Desensitization of P_{2U} receptor in neuronal cell line

Different control by the agonists ATP and UTP, as demonstrated by single-cell Ca^{2+} responses

Uwe CZUBAYKO and Georg REISER*

Institut für Neurobiochemie der Otto-von-Guericke-Universität Magdeburg, Leipziger Strasse 44, 39120 Magdeburg, Federal Republic of Germany

The neuronal cell line NG108-15 (180CC15) responds to extracellular stimuli of ATP or UTP with a transient increase in the level of cytosolic Ca^{2+} . Desensitization was investigated by recording single-cell Ca^{2+} responses induced by consecutive, regularly spaced (100 s intervals), brief pulses of the nucleotides. The two natural ligands of the P_{2U} receptor, ATP and UTP, were applied at a concentration that evoked responses of a comparable size. With two pulses of UTP (10 μ M), a substantial decrease (of 43 %) was observed in the size of the second response. The magnitude of response was determined by measuring either the maximal amplitude or the total response, represented by the area of the Ca^{2+} transient. The analogous studies with ATP pulses showed a much smaller decrease (of 12 %). Comparable experiments performed to investigate the mutual interaction between ATP and UTP revealed that after stimulation with ATP the

response to UTP was slightly (12 %) diminished, whereas the response to ATP after UTP was greatly (52 %) decreased. The different degree of desensitization by either UTP or ATP of P_{2U} receptors could be due to (1) a difference in the mode of activation of the receptor by the two ligands or (2) recruitment of another effector mechanism besides elevated Ca^{2+} . Our results indicate the existence of a novel mechanism of receptor control, involving different modes of the receptor, that are induced by two different, activating ligands. We also investigated the cross-talk between the bradykinin B_2 receptor and the nucleotide receptor. ATP and UTP, even when eliciting responses of comparable size in the neuronal cell line, affect the desensitization of the bradykinin receptor differently. This suggests regulatory binding sites for the nucleotides on either the nucleotide receptor or the peptide receptor.

INTRODUCTION

ATP is not normally a hormone because its lifetime in circulation is too short. It has important roles as a neurotransmitter in the autonomic system and the central nervous system [1]. Extracellular ATP acts on cell surface receptors in many different cell types by elevating the level of cytosolic Ca^{2+} ($[Ca^{2+}]_i$) [1–3]. Receptors for extracellular nucleotides have been designated P_2 receptors (specific for ATP or ADP) and P_1 receptors (specific for adenosine and AMP). P_2 receptors have been classified by using pharmacological criteria of affinity sequences of nucleotides and their analogues [4].

We have previously characterized the pharmacology of the nucleotide receptor in the neuronal neuroblastoma–glioma hybrid cell line 108CC15 (NG108-15) [5] by studying the response of $[Ca^{2+}]_i$ to various purine nucleotides [6]. We obtained the following order of potency: ATP = ATP[S] > ADP $\geq$ 2MeSATP > α,β -methylene-ATP (where ATP[S] is adenosine 5'-[γ -thio]triphosphate and 2MeSATP is 2-methylthioadenosine triphosphate). This sequence was consistent with results obtained from concentration–response curves determining the rise in level of the second messenger cGMP induced by nucleotide agonists and analogues [7]. Moreover, the potency of UTP proved the receptor to be of the P_{2U} type.

The purinoceptor present in the neuronal cell line is coupled to activation of phospholipase C and Ca^{2+} influx – the nature of this influx pathway [8] and even its unequivocal existence is still unclear – and has been characterized molecularly by cloning the corresponding cDNA [9]. The protein has structural similarities and homologies with other members of the family of G-protein-coupled receptors that have seven putative transmembrane domains. The receptor has been functionally expressed in stably transfected human leukaemia cells [10]. Low micromolar concentrations of both ATP and UTP activated the receptor and caused

mobilization of intracellular Ca^{2+} . A protein of 53 kDa, possibly corresponding to the P_{2U} receptor, was photolabelled by radioactive 3'-O-(4-benzoylbenzoyl)ATP. Labelling of the protein was inhibited by 10 μ M ATP, but was not affected by UTP at concentrations as high as 100 μ M [10]. This raises the possibility that the receptor has distinct binding sites for each nucleotide.

