Control of the cardiac muscarinic K⁺ current (iK,ACh) by β-arrestin 2 has been studied. In Chinese hamster ovary cells transfected with m2 muscarinic receptor, muscarinic K⁺ channel, receptor kinase (GRK2), and β-arrestin 2, desensitization of iK,ACh during a 5-min application of 10 μM ACh was significantly increased as compared with that in cells transfected with receptor, channel, and GRK2 only (fade in current increased from 45 to 78%). The effect of β-arrestin 2 was lost if cells were not co-transfected with GRK2. Resensitization (recovery from desensitization) of iK,ACh in cells transfected with β-arrestin 2 were significantly slowed (time constant increased from 34 to 232 s). Activation and deactivation of iK,ACh on application and wash-off of ACh in cells transfected with β-arrestin 2 were significantly slowed from 0.9 to 3.1 s (time to half peak iK,ACh) and from 6.2 to 13.8 s (time to half-deactivation), respectively. In cells transfected with a constitutively active β-arrestin 2 mutant, desensitization occurred in the absence of agonist (peak current significantly decreased from 0.4 ± 0.05 to 0.1 ± 0.01 nA). We conclude that β-arrestin 2 has the potential to play a major role in desensitization and other aspects of the functioning of the muscarinic K⁺ channel.

The cardiac muscarinic K⁺ current (iK,ACh) is responsible, at least in part, for the negative chronotropic, inotropic, and dromotropic effects of vagal stimulation on the heart (1–3). In the heart, ACh released from vagal nerves binds to the m2 muscarinic receptor (a G protein-coupled receptor) causing the dissociation of a trimeric G protein into α and βγ subunits and the free βγ subunits bind to and activate the muscarinic K⁺ channel (4). As in other G protein-coupled receptor systems, the free βγ subunits also bind to and activate receptor kinase and the activated receptor kinase binds to and phosphorylates the agonist-bound receptor (on the third intracellular loop in the case of the m2 muscarinic receptor) (5–7). The phosphorylation of G protein-coupled receptors, including the m2 muscarinic receptor, leads to receptor desensitization (5–7). The chronotropic, inotropic, and dromotropic effects of ACh on the heart fade in the presence of ACh (2, 8–10) and this is likely to be, in part at least, the result of a fade of iK,ACh as a result of desensitization (2, 11). In rat atrial cells and in a mammalian cell line (transfected with m2 muscarinic receptor, muscarinic K⁺ channel, and receptor kinase) we have previously obtained evidence that the phosphorylation of the receptor by receptor kinase is responsible for short-term desensitization of iK,ACh (12, 13).

Arrestins act in concert with receptor kinase to bring about desensitization. Receptor kinase-mediated phosphorylation of the receptor promotes the binding of an arrestin to the agonist bound receptor and this causes desensitization by (i) preventing receptor-G protein interaction and (ii) for the nonvisual arrestins (β-arrestin, β-arrestin 2) only, promoting internalization of the receptor via clathrin-coated pits (the nonvisual arrestins act as adaptor proteins and bind both the receptor and clathrin) (5). For the m2 muscarinic receptor specifically, there is some evidence that arrestins are involved in desensitization: β-arrestin and β-arrestin 2 bind to the m2 muscarinic receptor in a phosphorylation-dependent manner (14). There is evidence that β-arrestin and β-arrestin 2 may be involved in m2 muscarinic receptor uncoupling: deletion of a cluster of serine/threonine residues (phosphorylation sites) in the C-terminal part of the third intracellular loop of the m2 muscarinic receptor greatly reduced the binding of arrestins and in HEK293 cells abolished desensitization as a result of receptor-G protein uncoupling (measured as a reduction in the carbachol inhibition of an isoproterenol-stimulated increase in cAMP as a result of pretreatment with carbachol) (14, 15). The evidence that β-arrestin and β-arrestin 2 may also be involved in m2 muscarinic receptor internalization is less clear: Pals-Rylaarsdam et al. (14) showed that overexpression of β-arrestin and β-arrestin 2 in HEK-tsA201 cells resulted in an internalization of the m2 muscarinic receptor via a dynamin-dependent mechanism (arrestin-mediated internalization is known to occur via a dynamin- and clathrin-dependent mechanism). However, in the same study Pals-Rylaarsdam et al. (14) showed that internalization of the m2 muscarinic receptor in HEK-tsA201 cells in the absence of arrestin overexpression occurred via an unknown pathway that does not involve arrestins or dynamin. Furthermore, in rat ventricular cells, Feron et al. (16) reported evidence that the m2 muscarinic receptor is internalized via caveolae (clathrin-independent pathway), although we have observed co-localization of the m2 muscarinic receptor and clathrin after CCh pretreatment in the same cell type (17).

