isoprenaline in guinea pig right ventricle papillary muscle. ○ Isoprenaline, ● isoprenaline after pretreatment with HOE 234 (closed circle). * $P < 0.05$; *** $P < 0.001$ statistically significant values compared to the corresponding values from the group treated with isoprenaline only. Each point represents the mean ± S.E.M. of six experiments.

Table 1
The pD_2 values ($-\log ED_{50}$) before and after activation of ATP-sensitive K^+ channels by HOE 234 for the positive inotropic effects of the different cardiotonic drugs

Drugs	n	pD_2 [a]	pD_2 [b]
Isoprenaline	6	7.06±0.11	5.48±0.57 [c]
Digoxin	6	5.78±0.17	6.22±0.03 [c]
Milrinone	5	4.56±0.04	5.22±0.18 [c]
Pimobendan	5	4.86±0.21	4.97±0.36
Amrinone	6	3.71±0.15	3.54±0.11

Results are expressed as means±S.E.M. n = number of experiments; [a] pD_2 values before and [b] pD_2 values after pretreatment with HOE 234 (1 μM). [c] Values obtained after pretreatment with HOE 234 significantly different from corresponding values before pretreatment, $P < 0.05$, paired t-test.

It should be noted that 1 μM of HOE 234, while shifting the concentration-response curve for isoprenaline to the right, lowered significantly the maximum response (E_{max}) to this agonist, but only that concerning the dF value. The relative efficacy of isoprenaline compared with isoprenaline plus HOE 234 was depressed by about 9.2-fold (Table 1). Activation of ATP-sensitive K^+ channels also significantly influenced the increase in dF/dt induced by isoprenaline. Most of the points were shifted significantly, except the one for the maximum value, which did not change significantly compared with the value obtained after treatment with isoprenaline alone, Fig. 2.

Fig. 3 illustrates the results of experiments performed with digoxin, milrinone, amrinone and pimobendan. The maximum effects of the examined cardiotonic drugs on the amplitude of force of contraction (dF) and rate of its rise (dF/dt) before and after pretreatment with HOE 234 are presented. Positive inotropic effects of digoxin and milrinone were enhanced after preincubation with HOE 234

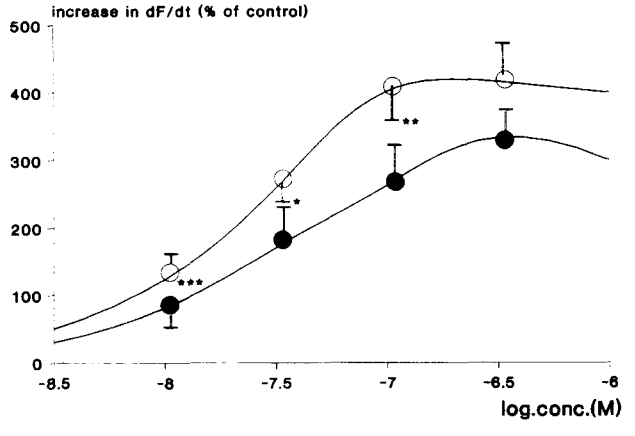

Fig. 2. Cumulative concentration-response curves for the rate of rise of force induced by isoprenaline before and after pretreatment with 1 μM of HOE 234 in the guinea pig papillary muscle. ○ Isoprenaline, ● isoprenaline after pretreatment with HOE 234 (closed circle) * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ statistically significant values compared to the corresponding values from the group treated with digoxin only. Each point represents the mean±S.E.M. of six experiments.

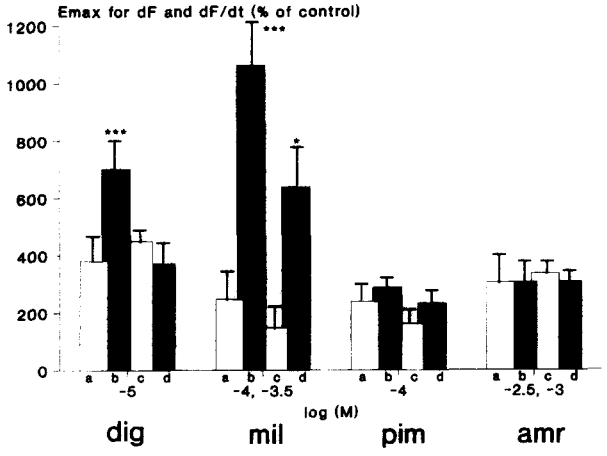

Fig. 3. The maximum effects (E_{max}) of digoxin, milrinone, pimobendan and amrinone on force of contraction (dF) and rate of rise of force (dF/dt) in the guinea pig papillary muscle before (light columns) and after (dark columns) activation of ATP-sensitive K^+ channels by HOE 234 (1 μM). dig, digoxin; mil, milrinone; pim, pimobendan; amr, amrinone; a, E_{max} for dF; b, E_{max} for dF after pretreatment with HOE 234; c, E_{max} for dF/dt; d, E_{max} for dF/dt after pretreatment with HOE 234. * $P < 0.05$, *** $P < 0.001$ statistically significant value compared to the corresponding value without HOE 234 pretreatment. Means± S.E.M. of six experiments.

(Table 1). The E_{max} of milrinone for both parameters was significantly increased after pretreatment with HOE 234. In the case of digoxin, only the E_{max} for the amplitude of force of contraction was greater in the presence of HOE 234. Additionally, the $-\log ED_{50}$ values (pD_2) for digoxin and milrinone were significantly increased in the groups treated with HOE 234.