Besides the P_{2U} receptor some related P_2 receptors have been identified by cloning cDNAs from several species [11–13]. Among these it is only the P_{2U} receptor that has the specific characteristics to be activated by the two natural ligands ATP and UTP. As for other G-protein-coupled receptors, a fast desensitization on continued exposure to agonist has also been found for metabotropic purinergic receptors of the P_{2Y} type [14]. In the present study we investigated whether the P_{2U} receptor is differently desensitized by its agonists ATP and UTP. We find that the response to UTP desensitizes strongly whereas that to ATP desensitizes only negligibly.

MATERIALS AND METHODS

Neuroblastoma–glioma hybrid cells (clone 108CC15, also designated NG108-15) of passage numbers between 14 and 32 were cultured as described [5]. Recording of $[Ca^{2+}]_i$ from single glioma cells was performed in accordance with published procedures [15,16] by microspectrofluorometry. In brief, cells grown on circular glass coverslips (12 mm in diameter) with 20–90 % confluency were removed from the culture dish and placed into 1 ml of Hepes-buffered saline [145 mM NaCl/5.4 mM KCl/1.8 mM $CaCl_2$ /1.0 mM $MgCl_2$ /25 mM glucose/20 mM Hepes (pH 7.4), adjusted with Tris] for 30 min at 37 °C supplemented with 2.5 μ M fura 2/AM. After the 30 min loading time, cells were washed with Hepes-buffered saline and placed into a recording chamber.

Abbreviations used: ATP[S], adenosine 5'-[γ -thio]triphosphate; $[Ca^{2+}]_i$, cytosolic Ca^{2+} ; HBS, Hepes-buffered saline; 2MeSATP, 2-methylthioadenosine triphosphate.

* To whom correspondence should be addressed.

By using a photon-counting dual excitation microfluorometric system (Newcastle Photometrics) fluorescence was determined from single cells loaded with the dye fura 2/AM. Cells were placed in a chamber (0.2 ml) on the stage of an inverted microscope (Nikon Diaphot). The emitted fura 2 fluorescence (510 nm) was corrected on-line for background and auto-fluorescence, and was sampled for time periods of 200 ms at a rate of 1 Hz with alternate excitations at 350 and 380 nm. The values were stored on a personal computer. To carry out the measurements of fura 2, the excitation light was passed through a neutral-density filter (ND2) and a dichroic mirror with 455 nm cut-off; emitted light was passed through a 510 nm barrier filter. $[Ca^{2+}]_i$ was assessed by converting the ratio F_{350}/F_{380} with a calibration curve established *in vitro*. Fura 2/AM was from

Chemie-Technik München (München, Germany); UTP was from Sigma (Deisenhofen, Germany). Analysis of data was performed with the software supplied by Newcastle Photometric System. The integral of the response was derived by calculating the area between the baseline (interpolation of averaged values before and after hormone-induced Ca^{2+} response) and the response curve.

RESULTS

In single cells of a neuronal tumour cell line (neuroblastoma–glioma hybrid cells) the responses to nucleotides ATP or UTP were determined by measuring the $[Ca^{2+}]_i$ signal derived from the fluorescence of fura 2. Application of brief pulses (12 s) of the nucleotides at concentrations from 10 nM to 10 μ M caused a fast rise in $[Ca^{2+}]_i$ with a duration between 47 and 53 s for ATP and between 52 and 86 s for UTP. The source for the Ca^{2+} rise is mainly Ca^{2+} release from internal stores. This can be concluded from the finding that the response to the nucleotide was nearly identical whether extracellular Ca^{2+} was

Figure 1 $[Ca^{2+}]_i$ responses induced by UTP in single neuroblastoma–glioma hybrid cells (A) and concentration–response curves for UTP or ATP (B, C)

