Activation of Calcium Currents in Cardiac Myocytes by Empty β-Adrenoceptors

Thorsten Mewes, MD; Silke Dutz, MD; Ursula Ravens, MD; Karl H. Jakobs, MD

Background. The cardiac calcium channel is known to be modulated by catecholamines via β-adrenoceptors acting through intermediary GTP-binding regulatory proteins (G proteins). In biochemical studies on isolated membranes and reconstituted systems, it has been demonstrated that various G protein–coupled receptors, including β-adrenoceptors, can activate G proteins and also intracellular second messengers like cyclic AMP (cAMP) even in the absence of an agonist and that antagonists can block this empty receptor action. We examined electrophysiologically whether agonist-free β-adrenoceptors can modulate L-type calcium currents (I_{Ca}) in intact cardiac myocytes.

Methods and Results. Cardiomyocytes were isolated from ventricles of guinea pig and human hearts and from human right atrial appendage. The patch-clamp technique was applied in the single electrode mode to measure whole-cell I_{Ca}. Modulation of calcium currents by β-adrenoceptor antagonists, without addition of an agonist, was studied in the absence and presence of the direct adenyl cyclase activator forskolin and the cAMP analog adenosine cyclic 3',5'-monophosphorothioate (Sp-cAMPS). In the presence of forskolin (0.5 μmol/L), an agent known to sensitize the adenyl cyclase signal transduction system for receptor regulation, addition of the β_{2}-selective antagonist atenolol and the nonselective antagonist propranolol (but not of the β_{1}-selective antagonist ICI 118,551) caused a marked reduction of I_{Ca} in a concentration-dependent and stereoselective manner. The inhibitory effect of atenolol was reversible after washing out and was found to be half maximal and maximal (50% reduction) at about 50 and 300 nmol/L, respectively. In the absence of forskolin, inhibition of I_{Ca} by atenolol was markedly less (18% at 10 μmol/L atenolol). Finally, in contrast to forskolin-stimulated currents, atenolol (1 μmol/L) did not reduce calcium currents activated by the protein kinase A activator Sp-cAMPS (0.1 mmol/L), causing by itself a similar increase in calcium currents as forskolin.

Conclusions. In isolated guinea pig and human cardiomyocytes, agonist-free β-adrenoceptors are functionally active and can stimulate L-type calcium currents, an effect blocked by receptor-specific antagonists. (Circulation. 1993;88:2916-2922.)

Key Words • cardiomyocytes • L-type calcium currents • β-adrenoceptors • β-blockers

According to classic models of drug-receptor interactions, agonist binding to receptors triggers the cellular response, whereas binding of antagonists merely blocks the signal transduction cascade initiated by agonist-receptor interaction, implicating that agonist-unliganded empty receptors are silent. For various receptors coupled to GTP-binding regulatory proteins (G proteins) such as δ-opioid, A_{2} adenosine and muscarinic acetylcholine receptors as well as β-adrenoceptors, biochemical studies in isolated membranes or reconstituted systems demonstrated that agonist-free receptors can activate the respective G proteins even in the absence of agonists.1-6 This empty receptor action was inhibited by receptor-specific antagonists. However, the physiological role of empty receptor activity in intact cells remains elusive.7,8

The cardiac L-type calcium channel plays a fundamental role in cardiac excitation and contraction and is known to be under major control by the sympathetic nervous system acting via β-adrenoceptors.9 Activation of these receptors activates the stimulatory G protein G_s, resulting in increased formation of cyclic AMP (cAMP) by the adenyl cyclase. This leads to activation of protein kinase A and, in cardiac myocytes, to phosphorylation and increased availability of calcium channels.10-12 The aforementioned biochemical data prompted us to study whether β-adrenoceptors can regulate cardiac calcium currents even in the absence of an agonist. Therefore, we measured the effects of various β-adrenoceptor antagonists on whole-cell I_{Ca}, using the patch-clamp technique, in isolated guinea pig and human cardiomyocytes. Calcium currents were measured in the absence and presence of forskolin, an agent known to directly activate adenyl cyclase and to sensitize this signal transduction system for receptor regulation,13 as well as of the cAMP analog adenosine cyclic 3',5'-monophosphorothioate (Sp-cAMPS), an activator of protein kinase A.14 We report here that β-adrenoceptor antagonists can markedly reduce calcium cardiac currents even in the absence of an agonist and that this inhibition is potentiated by forskolin and prevented by Sp-cAMPS.

