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Effect of Nonconventional β-adrenoceptor Agonist CGP 12177 on L-type Calcium Current and Contraction Force in Human Myocardium

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Summary. The aim of this study was to evaluate the effect of CGP 12177, a nonconventional partial agonist of β-adrenoceptors, on the L-type Ca^{2+} current (I_{Ca}) in whole-cell patch-clamped atrial myocytes and the contraction force of the atrial trabeculae from failing human heart. It was established that CGP 12177 stimulated I_{Ca} in a dose-dependent manner and the maximal effect was obtained with a 1-mmol/l concentration. In 14 atrial cells, I_{Ca} increased from 309.0±49.4 pA at control to 549.5±95.9 pA at 1-mmol/l CGP 12177 (n=14) ($p<0.05$). This represented an increase in Ca^{2+}-current density (I_{Ca}/membrane capacitance) from 2.38±0.26 pA/pF to 4.07±0.54 pA/pF (n=14). The concentration of agonist required to produce 50 percent of the maximal increase of I_{Ca} (EC_{50}) was 0.016±0.007 μmol/l (n=14). I_{Ca} density under the influence of 1-mmol/l isoprenaline was 6.78±1.07 pA/pF (n=5), i.e. more than twofold that documented with stimulation by CGP12177 ($p<0.05$). These data confirm that CGP12177 is a partial β-adrenoceptors agonist. The concentration of CGP 12177 that induced the maximal increase of I_{Ca}, i.e. 1 μmol/l, evoked an increase of contraction force 111.66±4.34 percent (n=6) as compared with its level at control conditions. Our data show that the nonconventional partial agonist CGP 12177 increases the L-type Ca^{2+} current in isolated atrial cells and the contraction force of atrial trabeculae from failing human heart. Finally, we provide a discussion on to which type of b-adrenoceptors the effect of CGP 12177 on the L-type Ca^{2+} current and contraction force might be attributed.

Introduction

The nonconventional partial agonist CGP 12177 was initially described as a high-affinity antagonist of β_1- and β_2-adrenoceptors [1, 2, 3, 4]. Later it was demonstrated that, at higher concentrations, this compound acts as an agonist activating β_3- and putative β_4-adrenoceptors [5, 6]. The existence of a putative β_4-adrenoceptor (previously called 'third β-adrenoceptor') was suggested some 20 years ago when cardiostimulant effects were observed in response to CGP 12177 and other nonconventional partial agonists [3, 7]. The putative β_4-adrenoceptors have been found in the heart of all mammalian species including mouse, rat, guinea pig, cat, ferret, and humans [5, 7, 8, 9, 10]. The cardiostimulant effects of CGP 12177 are unaffected by propranolol and are blocked, with moderate affinity, by bupranolol, a nonselective blocker of β_1-, β_2-, β_3- and β_4- adrenoceptors [7], and CGP 20712A, a selective blocker of β_1-adrenoceptors [11]. There are controversal data, however, about the effects of the nonconventional agonist CGP 12177 in human heart. As reported by Gauthier et al., at higher concentrations CGP 12177, BRL 37344, SR 58611, and CL 316243 produce a negative inotropic response through β_3-adrenoceptor in isolated preparations of human ventricle obtained from endomyocardial biopsies in cardiac transplant patients or during open heart surgery [6, 12]. Responses to BRL 37344 were inhibited by pertussis toxin treatment suggesting that the receptors are coupled to G_i protein. Kaumann and Molenaar [5] established that CGP 12177 increased contractile force in isolated human right atrial and ventricular trabeculae from failing human hearts. Furthermore, CGP 12177 increases the cyclic AMP level, activity of cyclic AMP-dependent protein kinase; and its effects are potentiated by the phosphodiesterase inhibitor IBMX in both rat [4] and human [13] atria. This suggests putative β_4-adrenoceptors be coupled positively to the G_s protein-adenylyl cyclase system [5, 13]. Interestingly, in recent studies it was established that CGP 12177 produced inotropic and chronotropic responses in β_3-adrenoceptors-knockout mice (β_3KO), similar to those observed in the wild mice (WT) [8]. It was also demonstrated that the ventricular putative β_4-adrenoceptors are expressed with the same density and possess the same affinity for [3H]CGP 12177 in WT and β_3KO [8]. These findings demonstrate that the cardiostimulant effects of CGP 12177 are mediated through the putative β_4-adrenoceptors which are not a splice variant of the β_3-adrenoceptors.