(A) Stimulation of one cell by successive pulses applied at the time points marked by arrows with U (10 μ M UTP). Here and in Figures 2–5, the traces show the ratio of fura 2 fluorescence intensities at 350 and 380 nm excitation wavelengths ($F_{350\text{ nm}}/F_{380\text{ nm}}$) and the conversion to $[Ca^{2+}]_i$ was as described in the Materials and methods section. The box above the trace indicates the length of the change to a Ca^{2+} -free superfusion medium supplemented with 10 μ M EGTA. (B, C) Concentration–response curves shown for amplitudes (B) or integrals of response (C) for various concentrations of UTP ($\square$) or ATP ($\blacktriangle$). For ATP, responses from at least five cells were analysed (at 10 μ M: 36 cells). For UTP, at 1, 10, 100, 1000, 10000 nM, 4, 10, 6 and 43 responses were analysed respectively. The size of the Ca^{2+} response was analysed by measuring either the amplitude (difference between maximal value and base line) or the integral of the Ca^{2+} response (area between base line, derived from interpolation of values before and after the transient rise and response curve). Error bars give S. D.

Figure 2 Homologous desensitization

(A, B) $[Ca^{2+}]_i$ recordings of responses of neuroblastoma–glioma hybrid cells to repetitive stimuli of 10 μ M ATP (A) or 10 μ M UTP (B). (C) Homologous desensitization with UTP given by the decrease in size of response. The amplitude (area) induced by the second of two pulses applied at an interval of 100 s is compared with that of the first response (set at 100%). For each cell, the desensitization (percentage decrease) is calculated. The results are an analysis from experiments with 12 cells.

Figure 3 Heterologous desensitization to the nucleotides ATP and UTP, shown by the influence of a UTP pulse to responsiveness to ATP

(A) [Ca²⁺]_i recording; U represents 10 μ M UTP and A 10 μ M ATP. (B) Stimulation of cells at intervals of 100 s by ATP (pulse 1), then UTP, then ATP (pulse 2). Histogram of decrease in size of response to pulse 2 of ATP in comparison with that induced by pulse 1 of ATP, analysed as in Figure 2(C). The results are from nine experiments.

Figure 4 Heterologous desensitization to the nucleotides ATP and UTP demonstrated by the influence of an ATP pulse to responsiveness to UTP pulses

(A) [Ca²⁺]_i recording; see Figure 3. (B) Stimulation of cells with UTP (pulse 1), then ATP, then UTP (pulse 2) at intervals of 100 s. Histogram of decrease in size of response to pulse 2 of UTP in comparison with that induced by pulse 1 of UTP, analysed as in Figure 2(C). The results are from 12 experiments.

present or not (Figure 1A). However, the duration of pre-exposure of cells to Ca²⁺-free medium was critical for the responsiveness of the cells to UTP or ATP. Incubation without extracellular free Ca²⁺ for 50 s did not affect the response of the hybrid cells, whereas incubation for 200 s strongly decreased the amplitude of the response to ATP or UTP by approx. 75%. Returning cells to Ca²⁺-containing medium caused a fast recovery of the size of the response (results not shown).

The sensitivity of the hybrid cells to the nucleotides ATP and UTP is shown in Figures 1(B) and 1(C). The concentration-response curves for both nucleotides were established with the following two parameters: the response amplitude (Figure 1B) and the response integral, which was determined by the area of the [Ca²⁺]_i rise (Figure 1C). With both methods the EC₅₀ for UTP was approx. 0.3 μ M and for ATP it was approx. 3 μ M. Because at 10 μ M the response caused by either nucleotide had approximately the same size, this concentration was used for the following experiments.