Methods
Preparation of Cardiomyocytes
Guinea pig ventricular myocytes were enzymatically isolated as described previously15,16 and were thoroughly

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From the Institut für Pharmakologie, Universität GH Essen, Essen, Germany.
Correspondence to Dr Jakobs, Institut für Pharmakologie, Universität GH Essen, Hufelandstrasse 55, D-4300 Essen, FRG.
washed to remove debris and cells other than myocytes. Human atrial myocytes were prepared as follows: Small tissue pieces excised from right atrial appendage during coronary bypass surgery were incubated in nominally calcium-free solution for 15 minutes. Composition of calcium-free solution was (mmol/L) KCl 10, NaCl 100, KH₂PO₄ 1.2, MgSO₄ 5, taurine 50, glucose 21, and MOPS (3-[N-morpholino]propanesulfonic acid) 5, pH 7.0. The tissue pieces were gently stirred for 45 minutes at 37°C in the calcium-free solution containing 0.66 mg/mL collagenase IA (Sigma) and 0.33 mg/mL protease XXIV (Sigma). The enzyme solution was discarded, and the remaining tissue was incubated for another 30 to 90 minutes at 37°C, depending on the cell yield, with 0.66 mg/mL collagenase IA in calcium-free solution. Every 10 minutes, the cells were separated by filtration through a Teflon grid and stored in KB medium for at least 1 hour before investigation. Only myocytes from the last three treatments were used for analysis.

Human ventricular myocytes were prepared according to the following procedure: Tissue chunks from donor hearts that could not be transplanted for technical reasons were cut into small pieces, washed five times in calcium-free solution, and stirred for 40 minutes at 37°C in calcium-free solution containing 1.5 mg/mL collagenase I (Sigma), 1 mg/mL trypsin III (Sigma), and 10 mg/mL bovine serum albumin 20/21 (Behring). For some preparations, 0.1 mg/mL DNase I (Boehringer Mannheim) was added. After discarding the enzyme solution, tissue pieces were incubated with albumin (1 mg/mL calcium-free solution) alone for 10 minutes. During the second enzymatic step, the tissue pieces were exposed to 0.5 mg/mL collagenase I (Sigma) in calcium-free solution for 20 to 150 minutes, depending on the cell yield. After filtration every 10 minutes, the cell suspension was placed in a plastic tube, calcium-free solution was added, and the myocytes were allowed to settle under gravity for 15 minutes. Finally, 0.5 mmol/L CaCl₂ was added, and the calcium concentration was increased in several increments to 1.8 mmol/L. The cells were stored at 4°C for 1 hour before examination.

**Measurement of Calcium Currents**

Membrane currents were recorded in the whole-cell single electrode voltage-clamp mode using an Axopatch 200 patch-clamp amplifier (Axon Instruments, Foster City, Calif.). Patch pipettes were pulled from glass capillaries to a resistance of 1.5 to 2.5 MΩ. Series resistance (3.5 to 10 MΩ) was uncompensated for by at least 75%. The capacitance of the membrane was calculated from the steady-state current in response to a ramp (5 mV/5 milliseconds) from −40 mV. The membrane capacitance was completely compensated for up to 100 pF.

The cell membrane was clamped to a holding potential of −40 mV to inactivate Na⁺ and T-type calcium currents. Potassium currents were suppressed by substituting K⁺ by Cs⁺, both in the modified Tyrode and the pipette solution. L-type calcium current was elicited by a clamp step from a holding potential of −40 mV to 0 mV for 450 milliseconds at a frequency of 0.1 Hz. Iₛ was measured as the difference between the inward peak and the current level at the end of the test pulse. Current-voltage relations were determined by clamping the myocytes from the holding potential of −40 mV to different test potentials between −30 mV and +40 mV for 450 milliseconds. Activation curves were achieved by dividing the currents measured at the respective potentials by the ion-driving force, i.e., the difference between measured potential and reversal potential. For measurement of potential dependency of current inactivation, a holding potential of −80 mV was chosen. In a first step, cells were depolarized to potentials between −70 mV and +10 mV for 450 milliseconds. After a 3-millisecond interpulse to −40 mV, the myocytes were clamped to 0 mV, and the remaining current was recorded.