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More recently, Konkar et al. demonstrated that the activation of adenylyl cyclase by CGP 12177 and LY 362884 in cells expressing rat or human β_1-adrenoceptors was mediated by the β_1-adrenoceptor and this response was resistent to blockade by β-adrenoceptor antagonist propranolol and CGP 20712A, in contrast to activation by catecholamines [14]. They suggest that these nonconventional agonists interact with distinct active conformation or state of the β_1-adrenoceptors. Kaumann et al. have demonstrated cardiostimulant effects of CGP 12177 in atria from β_2-adrenoceptor knockout and wild-type mice but these effects were absent in β_1/β_2-adrenoceptor double knockout mice [15]. These findings also indicated that the presence of β_1-adrenoceptors is obligatory for the cardiostimulant effects of CGP 12177 and that an atypical state of the β_1-adrenoceptor contributes to the mediation of the cardiostimulant effects. These results demonstrated that the cardiostimulant effects of CGP 12177, previously attributed to activation of the putative β_4-adrenoceptor, actually occur through its interaction with β_1-adrenoceptor. In contrast, our studies [16] showed that CGP 12177 had no effect on I_{Ca} in rat ventricular myocytes, in which β_1-adrenoceptor was predominant, and completely blocked the effect of β_1- and β_2-adrenoceptors agonist isoprenaline, as expected from an antagonist of these adrenoceptors. On the other hand, it is not clear in which extent the experimental results and their interpretation obtained on mice heart are suitable for human heart. Also, it is interesting to establish the action of the nonconventional agonist CGP 12177 in failing human heart because it is well known that failing human hearts lose β_1-adrenoceptors [17]. All data on the CGP 12177 action in human heart tissues reported by other authors had been obtained from contraction-measurement experiments without recording the calcium current. Since the L-type calcium current (I_{Ca}) is a major determinant of myocardial contraction force, in the present study we demonstrate the action of the nonconventional partial agonist CGP 12177 on I_{Ca} in human atrial cells comparing it with the effect of CGP 12177 on contraction force in human atrial trabeculae.

Materials and Methods

Atrial cells and trabeculae were isolated from human atrial pieces obtained from patients undergoing coronary bypass surgery in Kaunas Medical University Clinic. The atrial cells were enzymatically dispersed by a combination of collagenase, protease, and elastase as described by Jurevicius et al. [18]. These investigations were approved by the institutional Ethics Committee. The following drugs were prescribed for the patients: calcium channel blockers, β-adrenoceptor blockers, angiotensin-converting enzyme inhibitors, nitrates.

Upon excision the atrial pieces were immediately placed in oxygenated cold (10-12 °C) cardioplegic St.Thomas solution containing (in mmol/l): 110 NaCl, 5.4 KCl, 16 $MgCl_2$, 1.2 $CaCl_2$ 0.5 D-glucose, 10 Hepes, pH adjusted to 7.4 with NaOH and transported to laboratory. For the preparation of human atrial myocytes, the ionic composition of modified Ca^{2+}-free, oxygenated (95% O_2, 5% CO_2) Tyrode solution was (mmol/l): 35 NaCl, 16 Na_2HPO_4, 25 $NaHCO_3$, 4.7 KCl, 1.2 KH_2PO_4, 134 sucrose, 10 D-glucose, 10 Hepes, pH 7.2 at room temperature (18-22° C). The dissociation medium was composed of a Ca^{2+}-free Tyrode solution containing 5 mg/ml BSA to which collagenase type 2 (200 U/ml, Worthington USA.), protease type XXIV (6 U/ml, Sigma, USA.) and elastase (13 U/ml, Boehringer Meinheim) were added. All steps of cell isolation were carried out at 37 °C with continuous gassing with 95% O_2 and 5% CO_2. Storage solution was (mmol/l): 120 NaCl, 5.4 KCl, 0.9 $CaCl_2$, 5 $MgCl_2$, 20 D-glucose, 10 Hepes, 5 sodium pyruvatic acid, pH 7.4, at room temperature (18-22 °C).