The aim of the present study was to study the possible role of arrestin in short-term desensitization of iK,ACh. β-Arrestin 2 was chosen for study (both β-arrestin and β-arrestin 2 are ubiquitously expressed in tissues (18)).

**EXPERIMENTAL PROCEDURES**

**Cell Culture and Transfection**—Chinese hamster ovary (CHO)-K1 cells were cultured and transiently transfected as described previously (13). Cells were cultured in Ham’s F-12 nutrient mixture supplemented with 10% fetal bovine serum, 100 units/ml penicillin G, 100 μg/ml streptomycin sulfate, and 0.25 μg/ml Fungizone at 37 °C in 95% air and 5% CO₂. 

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*The abbreviations used are: iK,ACh, cardiac muscarinic K⁺ current; CHO, Chinese hamster ovary; GTPγS, guanosine 5’-3-O-(thio)-triphosphate; ACh, acetylcholine.*
5% CO₂ (all media and chemicals from Life Technologies Ltd., Paisley, United Kingdom). In all experiments, cells from one of two cell lines were transiently transfected with plasmid vectors for Kir3.1 (pEF-GIRK1) and Kir3.4 (pEF-CIR) to form the muscarinic K⁺ channel heteromultimer using the calcium phosphate method (13). One cell line was already stably transfected with plasmid vector for human m2 muscarinic receptor (pEF-Myc-hm2) and the G protein-coupled receptor kinase, GRK2, (pEF-GRK2). GRK2 is known to be present in the heart (19) and it phosphorylates the m2 muscarinic receptor both in vitro and in vivo (20, 21). The cell lines will be referred to as clones 1 and 2, respectively. To test whether the use of a particular clone influenced the results obtained, experiments were repeated using both clones. The results obtained with the two clones were indistinguishable and they have been combined, although the clones used are identified in figure legends. Depending on the experiment, cells of clone 1 were transiently co-transfected with neither, either, or both GRK2 and wild-type β-arrestin 2 (pCMVβ-β-arrestin 2). Depending on the experiment, cells of clone 2 were transiently co-transfected with either or either wild-type β-arrestin 2 or constitutively active mutant β-arrestin 2 (pCDNA3-β-arrestin CAM). Finally, all cells were transiently co-transfected with plasmid vector for the β6ST point mutation of green fluorescent protein (pGFP-β6ST; CLONTECH) as a marker for successfully transfected cells. The final concentrations of each of the plasmid vectors added during transient transfections were as follows (in ng/ml): Kir3.1, 400; Kir3.4, 400; GRK2, 400; β-arrestin 2, 400; green fluorescent protein, 200. 10 ml of the transfecting solution was added to ~1 – 2 × 10⁶ cells in a 100-mm diameter plastic tissue culture dish. The expression levels of the receptor and GRK2 in the stably transfected cells were measured and are given in Shui et al. (13). A few hours before electrophysiological experiments, 0.02% EDTA solution was used to remove the adherent cell layer from the dish. The cells were then centrifuged for 3 min at 100 × g and resuspended in fresh medium on fragments of glass coverslip.

Atrial Cell Isolation—Adult rats were killed by stunning and cervical dislocation. Atrial cells were prepared as described previously (22).