**Drugs and Solutions**

The pipette solution contained (mmol/L) CsCl 140, EGTA (ethylene glycol-bis[β-aminoethyl ether] N,N,N',N'-tetraacetic acid) 10, MgCl₂ 4, HEPES (N-[2-hydroxyethyl]piperazine-N'-[2-ethanesulfonic acid]) 10, and Na₂ATP 3 (pH 7.3). Composition of the modified Tyrode solution was (mmol/L) CsCl 5.4, CaCl₂ 1.8, NaCl 137, HEPES 10, MgCl₂ 1.25, and glucose 10 (pH 7.4 adjusted with CsOH). The temperature of the perfusate was 21 to 23°C (room temperature). Forskolin (gift of Hoechst AG, Frankfurt, Germany) was dissolved in dimethyl sulfoxide at a 1 mmol/L stock solution, which was diluted with the modified Tyrode solution to the final concentration. (-)-Atenolol and (-)-propranolol (both from Sigma), (-)-propranolol, (+)-propranolol, and (-)-ICI 118,551 (gifts of ICI Pharma, Plankstadt, Germany) were dissolved in Tyrode solution. Sp-cAMPs (from BioLog Life Science Institute, Bremen, Germany) was dissolved in the pipette solution.

**Statistical Analysis**

Averaged data are presented as mean±SEM. ANOVA, Bonferroni test, or Mann-Whitney test was used where appropriate (Instat V.1.14, GraphPad, San Diego, Calif). Differences were considered significant when P<.05.

**Results**

To make the myocytes more sensitive to receptor influences on calcium currents, the cells were superfused in the cell bath with the modified Tyrode solution supplemented with 0.5 μmol/L forskolin. At this concentration, forskolin increased Iₛ about twofold in all three types of myocytes (see Fig 1b for a human atrial myocyte) and, as recently demonstrated, increases cAMP levels in guinea pig ventricular myocytes to a similar extent. Fig 1 illustrates the effects of the β₁-selective antagonist (-)-atenolol and the nonsensitive antagonist (-)-propranolol on L-type calcium currents in the different myocytes. Typical recordings for human and guinea pig cardiac myocytes pretreated with forskolin and after addition of the antagonists are shown in Fig 1a. The time course of the effects of forskolin (0.5 μmol/L) and (-)-atenolol (1 μmol/L) in a human atrial cell is depicted in Fig 1b. With forskolin present throughout, both (-)-atenolol (0.6 or 1 μmol/L) and (-)-propranolol (1 μmol/L) distinctly reduced calcium currents in the three myocyte preparations within a few minutes (maximum after about 5 minutes). The inhibi-
Fig 1. Influence of atenolol and propranolol on L-type calcium currents in isolated guinea pig and human cardiomyocytes. a, Typical recordings from a guinea pig ventricular, human atrial, and human ventricular myocyte. Cells were kept at a holding potential of −40 mV to inactivate Na+ currents and were depolarized to 0 mV for 450 milliseconds to activate I\textsubscript{Ca}. Shown are control currents (0.5 μmol/L forskolin alone) and currents 5 minutes after the addition of (±)-atenolol and (±)-propranolol at the indicated concentrations. Horizontal bars represent 100 milliseconds. b, Time course of changes in L-type calcium currents in a human atrial myocyte after addition of forskolin (0.5 μmol/L) and (±)-atenolol (1 μmol/L). Wash, superfusion with a solution containing forskolin alone. c, Time course of changes in L-type calcium currents in a human ventricular myocyte after addition of forskolin (0.5 μmol/L) and three consecutive exposures to (±)-atenolol (10 μmol/L). The dotted line represents the extrapolated rundown of I\textsubscript{Ca} in this cell. Note the reversal of effects during wash of (±)-atenolol.

tory effect of the hydrophilic antagonist (±)-atenolol was readily reversible by washing out. Furthermore, in long-lasting recordings the effect of (±)-atenolol could be repeated several times (Fig 1c), indicating that the antagonist exhibits an activity even after displacement and washout of a possibly receptor-bound agonist, i.e., when the receptor is reliably agonist free. Because of limited availability of human cardiac tissue, most of the experiments were performed with guinea pig ventricular myocytes. In these cells, in contrast to (±)-atenolol and (±)-propranolol, the β\textsubscript{2}-selective antagonist (±)-ICI 118,551, used at a concentration at which most of the β\textsubscript{2}-adrenoceptors should be occupied (50 nmol/L), did not significantly reduce forskolin-stimulated calcium currents (88.6±10.5% of predrug control, n=9).