For electrophysiology, the control external solution contained (in mmol/l): 127 NaCl, 20 CsCl, 4 $NaHCO_3$, 0.8 NaH_2PO_4, 1.8 $MgCl_2$, 1.8 $CaCl_2$, 5 sodium pyruvatic acid, 5 D-glucose, 10 Hepes, pH 7.4 adjusted with NaOH. Patch electrodes (0.7-1.2 MW) were filled with a control internal solution which contained (mmol/l): 140 CsCl, 0.062 $CaCl_2$, 4 $MgCl_2$, 5 EGTA, 10 Hepes, 5 creatine phosphate disodium salt, 3.1 Na_2ATP, 0.42 Na_2GTP, pH 7.3 adjusted with CsOH.

Tetrodotoxin was from Latoxan (Rosans, France). Nonconventional agonist CGP 12177 (4-[3-t-butylamino-2-hydroxypropoxy]benzimidazol-2-one) was a gift from CIBA-Geigy (Basel, Switzerland). All other drugs were from Sigma (USA).

Reccording L-type calcium current (I_{Ca}) in human atrial myocytes. The whole-cell configuration of the patch-clamp technique was used to record the L-type calcium current in human atrial myocytes. In the routine protocol, the cells were depolarized every 8 s from a holding potential of -80 mV to 0 mV for 400 ms, after a brief prepulse (50 ms) to -50 mV. The prepulse and application of tetrodotoxin (1 μmol/l) were used to eliminate the fast sodium current. K^+ currents were blocked by replacing all K^+ ions with intracellular and extracellular Cs^+. Voltage-clamp protocols were generated and currents recorded by means of the patch-clamp amplifier VP-500 (Biologic, France). Computer software Visual-Patch v.1.30 was used to control all experimental parameters, cell stimulation, and current recording. Recordings were low-pass filtered at 2 kHz and data were stored on the hard disk of an IBM-compatible computer.

Control or drug-containing solutions were applied onto the exterior of the cell – by placing the cell at the opening of 300-μm inner diameter capillary tubings – flowing at a rate of ≈ 50 μl min^{-1} (Skeberdis et al., 1997). Changes in external solutions were automatically achieved by a rapid solution changer (RSC200, Bio-logic, France). All experiments were done at room temperature (18-22 °C), and the temperature did not change by more than 1 °C in a given experiment. The peak of I_{Ca} was measured with respect to the steady-state current at the end of the 400-ms pulse.

Recording contraction force in human atrial trabeculae. The isolated trabeculae were placed in an experimental chamber and superfused, at a flow rate 5 ml/min, with oxygenated (100% O_2) Tyrode's solution (pO_2 580-600 mmHg) composed of as follows (in mmol/l): NaCl 137, KCl 5.4, $CaCl_2$ 1.8, $MgCl_2$ 0.9, Hepes 10, at 36±0.5 °C, pH 7.4. The trabeculae were stimulated through two silver chloride electrodes with

2-3-fold-threshold pulses of 5-10-ms duration following at 1.5 Hz. Isometric contraction was recorded using a linear force-displacement transducer. The contraction force was expressed in percent of control level. The data presented are expressed as M±S.E.M. The significance was assessed using Student's test and results were considered significant at $p<0.05$.

Results

In the first set of experiments we explored the effects of cumulative concentrations of CGP 12177 on the L-type Ca^{2+} current in human right atrial myocytes.