Electrophysiology—CHO cells were placed in a recording chamber mounted on a Nikon Diaphot microscope. 470–490-nm light was used to excite the green fluorescent protein in successfully transfected cells. The green fluorescent light was passed through a 515-nm filter for observation. Cells with a middle level of green fluorescence were chosen for study. Experiments were carried out in the whole cell configuration of the patch clamp technique at room temperature (22–25 °C). Extracellular solution contained (in mM): KCl, 140; MgCl₂, 1.8; EGTA, 5; HEPES, 5; pH 7.4. 10 μM ACh was added to the extracellular solution when required. Pipette solution contained (in mM): potassium aspartate, 120; KCl, 20; KH₂PO₄, 1; MgCl₂, 2.8 (free Mg²⁺, 1.8); EGTA, 5; HEPES, 5; Na₂GTP, 0.1; Na₃ATP, 3; pH 7.4. Whole cell currents were recorded with an Axopatch-1D amplifier and acquired with pClamp software (Axon Instruments Inc., Foster City, CA). Currents were filtered at 2 kHz with an 8-pole Bessel filter and sampled every 1 ms. The software (Axon Instruments Inc., Foster City, CA). Currents were filtered at 2 kHz with an 8-pole Bessel filter and sampled every 1 ms. The software (Axon Instruments Inc., Foster City, CA). Currents were filtered at 2 kHz with an 8-pole Bessel filter and sampled every 1 ms. The software (Axon Instruments Inc., Foster City, CA). Currents were filtered at 2 kHz with an 8-pole Bessel filter and sampled every 1 ms.

RESULTS

Effect of Expression of β-Arrestin 2 on Desensitization of iK,ACh

In the present study, as in our previous study (13), we have recorded iK,ACh in CHO cells transfected with the m2 muscarinic receptor and the muscarinic K⁺ channel (as well as other proteins). In the previous study, the cell-attached and inside-out configurations of the patch clamp technique were used and the basic properties of the channel (single channel conductance, current-voltage relationship, single channel open time, dependence on receptor and agonist) were the same as those of the expressed K⁺ channel in heart cells (13). In the present study, the whole cell configuration of the patch clamp technique was used; 10 μM ACh was applied using a rapid solution changer for 3 min to activate iK,ACh maximally and current was recorded at a holding potential of ~60 mV in a bathing solution containing 140 mM K⁺. Fig. 1, A and C, shows examples of iK,ACh recorded from CHO cells during an application of ACh. Mean traces from ≥8 cells are shown. In both cases, iK,ACh was activated and deactivated on application and wash-off of ACh, respectively. During the 3-min application of ACh, there was a fade of iK,ACh as a result of short-term desensitization. In the present study, iK,ACh was recorded from rat atrial cells under conditions identical to those used for the recording of iK,ACh from CHO cells in order that the muscarinic K⁺ channel system reconstructed in CHO cells can be compared with the native system in heart. Fig. 6A shows a typical recording of iK,ACh in a rat atrial cell. iK,ACh in CHO cells was qualitatively similar to that in rat atrial cells. However, there were differences. The semi-logarithmic plots in Fig. 1, B and D, show that the fade of iK,ACh in CHO cells as a result of desensitization was monoeponential, whereas it can be seen from Fig. 6A that in rat atrial cells it is biexponential. It is known that in heart cells, short-term desensitization is comprised of two independent phases: a fast phase that develops over ~20 s and a slower phase that develops over several minutes (23).
Both of these phases are evident in Fig. 6A. It has been shown that the fast phase is the result of a change in the channel, whereas the slower phase is the result of a change in the receptor (23, 24). The fast phase of desensitization may involve a dephosphorylation of the muscarinic K+ channel (23–26), and a cytosolic protein and a G protein-independent pathway (27). Alternatively, it may be caused by the nucleotide exchange and hydrolysis cycle of the G protein (28). Based on the time course of desensitization (Fig. 1), the fast phase of desensitization observed in heart cells is absent in CHO cells (perhaps the underlying cellular machinery is absent) and the desensitization of iK,ACh in CHO cells is equivalent to the slower phase in heart cells.