The effect of suramin, an antagonist at P₂ receptors, was tested in the following way. The response that could be elicited by a brief pulse of ATP (UTP) after a superfusion with suramin for 200 s was compared with the control response obtained before application of suramin. In comparison with the area (amplitude) of the control response we observed in the presence of 100 μ M suramin a decrease in the response caused by 10 μ M ATP by 81 $\pm$ 14% (66 $\pm$ 23%; mean $\pm$ S.D., *n* = 7). The effect of UTP was blocked by suramin to a comparable degree. The recovery determined by measuring the Ca²⁺ rise induced by the ATP (UTP) pulse after a subsequent 200 s wash-out period amounted to 77% (70%) of the original response.

Homologous desensitization of the purinoceptor was investigated by repetitive stimuli of 12 s duration, which were usually separated by intervals of 100 s. Two of these experiments are shown in Figure 2. The consecutive ATP pulses in Figure 2(A) caused [Ca²⁺]_i rises of comparable size. This constant responding to ATP has also been shown in our previous experiments [15]. With UTP, however, the second response was greatly decreased (Figure 2B). For the analysis of such experiments the degree of desensitization was classified into three categories: strong, with more than 40% decrease; moderate, with 40–20% decrease; and modest, with less than 20% decrease. A strong desensitization was observed with two pulses of UTP in most experiments, as displayed in the histogram in Figure 2(C). The mean decrease in the integral (amplitude) was 43 $\pm$ 19% (33 $\pm$ 14%; mean $\pm$ S.D., *n* = 12).

The mutual influence of the two nucleotides ATP and UTP was studied by a similar experimental protocol, as demonstrated in Figure 3(A). After a control response induced by a pulse of ATP, a pulse of UTP was applied. Then the response to a test pulse of ATP was measured and compared with the original control response. The histogram in Figure 3(B) shows that the size of the response to the ATP test pulse was greatly decreased. In most cells, both amplitude and integral of response to the test pulse of ATP showed strong desensitization. The mean decrease in amplitude (area of response) was 52 $\pm$ 16% (51 $\pm$ 20%; *n* = 9). Surprisingly, changing the sequence of applications of UTP and ATP, as demonstrated in Figure 4(A), with a pulse of ATP between the control and the test pulse of UTP, gave a

Figure 5 Heterologous desensitization of neuroblastoma–glioma hybrid cells by bradykinin and UTP

(A, B) $[Ca^{2+}]_i$ responses induced by stimuli of UTP ($10 \mu M$) or bradykinin ($10 nM$), indicated by U and B. Bradykinin caused a small decrease in a subsequent response to UTP (A) whereas UTP led to a substantial decrease in a following response to bradykinin (B). (C) Histogram of experiments demonstrated in (B) with a stimulus sequence of bradykinin (pulse 1), then UTP, then bradykinin (pulse 2). Desensitization was measured by determining the size of the second response to bradykinin relative to response to pulse 1 of bradykinin. The results are from 10 experiments.

completely different result. Under these conditions, in most cells response number 2 showed only a weak desensitization, but in none of the 12 experiments was it strongly decreased, as can be seen in the histogram in Figure 4(B). Thus the mean decrease in the amplitude (area) of response was only 12% (8%; mean $\pm$ S.D., $n = 12$). The lack in desensitization to consecutive UTP pulses (Figure 4A) after the ATP pulse was seen only occasionally.

Examples of heterologous desensitization with the peptide bradykinin and the nucleotide UTP are given in Figure 5. The Ca^{2+} response to bradykinin in the neuroblastoma–glioma hybrid cells has already been characterized in detail [17]. The influence of a bradykinin pulse on responses to UTP, which is demonstrated in Figure 5(A), is negligible. UTP, in contrast, had a decisive impact on the responses to bradykinin (Figure 5B). The analysis given in the histogram (Figure 5C) proves that most cells reacted with a strong desensitization. The amplitude (integrated response) was decreased by $40 \pm 26\%$ ($45 \pm 17\%$; mean $\pm$ S.D., $n = 10$).