To examine whether the inhibitory effects of the antagonists on forskolin-stimulated I\textsubscript{Ca} are caused by binding to β-adrenoceptors, stereoselectivity and concentration response studies were performed. In guinea pig myocytes, addition of 1 μmol/L (−)-propranolol reduced I\textsubscript{Ca} to 60.9±4.7% of the predrug control (n=12) (Fig 2a), which was significantly different (P<.001) from the spontaneous rundown in time-matched control cells (n=16). After a similar exposure time (5 minutes) to 1 μmol/L (−)-propranolol, forskolin-stimulated I\textsubscript{Ca} was decreased to only 84.0±9.3% of predrug control (n=4, not statistically different from the time-matched control cells) (Fig 2b).

Concentration dependency of the inhibitory action of the antagonists was determined with (±)-atenolol. For this, calcium currents in guinea pig cardiomyocytes exposed to forskolin (0.5 μmol/L) were first recorded for 4 minutes in the absence of the antagonist to ensure stable recording conditions. Inhibition of I\textsubscript{Ca} by (±)-atenolol was determined 5 minutes after addition of the drug. Quantification of (±)-atenolol-induced inhibition of calcium currents was corrected for rundown compared with 32 control cells exposed to forskolin alone. (±)-Atenolol reduced I\textsubscript{Ca} of guinea pig ventricular myocytes in a concentration-dependent manner. Threshold inhibition (5%) was observed at 10 nmol/L and half-maximal and maximal inhibition at about 50 nmol/L and 300 nmol/L (±)-atenolol, respectively (Fig 3). Maximal inhibition of forskolin-activated calcium current by (±)-atenolol amounted to 50%. Thus, atenolol apparently completely blocked the increase in
calcium current caused by forskolin. In guinea pig myocytes not treated with forskolin, the inhibitory effect of (+)-atenolol on calcium currents was less pronounced. Additionally, a significant reduction (by 18%) of $I_{Ca}$, corrected for rundown in 13 control myocytes and forskolin, was observed only at a concentration of 10 μmol/L (+)-atenolol.

(+)-Atenolol reduced L-type calcium currents by affecting the activation of the channels. Fig 4 shows that the current-voltage relation, with 0.5 μmol/L forskolin present, exhibited a maximum when the cells were depolarized from a holding potential of -40 mV to 0 mV, which is in the same range as reported for forskolin-exposed frog ventricular myocytes. Forskolin did not alter the shape of the current-voltage plot compared with drug-free cells but enhanced the amplitude at all tested potentials (not shown). Addition of (+)-atenolol (0.1, 1, and 10 μmol/L) in combination with forskolin reduced the current amplitudes and conductances at all tested potentials. The maximum of the current-voltage plot was slightly but not significantly shifted to more negative potentials. Forskolin-treated cells had an apparent reversal potential of about +55 mV. After addition of (+)-atenolol (0.1, 1, and 10 μmol/L), the

Fig 3. Concentration-dependent inhibition of L-type calcium currents in guinea pig ventricular myocytes by atenolol. Inhibition of calcium currents was determined 5 minutes after the addition of (+)-atenolol at the indicated concentrations to cells preincubated (10 minutes) and superfused without (△) and with 0.5 μmol/L forskolin (●). Quantification of (+)-atenolol-induced inhibition of calcium currents was corrected for rundown compared with 32 control cells exposed to forskolin alone and 13 control cells without forskolin. Numbers of cells are given in brackets; error bars are SEM. $I_{Ca}$ is given on the ordinate as percentage of 32 and 13 controls in the absence and presence of forskolin, respectively.

Fig 4. Effects of atenolol on current-voltage relations and calcium channel conductance in forskolin-treated guinea pig cardiomyocytes. The cells were clamped to a holding potential of -40 mV and then depolarized to different potentials between -30 mV and +40 mV. a, Current amplitudes corrected for cell size observed in the presence of 0.5 μmol/L forskolin alone (control, ○) and 0.5 μmol/L forskolin plus 1 μmol/L (+)-atenolol (●) are plotted against the test potentials. b, Conductance of calcium currents observed in the presence of 0.5 μmol/L forskolin (control, ○) and 0.5 μmol/L forskolin plus 1 μmol/L (+)-atenolol (●). Qualitatively similar data were obtained with 0.1 and 10 μmol/L (+)-atenolol. Error bars are SEM.
reversal potential remained unchanged, indicating no influence of this agent on the ion driving force. With forskolin alone, the activation curve had a maximum conductance of about 0.10 nS/pF. Addition of (-)-atenolol reduced the conductances at all potentials by about 50%. In contrast to current activation, steady-state inactivation of guinea pig myocyte calcium currents was not influenced by the addition of (-)-atenolol to forskolin-treated myocytes (not shown).