Fig. 1, A and C, shows iK,ACh in CHO cells transfected with (in addition to the receptor and channel) GRK2 alone (Fig. 1A) or GRK2 and β-arrestin 2 (Fig. 1C). As compared with cells transfected with GRK2 alone (Fig. 1A), in cells transfected with GRK2 and β-arrestin 2 (Fig. 1C), activation and deactivation of iK,ACh was slowed (see below), the peak amplitude of iK,ACh, was not significantly different (p = 0.8) and the fade of iK,ACh as a result of desensitization was greater. The amplitude of desensitization (see Fig. 1 legend for measurement) in four groups of CHO cells is shown in Fig. 1E: (i) CHO cells not transfected with either GRK2 or β-arrestin 2, (ii) CHO cells transfected with β-arrestin 2, (iii) CHO cells transfected with GRK2, and (iv) CHO cells transfected with GRK2 and β-arrestin 2. Desensitization was least in cells not transfected with either GRK2 or β-arrestin 2. Desensitization was significantly greater in cells transfected with GRK2 alone (p < 0.05), but not with β-arrestin 2 alone (p = 0.3). Desensitization was greatest in CHO cells transfected with both GRK2 and β-arrestin 2, in this cell group desensitization was significantly greater than that in other cell groups (p < 0.05 in each case). In summary, the results show that desensitization was increased by transfection with GRK2 and β-arrestin 2, but not with β-arrestin 2 alone. For comparison, Fig. 1E also shows the amplitude of the equivalent phase of desensitization in rat atrial cells (see Fig. 1 legend for measurement).

The semi-logarithmic plots in Fig. 1, B and D, show that in CHO cells transfected with GRK2 alone (Fig. 1B) and GRK2 and β-arrestin 2 (Fig. 1D) the fade of iK,ACh, as a result of desensitization occurred with similar time constants of 69.5 and 53.7 s, respectively (based on recordings from 15 and eight cells, respectively). The time constants of desensitization were also similar for CHO cells not transfected with either GRK2 or β-arrestin 2 (54.5 s; based on recordings from nine cells) or CHO cells transfected with β-arrestin 2 alone (40.9 s; based on recordings from eight cells). The time constant of the equivalent phase of desensitization in rat atrial cells was 144.0 s (based on recordings from 10 cells).

Effect of Expression of β-Arrestin 2 on Activation and Deactivation of iK,ACh—Close inspection of Fig. 1 shows that activation and deactivation of iK,ACh on application and wash-off of ACh was slowed in CHO cells transfected with GRK2 and β-arrestin 2 as compared with activation and deactivation in CHO cells transfected with GRK2 alone. This is clearly shown by Figs. 2 and 3.

Fig. 2A shows the time to half-peak iK,ACh in CHO cells transfected with GRK2 alone (p = 0.08). Fig. 2B also shows the mean time to half-peak iK,ACh in CHO cells not transfected with either GRK2 or β-arrestin 2 and in CHO cells transfected with β-arrestin 2 only, these data show that the effect of β-arrestin 2 on activation was independent of the absence or presence of overexpressed GRK2 (they also show that overexpressed GRK2 had no effect on activation).

The effect of transfection of GRK2 and β-arrestin 2 on deactivation of iK,ACh in CHO cells is shown in Fig. 3. Fig. 3A compares the deactivation of iK,ACh on wash-off of ACh in CHO cells, transfected with GRK2 alone or GRK2 and β-arrestin 2, and rat atrial cells. Mean traces from 5 to 15 cells are shown. Fig. 3B shows the mean time to half-deactivation of iK,ACh on wash-off of ACh. The time to half-deactivation of iK,ACh in CHO cells transfected with GRK2 and β-arrestin 2 was significantly longer than in CHO cells transfected with GRK2 alone (p < 0.001), whereas the time to half-deactivation of iK,ACh in atrial cells was significantly shorter than in CHO cells transfected with GRK2 alone (p < 0.001). Fig. 3B also shows the mean time to half-deactivation of iK,ACh in CHO cells not transfected with either GRK2 or β-arrestin 2 and in CHO cells transfected with β-arrestin 2 only, these data show that the effect of β-arrestin 2 on deactivation was independent of the absence or presence of β-arrestin 2 alone (p = 0.08).
overexpressed GRK2 (they also show that overexpressed GRK2 had no effect on deactivation).