CGP 12177 produced a dose-dependent increase in I_{Ca}, the maximal effect being obtained with a 1-μmol/l concentration (Fig. 1). In 14 atrial cells, I_{Ca} increased from 309.0±49.4 pA at control to 549.5±95.9 pA at 1 μmol/l CGP 12177 (n=14) ($p<0.05$). This represented an increase in Ca^{2+}current density (I_{Ca}/membrane capacitance) from 2.38±0.26 to 4.07±0.54 pA/pF (n=14). The cumulative dose-response curve for the effect of CGP 12177 (0.001-1 μmol/l) on I_{Ca} is presented in Figure 2. For each concentration of the agonist, a percentage increase in I_{Ca} amplitude with respect to its basal level in the absence of the agonist was calculated: (percent increase in I_{Ca}) = 100[(I_{Ca} with agonist) - (basal I_{Ca})]/(basal I_{Ca}). The dependence of I_{Ca} upon CGP 12177 was described well enough by the Michaelis equation (the continuous line in Fig.2). Thus, CGP 12177 increased I_{Ca} with an EC_{50} value (i.e. the concentration of agonist required to produce 50 percent of the maximal increase in I_{Ca}) of 0.016±0.007 μmol/l and E_{max} = 61.7±5.8 percent (n=14).

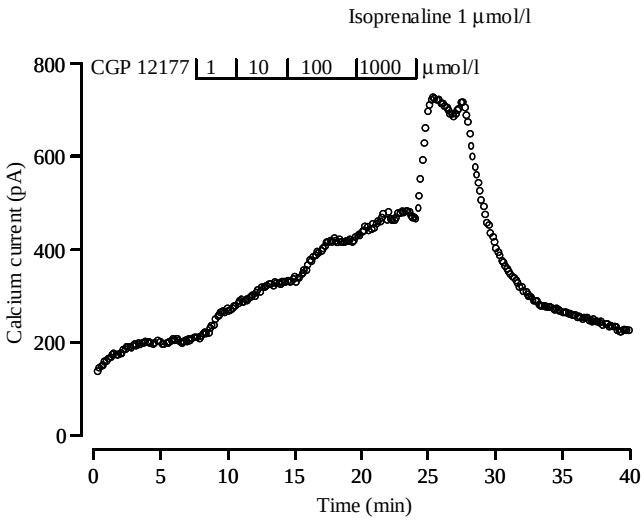

Fig. 1. **Time course of the effect of CGP 12177 and isoprenaline on L-type Ca^{2+} current in human atrial myocyte.** A human atrial myocyte was initially superfused with control external solution and internally dialyzed with control intracellular solution. During the periods indicated by the horizontal lines the cell was successively exposed to concentrations of CGP 12177 ranging from 1 nmol/l to 1 bmol/l of CGP 12177 and to 1 μmol/l of isoprenaline.

The maximal effect of CGP12177 was compared with the action of isoprenaline (Fig.1), a nonselective β-adrenoceptor agonist, on the L-type Ca^{2+} current. The I_{Ca} density under the effect of 1 mmol/l of isoprenaline was 6.78±1.07 pA/pF (n=5), i.e. more than two-fold that documented with stimulation by CGP12177 ($p<0.05$) (Fig. 3). These data confirm that CGP12177 is a partial β-adrenoceptors agonist.

In another set of experiments we investigated the action of 1μmol/l CGP 12177 (a maximally effective concentration) on the contraction force in atrial trabeculae from human fail-