DISCUSSION

Fading of physiological responses during persistent activation of cellular receptors by biologically active compounds such as hormones or neurotransmitters is a feature common to many transmembrane signalling events. This phenomenon, known as desensitization, limits the responsiveness of cells to one or more external stimuli and enables fast adaptation in response to changing extracellular signals. Desensitization could be mediated by a decrease in the amount of intracellular messenger or by the disruption of interactions between receptors and transducers. This disruption is caused by uncoupling the receptor from intracellular G-proteins. Receptors such as β -adrenergic receptors are down-regulated by a specific receptor kinase. The slow phase of desensitization frequently involves receptor internalization [18,19]. P_{2U} receptors in turkey erythrocytes coupled to phospholipase C have already been shown to be desensitized owing to a modification at the receptor level [14]. This modification is possibly a phosphorylation by a receptor kinase specific for phospholipase C-coupled receptors [20–22].

Our previous study of receptor cross-talk in neuroblastoma–glioma hybrid cells [15] involved the characterization of the interaction of the receptors for the peptide bradykinin, for the amine serotonin and also for ATP. The most interesting question here was to what degree the two nucleotides ATP and UTP induce ‘heterologous’ desensitization at the same receptor. We therefore applied repetitive stimuli of nucleotide agonists and determined the responsiveness of the neuronal cells by recording the $[Ca^{2+}]_i$ transients in single cells. Even the short (12 s duration) successive stimuli of UTP caused a substantial desensitization. ATP, however, applied under the same conditions (pulse length, interval and agonist concentration), despite eliciting nearly identical initial responses caused hardly any desensitization.

In aortic endothelial cells, which express both types of receptor, P_{2Y} and P_{2U} , multiple desensitization events occur [23]. Wilkinson et al. [23] investigated the desensitization of P_{2Y} receptors and nucleotide (P_{2U}) receptors coexisting on bovine aortic endothelial cells by measuring the $Ins(1,4,5)P_3$ levels caused by short-pulse stimulations. Preincubation with the P_{2Y} -selective agonist 2MeSATP did not affect a subsequent response to UTP, whereas pretreatment with UTP attenuated the response to 2MeSATP. Thus homologous desensitization occurred with both P_{2Y} and P_{2U} receptors but heterologous desensitization followed only from activation of the P_{2U} receptor. However, in such preparations the occurrence of different cell types and the mixture of various receptors did not permit the study of solely the interaction of two different nucleotides at the same receptor. We therefore used a homogeneous cell preparation, neuroblastoma–glioma hybrid cells, which express the P_{2U} receptor, as identified by molecular cloning [9]. This cloned receptor is stimulated equally by the nucleotides ATP and UTP [10].

It has been shown [24] that diadenosine polyphosphates, for which the existence of specific P_{2D} receptors had been postulated, activate with comparable potency the P_{2U} receptor in astrocytoma cells transfected with a vector carrying the P_{2U} receptor nucleotide sequence [25]. In the neuroblastoma–glioma hybrid cell line, however, the P_{2U} receptor was only weakly activated by the dinucleotides when measuring the Ca^{2+} responses (B. Stix and G. Reiser, unpublished work). A further slight difference in the affinity profile was found between the P_{2U} receptor (derived from NG108-15 = 108CC15) expressed in K-562 human leukaemia cells [25] and the P_{2U} receptor studied in the neuroblastoma–glioma hybrid cells [6,7]. In contrast with the results obtained with NG108-15 cells there was no effect of 2MeSATP and ADP in the transfected K-562 cells. This does not necessarily imply the

existence of two different types of nucleotide receptor on the NG108-15 cells. It raises the interesting possibility that interaction of the nucleotide receptor with other proteins in different cell types might affect its affinity. However, at present we cannot yet definitively exclude the possibility of the occurrence of two receptor types in the neuronal hybrid cells.

It is not even yet clear whether, besides the P_{2U} receptor, a receptor uniquely responsive to pyrimidine nucleotides exists on the hybrid cells. A uridine nucleotide-selective receptor activating phospholipase C was described in C6-2B cells [26]. In HL-60 cells an equally strong cross-desensitization to either UTP or ATP was found. Cloning of P_{2Y}-related receptors revealed the first indication for receptors stimulated by UTP and UDP, and partly by ATP [27,28].