Effect of Expression of β-Arrestin 2 on Resensitization of \( i_{K,ACH} \)—Resensitization of \( i_{K,ACH} \) (i.e., the recovery of \( i_{K,ACH} \) from desensitization) was studied by applying test applications of \( \text{ACh} \) at various test intervals after control applications of \( \text{ACh} \). Fig. 4 shows typical traces of \( i_{K,ACH} \) in response to control (Fig. 4, A and C) and test (Fig. 4, B and D) applications of \( \text{ACh} \) in CHO cells transfected with GRK2 alone (Fig. 4, A and B) or GRK2 and β-arrestin 2 (Fig. 4, C and D). When the test application of \( \text{ACh} \) was applied soon after the control application, \( i_{K,ACH} \) during the test application of \( \text{ACh} \) was smaller than the control as a result of insufficient time for recovery from the desensitization that developed during the control application of \( \text{ACh} \). As the recovery interval between the two applications was increased, \( i_{K,ACH} \) during the test application of \( \text{ACh} \) increased toward the control as a result of resensitization. The test intervals for full recovery of \( i_{K,ACH} \) in CHO cells transfected with GRK2 alone or GRK2 and β-arrestin 2 were 3 and 10 min, respectively (Fig. 4, B and D). The time courses of \( i_{K,ACH} \) resensitization in CHO cells transfected with GRK2 alone (squares) or GRK2 and β-arrestin 2 (circles) are shown in Fig. 5 from three or four cells, the peak amplitude of \( i_{K,ACH} \) in response to the test application of \( \text{ACh} \) has been normalized to the peak amplitude during the previous control application. The peak currents are indicated by the arrows with the intervals between control and test applications of \( \text{ACh} \). C and D, \( i_{K,ACH} \) recorded from a CHO cell transfected with GRK2 only (clone 2). Four test responses are shown superimposed. The data are fitted with single exponential functions with time constants of 34 s (GRK2) and 232 s (GRK2 and β-arrestin 2). The CHO cells were also transfected with the m2 muscarinic receptor and muscarinic K⁺ channel.

overdesensitization of \( i_{K,ACH} \) was slower in CHO cells transfected with both GRK2 and β-arrestin 2 than in CHO cells transfected with GRK2 alone. The time constant of resensitization in the CHO cells transfected with GRK2 alone or GRK2 and β-arrestin 2 was 34 and 232 s, respectively.

Fig. 6 shows resensitization of \( i_{K,ACH} \) in rat atrial cells. A typical trace during a control application of \( \text{ACh} \) is shown in Fig. 6A and superimposed traces of \( i_{K,ACH} \) during test applications of \( \text{ACh} \) are shown in Fig. 6B. As discussed above, in rat atrial cells, unlike in CHO cells, there are two phases of desensitization of \( i_{K,ACH} \) during a 3-min application of \( \text{ACh} \). The two phases can be seen in Fig. 6, A and B. Resensitization of \( i_{K,ACH} \) will be influenced by both the fast and slower phases, whereas in this study we are concerned with the slower phase only. For this reason, resensitization of \( i_{K,ACH} \) was calculated in two ways: (i) peak \( i_{K,ACH} \) during the test application of \( \text{ACh} \) was expressed as a percentage of peak \( i_{K,ACH} \) during the previous control application of \( \text{ACh} \).
control application of ACh and plotted against the recovery interval between the two applications; (ii) the amplitude of \( i_{K,ACh} \) after the fast phase of desensitization (20 s after the start of the ACh application) was expressed as a percentage of the amplitude of \( i_{K,ACh} \) during the control application in rat atrial cells as determined by the two methods is shown in Fig. 6C and is roughly similar. The time constant of resensitization of \( i_{K,ACh} \) in rat atrial cells was 64 and 75 s for methods i and ii, respectively. This is intermediate between the time constants of resensitization of \( i_{K,ACh} \) in CHO cells with and without α-arrestin 2 (Fig. 5).