To examine whether the antagonists reduce any increased calcium current, the cAMP analog Sp-cAMPS (100 μmol/L) was applied intracellularly via the pipette for direct activation of protein kinase A.14 I_{Ca} was of a similar magnitude as with forskolin treatment. However, in contrast to forskolin treatment, (-)-atenolol (1 μmol/L) did not significantly reduce Sp-cAMPS-activated I_{Ca} (Fig 5). After 5-minute exposure to the B-adrenoceptor blocker, calcium current was 85.8±9.7% (n=8) of predrug control compared with 93.0±6.8% in 6 time-matched control cells (internal application of Sp-cAMPS only). In 4 human atrial cells (from 2 hearts), I_{Ca} measured under similar conditions decreased to 82.0±22.7% of the preatenolol value, which was also not significantly different from the spontaneous current decline in Sp-cAMPS-exposed cells without B-adrenoceptor blocker.

Discussion

Using intact cardiomyocytes, we investigated whether empty cardiac B-adrenoceptors are silent or functionally active. Therefore, the effects of various B-adrenoceptor antagonists on cardiac calcium currents were studied in isolated cardiomyocytes from guinea pig and human ventricle and human right atrial appendage. It is demonstrated herein that in the absence of a receptor agonist application of the B1-selective antagonist atenolol, as well as of the nonselective antagonist propranolol, to the three types of cardiomyocytes effectively reduced L-type calcium currents. No significant inhibitory effect could be detected with the B2-selective antagonist ICI 118,551, used at a concentration at which most of the B2-adrenoceptors should be blocked.21 The reason for this apparent ineffectiveness may be due to the small number of B2 versus B1-adrenoceptors present in guinea pig and human heart muscle.22,24 Accordingly, a small effect of ICI 118,551 may have been undetected in the data scatter.

The following considerations support the hypothesis that the inhibitory effects of atenolol and propranolol on cardiomyocyte calcium currents are caused by an action at agonist-free, empty B-adrenoceptors: First, the measurements were performed on isolated, superfused single myocytes. Second, the inhibitory action of the hydrophilic antagonist atenolol was readily reversible by washing out, thus strongly suggesting that the effect of the antagonists is not due to competition with a possibly contaminating endogenous agonist. Third, as demonstrated with (--)- and (+)-propranolol, there was a clear stereoselectivity in the inhibitory action of the antagonists. Finally, the negative effect of the two antagonists was observed at concentrations of these blockers being specific for binding to B-adrenoceptors. Specifically, the concentrations of atenolol causing inhibition of forskolin-stimulated calcium currents are in good agreement with the known affinity of this agent for cardiac B-adrenoceptors.25 Based on all of these arguments, it is feasible to assume that cardiac B-adrenoceptors, at least the B1-adrenoceptor population, are functionally active even in the absence of an agonist and can regulate L-type channel activity, and that this empty B-adrenoceptor action is blocked by binding of receptor-specific antagonists.

In the absence of forskolin, significant reduction of calcium currents by atenolol was observed only at a concentration of 10 μmol/L and amounted to maximally 18%. Since, because of this rather small difference, the B-adrenoceptor specificity of the antagonist action could not be studied in a reliable manner, it cannot be excluded that under this condition, calcium current inhibition by atenolol (10 μmol/L) may be caused by direct interference with the L-type calcium channels. Addition of forskolin markedly increased the inhibitory efficacy of atenolol and apparently largely amplified the inhibitory potency of atenolol on calcium currents. A similar apparent forskolin requirement has been reported for cAMP-lowering effects of B-adrenoceptor antagonists in parotid acinar cells.26 At the concentration used in our studies (0.5 μmol/L), forskolin does not directly interfere with Na\(^+\), K\(^+\), or Ca\(^{2+}\) currents. At a high micromolar concentration, forskolin is able to inhibit voltage-dependent Na\(^+\) and K\(^+\) currents in amphibian nodes of Ranvier.27 In our experiments, Na\(^+\) current was inactivated at the holding potential of ~40 mV, and K\(^+\) current was largely suppressed by Cs\(^+\) substitution. Therefore, it appears unlikely that forskolin-induced modulation of these currents interferes with the effects described herein.