In the NG108-15 cells the low potency and efficacy of 2MeSATP do not suggest the occurrence of a large proportion of P_{2Y} receptors; moreover homologous desensitization induced by ATP was only weak. Based on the most likely assumption that the NG 108-15 cells express mainly one P₂ receptor type the results presented here are explained by the conclusion that ATP and UTP, despite eliciting responses of comparable amplitude, induce different receptor conformations leading to different degrees in desensitization. ATP affected only slightly the response to a subsequent pulse of UTP, whereas UTP greatly decreased the response caused by a subsequent pulse of ATP. Therefore the two agonists, which obviously elicit identical cellular effects, induce distinct states of the receptor, which could conceivably result in different modes of interaction with G-protein-coupled receptor kinase [29], possibly causing different degrees of phosphorylation. We do not yet know whether another effector system besides [Ca²⁺]_i might control the desensitization of P_{2U} receptors in the neuronal cell line studied here.

The results presented here suggest that the sites and modes of interaction of the P_{2U} receptor with the natural ligands ATP and UTP do not coincide. This conclusion is consistent with the finding that binding of the photoaffinity label 3'-*o*-(4-benzoyl benzoyl) ATP to the P_{2U} receptor was displaced by ATP but not by UTP [10]. The displacement of 3'-*o*-(4-benzoyl benzoyl) ATP labelling by ATP, but not UTP, was similarly seen in PC12 cells [30]. Also in HL-60 cells binding of [³⁵S]ATP[S] was displaced by ATP with 40-fold higher affinity than by UTP [31]. The model for partly overlapping binding sites for ATP and UTP on the P_{2U} receptor proposed by Conigrave and Jiang [31] has yet to be substantiated by experimental evidence.

Another G-protein-coupled receptor that can be activated by two 'natural' ligands is the thrombin receptor. Interestingly, in osteoblast cells Ca²⁺ responses to thrombin receptor activation induced either by the cognate ligand thrombin or by the short peptide 'thrombin receptor activating peptide' (TRAP) had different kinetics and desensitization characteristics [32]. We reported that the thrombin receptor on rat glioma cells is activated similarly by thrombin and TRAP [16].

A further remarkable finding is the difference in proper heterologous desensitization between ATP/UTP receptors and bradykinin receptors in the neuronal cell line studied here. Activation of the P_{2U} receptor with ATP caused a weak desensitization of the bradykinin B2 receptor (see Fig. 4 in [15]), whereas UTP elicited a strong desensitization. This distinction could be due to the existence of additional regulatory binding sites on the

nucleotide receptor besides the ligand-binding site required for receptor activation.

We thank Dr. R. Schäfer for helpful comments on the manuscript. Experiments were performed as part of the diploma thesis work of U. C. at the Physiologisch-chemisches Institut der Universität Tübingen. This work was supported by a project grant from Deutsche Forschungsgemeinschaft (Re 563/2-4), by Fonds der Chemischen Industrie, and by Land Sachsen-Anhalt.