**Effect of Expression of a Constitutively Active β-arrestin 2 Mutant on \( i_{K,ACh} \)—Arrestins preferentially bind to the receptor once the receptor is agonist-bound and has been phosphorylated by receptor kinase. However, truncation of the C terminus of arrestins enhances binding to the dephosphorylated receptor, perhaps because the C terminus via an intramolecular interaction with the N terminus maintains arrestins in an inactive conformation (29, 30). With the phosphorylated but not dephosphorylated receptor, arrestins result in a high affinity agonist-binding state, which has been postulated to correlate with the desensitization process (31). The C-terminal truncation mutant, β-arrestin 2 (1–393), is able to result in such a state even in the case of the dephosphorylated receptor (31). This suggests that β-arrestin 2 (1–393) is constitutively active and binds regardless of the phosphorylation state of the receptor. Expression of such a constitutively active β-arrestin 2 mutant might be expected to result in desensitization in the absence of the agonist, although this has never been tested. Fig. 7A shows \( i_{K,ACh} \) recorded from CHO cells transfected with GRK2 only or GRK2 and the constitutively active β-arrestin 2 mutant. Mean traces (from number of cells above) are shown. B, mean + S.E. value of the peak amplitude of \( i_{K,ACh} \) in CHO cells transfected with GRK2 and β-arrestin 2, with GRK2 only or GRK2 and the constitutively active β-arrestin 2 mutant. n numbers are shown in parentheses.

**DISCUSSION**

In the present study, evidence has been obtained to show that β-arrestin 2 is a multifunctional protein and it affects activation, desensitization, deactivation, and resensitization of \( i_{K,ACh} \) when β-arrestin 2 was expressed in CHO cells, activation of \( i_{K,ACh} \) on application of ACh was slowed, short-term
desensitization of \(i_{K\text{ACH}}\) during the application of ACh was enhanced, deactivation of \(i_{K\text{ACH}}\) on wash-off of ACh was slowed, and resensitization of \(i_{K\text{ACH}}\) after the wash-off of ACh was slowed.

**Short-term Desensitization of \(i_{K\text{ACH}}\)**—In the present study we have studied desensitization of \(i_{K\text{ACH}}\) during a 5-min application of ACh, in CHO cells there was a fade of \(i_{K\text{ACH}}\) with a time constant of 40.9–69.5 s depending on the components transfected (Fig. 1). This is equivalent to the short-term desensitization that develops over minutes in heart cells, e.g., rat atrial cells as used in the present study. Kobrinsky et al. (32) have recently studied desensitization of \(i_{K\text{ACH}}\) in neonatal rat atrial cells during a 2-min application of 100 \(\mu M\) ACh (same phase of desensitization investigated as in the present study). They have proposed a radical new hypothesis to explain the desensitization of \(i_{K\text{ACH}}\) in the heart (32). They have suggested that ACh, as well as activating \(i_{K\text{ACH}}\), activates phospholipase C and this results in the hydrolysis of phosphatidylinositol bisphosphate and a consequent decrease in phosphatidylinositol bisphosphate binding to the muscarinic K\(^+\) channel and, thus, channel activity. In part this hypothesis was based on the observation that addition of the aminosteroid, U-73122, to inhibit phosphatidylinositol bisphosphate hydrolysis, abolished the desensitization of \(i_{K\text{ACH}}\) (32). According to this hypothesis the desensitization of \(i_{K\text{ACH}}\) is the result of a change in the receptor rather than the channel. However, Zang et al. (23) showed that in guinea pig atrial cells, if the muscarinic K\(^+\) channel was activated by GTP\(\gamma\)S, which is known to bypass the receptor and activate the G protein and thus the channel directly, then the phase of desensitization of \(i_{K\text{ACH}}\) that develops over minutes was abolished, suggesting that this phase of desensitization is a receptor, rather than channel, phenomenon. Furthermore, Shui et al. (12) showed that this phase of desensitization of \(i_{K\text{ACH}}\) was observed in whole cell, cell attached, and perforated outside-out recordings (in which the cytoplasm is retained) but was lost in inside-out and conventional outside-out recordings (in which the cytoplasm is lost). It was conjectured that receptor kinase was lost on loss of the cytoplasm and, consistent with this explanation, in inside-out recordings desensitization of \(i_{K\text{ACH}}\) was restored on adding exogenous GRK2 (12). This work suggested that, in heart, this phase of desensitization of \(i_{K\text{ACH}}\) is the result of phosphorylation of the receptor by receptor kinase (12). Subsequently Shui et al. (13) showed that in CHO cells transfected with the m2 muscarinic receptor, muscarinic K\(^+\) channel, and GRK2 short-term desensitization of \(i_{K\text{ACH}}\) was comparable to that in heart cells, but short-term desensitization was lost if the cells were not transfected with GRK2 or were transfected with a mutant m2 muscarinic receptor lacking the third intracellular loop containing the phosphorylation sites. The present study confirms that if CHO cells are not expressed with GRK2 (with or without \(\beta\)-arrestin 2) then desensitization is reduced (Fig. 1E).