Forskolin activates the cAMP-forming enzyme adenyl cyclase directly. Additionally, at least in intact cells, G\(_i\) proteins apparently play an important role in forskolin activation of the cyclase.13,28 Thus, this agent has been shown to sensitize the adenyl cyclase signal transduction system for stimulation by G\(_i\)-coupled receptors, including B-adrenoceptors.13,28 In our studies on cardiomyocytes, forskolin was applied at a concentration (0.5 μmol/L) that is rather a threshold concentration for increasing cAMP levels,20 although being already half maximal for increasing calcium currents.21 Addition of the B-adrenoceptor antagonists almost
completely reversed the increase in calcium current caused by forskolin. In contrast, when currents were stimulated by intracellular application of the cAMP analog 8-cAMPs, directly activating protein kinase A,14 addition of the β-adrenoceptor antagonistatenol did not alter ICa. From these data and the known interaction of forskolin and Gi proteins in intact cells,13,28 it may thus be concluded that agonist-free, empty β-adrenoceptors induce some activation of Gi protein and the adenyl cyclase, which is potentiated by the presence of forskolin, and that the antagonists block this empty receptor-induced Gi protein activation.

Consistent with this hypothesis, muscarinic acetylcholine receptor antagonists were very recently reported to stimulate calcium currents in frog and rat cardiac myocytes in the absence of an agonist.29 As cardiac muscarinic receptors convey their effects via the inhibitory G protein Gi, it has been proposed that agonist-free, empty muscarinic receptors can spontaneously activate Gi proteins and the subsequent signal transduction cascade, and that receptor antagonists block this empty receptor action and thus enhance ICa.29

Extracellularly applied agents such as β-adrenoceptor agonists and forskolin, which promote formation of cAMP, and intracellularly applied cAMP or catalytic subunit of protein kinase A have been shown to cause a similar, nonadditive increase in calcium currents in cardiomyocytes, which is most likely due to L-type calcium channel phosphorylation.12 In our experiments, 0.5 μmol/L forskolin doubled ICa and enhanced conduc-
tance twofold in the potential range of −25 mV to +40 mV. Addition of the β-adrenoceptor antagonists blocked this increase in calcium current and reduced the conductance at all tested potentials to levels ob-
served in the absence of forskolin. As discussed above, this may be interpreted to mean that the antagonists decreased the extent of channel phosphorylation by reducing the level of activated Gi proteins and conse-
sequently of cAMP. L-type calcium channels have also been proposed to be under direct Gi protein control, ie, independent of channel phosphorylation.30 Thus, although not very likely, it may be considered that antagonist-ligated β-adrenoceptors reduce myocyte calcium current by decreasing direct Gi protein activation of L-type calcium channels.

The hypothesis that empty β-adrenoceptors can be functionally active in intact cardiomyocytes is supported by data obtained with cultured chicken cardiomyocytes. Treatment of these cells, cultured under serum-free conditions in which the β-adrenoceptors are agonist-free, with the agonist propranolol resulted in a considerable increase in β-adrenoceptor density and functional responsiveness of these receptors (cAMP, inotropy).31 These findings, obtained in the absence of forskolin, could be interpreted to be a consequence of the blockade of empty receptor action, including receptor downregulation, by the antagonist. The data additionally suggest that, depending on the species, empty β-adrenoceptor action in cardiac myocytes may even be relevant under basal conditions, ie, in the absence of forskolin. Furthermore, the apparent forskolin requirement reported herein for regulation of cardiac myocyte calcium channels by empty β-adrenoceptors may be accomplished physiologically by hormonal agents or neurotransmitters, eg, histamine, serotonin, and glucagon, activating the system via distinct receptors but sharing a common pool of Gi proteins with β-adrenoceptors.

Conclusions

We have provided evidence, in that in addition to the accepted action of displacing agonist from its binding sites, β-adrenoceptor antagonists can also impair the spontaneous Gi protein activation by the agonist-unliganded receptor. The possible physiological and clinical significance of this negative intrinsic activity of β-adrenoceptor blocking drugs remains to be elucidated.

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T Mewes, S Dutz, U Ravens and K H Jakobs

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