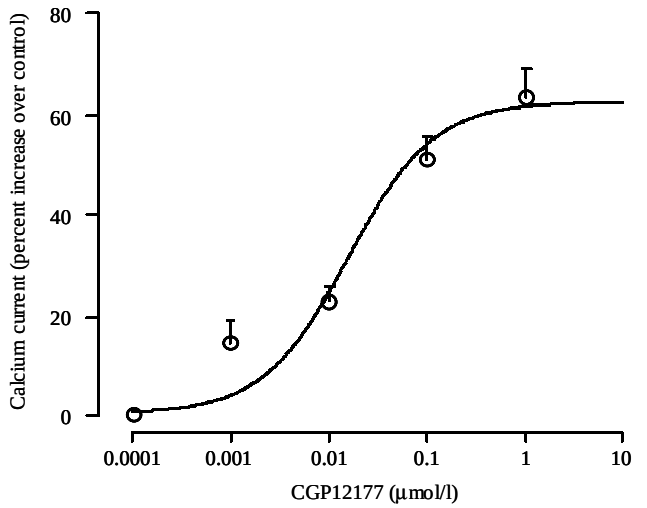

Fig. 2. **Concentration-response curve for the stimulating effect of CGP 12177 on L-type Ca^{2+} current in human atrial cells.** EC_{50} and E_{max} values for the effect of CGP 12177 on I_{Ca} were 0.016±0.007 μmol/l and 61.7±5.8 percent (n=14) respectively. The open circles show the mean and the vertical lines the s.e.m. The dependence of I_{Ca} upon CGP 12177 was described well enough by the equation of Michaelis (the continuous line). The data are presented as percentage of the control values.

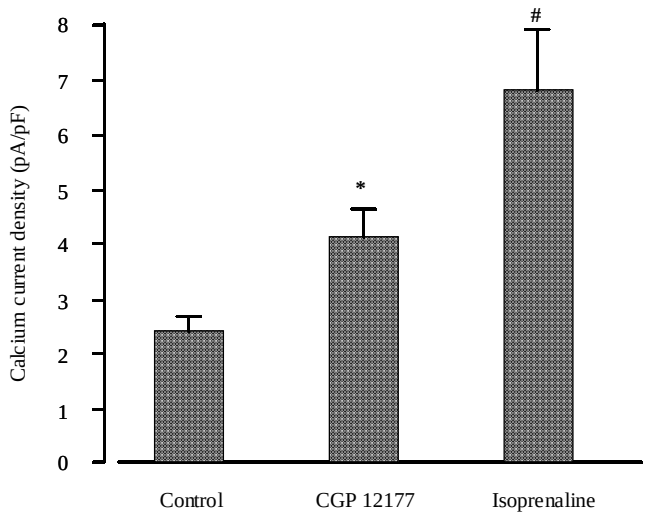

Fig. 3. **Effects of 1-μmol/l CGP 12177 and 1-mmol/l isoprenaline on L-type Ca^{2+} current density (n=5).** * indicates significant difference as compared with control ($p<0.05$), # - as compared with CGP 12177 ($p<0.05$).

ing hearts. Figure 4 demonstrates the original traces from a typical experiment showing the contraction of trabecula in control (*a* and *d*), after 5 min and after 15 min in 1μmol/l CGP 12177 (*b* and *c*, respectively). Upon these conditions, CGP 12177 evoked an increase of contraction force to 123.1±7.4 percent (n=6) and 111.7±4.3 percent (n=6) after 5 min and 15 min, respectively, as compared with its level in control (Fig.4).

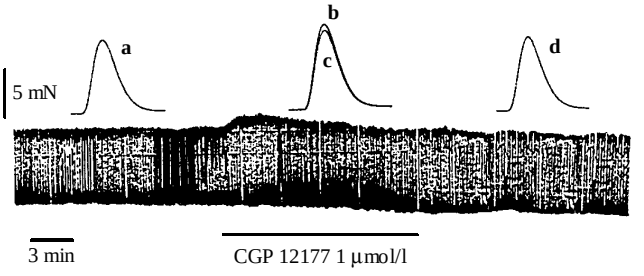

Fig. 4. **Time course of the effect of CGP 12177 on contraction force of human atrial trabeculae.** The upper traces show contraction at baseline (a and d), after 5-min (b) and 15-min (c) perfusion with 1-μmol/l CGP 12177. In the lower part of the figure the kinetics of contraction force is shown at low recording rate.

Discussion

Our experiments performed on atrial myocytes isolated from human failing hearts demonstrated that the nonconventional agonist CGP 12177 increased the L-type Ca^{2+} current. We also determined that CGP 12177 at concentration which induced the maximal increase of I_{Ca} increased the contraction force in isolated human atrial trabeculae.