The present study has shown that desensitization of \(i_{K\text{ACH}}\) in atrial and CHO cells—while a comparison of \(i_{K\text{ACH}}\) in atrial and CHO cells may be useful, such a comparison must be considered cautiously, because of the absence/presence of additional regulatory components in the two cell types as well as the effects of protein overexpression on the relative stoichiometries of the different components. Fig. 1E shows that the amplitude of desensitization in rat

**Activation and Deactivation of \(i_{K\text{ACH}}\)**—Overexpression with \(\beta\)-arrestin 2 slowed both the activation and deactivation of \(i_{K\text{ACH}}\) on application and wash-off, respectively, of ACh (Figs. 2 and 3). Activation of \(i_{K\text{ACH}}\) involves ACh-receptor binding, G protein activation, and the subsequent activation of the channel by the G protein \(\beta\gamma\) subunits. It is known that binding of a \(\beta\)-arrestin to the receptor inhibits G protein interaction (5). Although arrestins preferentially bind to the agonist-occupied receptor kinase-phosphorylated receptor, arrestins still bind but with reduced efficacy to the agonist-unoccupied, dephosphorylated receptor (29). Perhaps with \(\beta\)-arrestin 2 overexpression, as in the present study, there is increased interaction of \(\beta\)-arrestin 2 with the agonist-unoccupied, dephosphorylated receptor and this is manifested as impaired coupling with the G protein and a consequent slowing of the activation of \(i_{K\text{ACH}}\) on application of ACh. Alternatively, it is possible that \(\beta\)-arrestin 2 has a direct effect on the muscarinic K\(^+\) channel to slow activation of \(i_{K\text{ACH}}\).

On wash-off of ACh, deactivation of \(i_{K\text{ACH}}\) involves the splitting of GTP by the G protein \(\alpha\) subunit, unbinding of the G protein \(\beta\gamma\) subunits from the channel and reformation of the G protein trimer. It is possible that interaction of the G protein trimer with the receptor is involved in the deactivation process, perhaps by stabilizing the trimer. In this case, with \(\beta\)-arrestin 2 overexpression as in the present study, there may be increased interaction of \(\beta\)-arrestin 2 with the agonist-unoccupied, dephosphorylated receptor as argued above and this may impair coupling of the receptor with the G protein and this may slow deactivation of \(i_{K\text{ACH}}\). Alternatively, it is possible that \(\beta\)-arrestin 2 has a direct effect on the G protein or channel.

**Comparison of \(i_{K\text{ACH}}\) in Atrial and CHO Cells**—While a comparison of \(i_{K\text{ACH}}\) in atrial and CHO cells may be useful, such a comparison must be considered cautiously, because of the absence/presence of additional regulatory components in the two cell types as well as the effects of protein overexpression on the relative stoichiometries of the different components.
potential to be involved in this desensitization process. In addition, it is concluded that 

\[ \text{beta-arrestin} \]

activation of \( \text{iK}, \text{ACh} \) in \( \text{CHO} \) cells transfected with or without 

\[ \text{Arrestin} \] 2 was intermediate between that in \( \text{CHO} \) cells in the absence and 

presence of \( \text{beta-arrestin} \) 2 (\( \tau \), 34 and 232 s, respectively). It is possible that transfection of \( \text{CHO} \) cells with 

\[ \text{beta-arrestin} \] 2 is the result of a change in the receptor and, on the basis 

of the present study, it is concluded that 

\[ \text{beta-arrestin} \] 2 has the 

potential to be involved in this desensitization process. However, whether \( \text{beta-arrestin} \) 2 is involved in regulation of the muscarinic \( K^+ \) channel in the heart remains to be elucidated.

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