It can be seen that there was no significant difference between the reactivity of the tissues to pimobendan and amrinone before and after preincubation with HOE 234. Neither was a significant difference observed between the $-\log ED_{50}$ values (Table 1).

HOE 234 had a dose-dependent negative inotropic action, which was reversed by glibenclamide in a dose-dependent manner, $pA_2 = 6.6$ (Kocić, 1994).

The effects of the investigated cardiotonic drugs after pretreatment with a higher concentration of HOE 234 were not significantly different from those obtained with 1 μM. Glibenclamide (1 μM), a known selective blocker of ATP-sensitive K^+ channels in the heart, prevented all the above-described modulations of cardiotonic drug activity induced by pretreatment with HOE 234.

4. Discussion

It is well known that coronary heart disease is a common cause of congestive heart failure and that positive inotropic drugs are the basic therapeutic agents used in the management of this malady (Notterma, 1991). One must remember that ischemic episodes, which can occur in

patients treated with positive inotropic drugs, activate ATP-sensitive K^+ channels (Noma, 1983; Kass and Freeman, 1993). This event could be very important as a modulator of the action of positive inotropic drugs.

The main goal of the present series of experiments was to investigate whether activation of ATP-sensitive K^+ channels by HOE 234 changes the positive inotropic effects of isoprenaline, digoxin, amrinone, milrinone and pimobendan. To solve this problem both the force (dF) and the velocity of myocardial contraction (dF/dt) were analysed. The latter parameter gives us useful information concerning the mechanism of action of drugs that enhance contractility.

In the present study activation of ATP-sensitive K^+ channels by HOE 234 modulated the positive inotropic action of the drugs. So, the effects of isoprenaline were strongly depressed but significantly increased for digoxin and milrinone. There was no significant influence of pretreatment with HOE 234 on the positive inotropic effects of amrinone and pimobendan. It is worth stressing that a higher concentration of HOE 234, which produced a stronger direct negative inotropic action, did not induce a stronger modulation of cardiotonic drug activity.

What is more, glibenclamide, a selective blocking agent of ATP-sensitive K^+ channels in heart muscle, prevented the modulatory action of HOE 234. This means that the above-mentioned changes in the positive inotropic activity of cardiotonic drugs are really due to the activation of ATP-sensitive K^+ channels.

It has already been found that the β-adrenoceptor signalling pathway is impaired in the ischaemic zone in the heart because of defective coupling of these receptors to G_S protein and loss of adenylate cyclase activity (Homcy, 1991). Furthermore, it is known that such ischaemic conditions lead to activation of ATP-sensitive K^+ channels. As this study has shown, activation of these channels strongly depresses the inotropic action of isoprenaline and probably is responsible for the disturbances of the β-adrenoceptor signalling pathway in the ischaemic myocardium. The fact that HOE 234 did not modify the action of amrinone (inhibitor of cAMP degradation) suggests that a defective coupling to G_S protein occurs rather than a loss of adenylate cyclase activity. It was noted that the inhibitory effect of HOE 234 on isoprenaline-induced increases in the rate of force rise was reversible. Such a phenomenon did not occur in the case of the amplitude of contraction. This difference suggests the existence of separate mechanisms involved in the genesis of these parameters.

In contrast to isoprenaline, the activation of ATP-sensitive K^+ channels with HOE 234 strongly augmented the positive inotropic effects of digoxin and milrinone. Cardiac glycosides increase the force of contraction of cardiac muscle by inhibition of Na^+,K^+-ATP-ase. Recent papers have reported that cardiac glycosides directly activate cardiac sarcoplasmatic reticulum channels (McGarry and Williams, 1993). Moreover, it has been previously established that ischaemic conditions increase the cardiotoxicity of digoxin (Kober et al., 1994). Our results suggest a possible role of ATP-sensitive K^+ activation as a cause of that phenomenon. It was noted that pretreatment with HOE 234 significantly enhanced the E_{max} of digoxin for dF but not for dF/dt. The E_{max} of milrinone for both parameters (dF and dF/dt) was significantly greater after activation of ATP-sensitive K^+ channels, as was the pD_2, which was enhanced 5.6-fold. There is a common point in the action of milrinone and digoxin, which could be the explanation for the similar reaction of these drugs after pretreatment with HOE 234. Namely, it has been reported that activation of sarcoplasmatic reticulum channels probably contributes to the positive inotropic action of milrinone, similarly to digoxin, as was mentioned above (Holmberg and Williams, 1991).

Positive inotropic effects induced by amrinone and pimobendan were not significantly changed after HOE 234 pretreatment. Comparing the results presented in this paper to those of current clinical trials, one can note that the cardiotonic drugs which induce a high mortality rate in clinical studies were shown to potentiate the positive inotropic action after activation of ATP-sensitive K^+ channels (digoxin, milrinone). The interaction between isoprenaline and HOE 234 suggests that the blunted cardiac β-adrenergic responsiveness in heart failure might result from the activation of ATP-sensitive K^+ channels.

In conclusion, this work has demonstrated that activation of ATP-sensitive K^+ channels is a very important event which can dramaticaly change the cardiac muscle response to positive inotropic drugs. This fact is worth taking into account in future clinical trials.

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