The atrial contraction force is augmented by the nonconventional partial agonists CGP 12177 and cyanopindolol or iodocyanopindolol in the rat and cat [2, 11] as well as in man [7, 13]. It was established that the cardiostimulant effects of CGP 12177 are unaffected by propranolol and are blocked by bupranolol [7]. CGP 12177 and cyanopindolol (in the presence of 3-isobutyl-1methyl-xantine) also increase the contraction force of human ventricular trabeculae obtained from failing hearts [5, 19]. The positive inotropic and lusitropic effects mediated by CGP 12177 are in agreement with those found in ferret ventricle [9]. Viard et al. demonstrated that CGP 12177 stimulated the L-type Ca^{2+} current in single myocytes isolated from rat portal vein. The stimulated Ca^{2+} current was blocked by SR 59230A, a β_3-adrenoceptor blocker and by cyclic AMP-dependent protein kinase A inhibitors [20]. It was postulated that nonconventional partial agonists caused cardiostimulation through as yet uncloned putative β_4-adrenoceptors [3, 5, 10, 19]. Also, there are controversial data on the effects mediated by CGP 12177 in human heart. Gauthier et al. have reported that CGP 12177, BRL 37344, and SR 58611 mediate the shortening of the action potentials and the negative inotropic effect in isolated preparations of human ventricle obtained from endomyocardial biopsies during open heart surgery [6, 12]. They postulated that these compounds caused cardiodepressant effects through human ventricular β_3-adrenoceptors. The negative inotropic effect was associated with a parallel increase in the production of NO and intracellular cGMP and was blunted in pertussis toxin-pretreated tissues. These results suggest the involvement of G_i proteins in the β_3-adrenoceptor signaling pathway. However, studies in human and rat myocardium show that CGP 12177 increases the cyclic AMP level and causes higher activity of cyclic AMP-dependent protein kinase, and these effects are potentiated by the phosphodiesterase inhibitor IBMX [4, 5]. This suggests a role of G_s protein in coupling the putative β_4-adrenoceptor to adenylate cyclase. So, the putative β_4-adrenoceptors are clearly distinct from the β_3-adrenoceptors in human heart. The cardiostimulant effect of CGP 12177 through the putative β_4-adrenoceptors was confirmed also in the recent studies of β_3-adrenoceptor knockout paradigm and in wild mice. It was established that CGP 12177 caused similar cardiostimulant effect in atria from both β_3-KO and WT mice [8].

More recently it was demonstrated that the presence of β_1-adrenoceptors in Chinese hamster ovary and HEK 293 cells expressing rat or human β_1-adrenoceptors [14] and in atria from double β_1- and β_2-adrenoceptor knockout transgenic mice [15] is obligatory for the cardiostimulant effect of CGP 12177 and that an atypical state of β_1-adrenoceptors contributes to the mediation of the cardiostimulant effect of CGP 12177. Nevertheless, it is not evident if these interpretations might be applied to human heart. Our studies [16] have not either shown any stimulatory effect of CGP 12177 on the rat ventricular myocytes possessing predominantly β_1-adrenoceptors. The majority of the studies are done on human atria or ventricular preparations obtained during heart surgery from the patients with heart failure, and it is well known that failing human hearts lose β_1-adrenoceptors [17]. Despite the chronic use of the β-adrenoceptor blocking drugs, usually selective for β_1-adrenoceptor, by such patients, in our and other studies [5, 19], the effect of CGP 12177 would be attributed to the cardiac putative β_4-adrenoceptor. The reasons for differences between the data and the interpretations on the effects mediated by the nonconventional agonist CGP 12177 in human heart are not obvious at present. Our results require verification against those in the myocardium from normal hearts of donors not treated with drugs, such as β-adrenoceptor blockers, ACE inhibitors and others. It is reasonable, however, to suggest that the nonconventional partial agonist CGP 12177 increases the L-type Ca^{2+} current in isolated atrium cells and the contraction force in atrial trabeculae from failing human heart through the β_4-adrenoceptors.

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