REFERENCES

- 1 Burnstock, G. (1993) *Drug Dev. Res.* **28**, 195–206
- 2 Gordon, J. L. (1986) *Biochem. J.* **233**, 309–319
- 3 Dubyak, G. R. and El-Moatassim, C. (1993) *Am. J. Physiol.* **265**, C577–C606
- 4 Fredholm, B. G., Abbracchio, M. P., Burnstock, G., Daly, J. W., Harden, T. K., Jacobson, K. A., Leff, P. and Williams, M. (1994) *Pharmacol. Rev.* **46**, 143–153
- 5 Reiser, G. (1992) in *Electrophysiological Methods for in vitro Studies in Vertebrate Neurobiology* (Kettenmann, H. and Grantyn, R., eds.), pp. 111–116, Wiley-Liss, New York
- 6 Reetz, G. and Reiser, G. (1994) in *Proceedings of the 22nd Göttingen Neurobiology Conference* (Elsner, N. and Breer, H., eds.), Thieme Verlag, Stuttgart
- 7 Reiser, G. (1995) *J. Neurochem.* **64**, 61–68
- 8 Lin, T.-A., Lustig, K. D., Sportiello, M. G., Weisman, G. A. and Sun, G. Y. (1993) *J. Neurochem.* **60**, 1115–1125
- 9 Lustig, K. D., Shiau, A. K., Brake, A. J. and Julius, D. (1993) *Proc. Natl. Acad. Sci. U.S.A.* **90**, 5113–5117
- 10 Erb, L., Lustig, K. D., Sullivan, D. M., Turner, J. T. and Weisman, G. A. (1993) *Proc. Natl. Acad. Sci. U.S.A.* **90**, 10449–10453
- 11 Parr, C. E., Sullivan, D. M., Paradiso, A. M., Lazarowski, E. R., Burch, L. H., Olsen, J. C., Erb, L., Weisman, G. A., Boucher, R. and Turner, J. T. (1994) *Proc. Natl. Acad. Sci. U.S.A.* **91**, 3275–3279
- 12 Henderson, D. J., Elliot, D. G., Smith, G. M., Webb, T. E. and Dainty, I. A. (1995) *Biochem. Biophys. Res. Commun.* **212**, 648–656
- 13 Tokuyama, Y., Hara, M., Jones, E. M. C., Fan, Z. and Bell, G. I. (1995) *Biochem. Biophys. Res. Commun.* **211**, 211–218
- 14 Martin, M. W. and Harden, T. K. (1989) *J. Biol. Chem.* **264**, 19535–19539
- 15 Reetz, G. and Reiser, G. (1994) *J. Neurochem.* **62**, 890–897
- 16 Czubyko, U. and Reiser, G. (1995) *Neuroreport* **6**, 1249–1252
- 17 Reiser, G., Binmöller, F.-J. and Donié, F. (1990) *Exp. Cell Res.* **186**, 47–53
- 18 Dohlmann, H. G., Thorner, J. C. M. G. and Lefkowitz, R. J. (1991) *Annu. Rev. Biochem.* **60**, 653–688
- 19 Premont, R. T., Inglese, J. and Lefkowitz, R. J. (1995) *FASEB J.* **9**, 175–182
- 20 Boarder, M. R., Weisman, G. A., Turner, J. T. and Wilkinson, G. F. (1995) *Trends Pharmacol. Sci.* **16**, 133–139
- 21 Tobin, A. B., Keys, B. and Nahorski, S. R. (1993) *FEBS Lett.* **335**, 353–357
- 22 Wojcikiewicz, R. J. H., Tobin, A. B. and Nahorski, S. R. (1993) *Trends Pharmacol. Sci.* **14**, 279–285
- 23 Wilkinson, G. F., Purkiss, J. R. and Boarder, M. R. (1994) *Mol. Pharmacol.* **45**, 731–736
- 24 Pintor, J., Diaz-Rey, M. A. and Miras-Portugal, M. T. (1993) *Br. J. Pharmacol.* **108**, 1094–1099
- 25 Lazarowski, E. R., Watt, W. C., Stutts, M. J., Boucher, R. C. and Harden, T. K. (1995) *Br. J. Pharmacol.* **116**, 1619–1627
- 26 Lazarowski, E. R. and Harden, T. K. (1994) *J. Biol. Chem.* **269**, 11830–11836
- 27 Communi, D., Piroton, S., Parmentier, M. and Boeynaems, J.-M. (1995) *J. Biol. Chem.* **270**, 30849–30852
- 28 Nguyen, T., Erb, L., Weisman, G. A., Marchese, A., Heng, H. H. Q., Garrad, R. C., George, S. R., Turner, J. T. and O'Dowd, B. F. (1995) *J. Biol. Chem.* **270**, 30845–30848
- 29 Inglese, J., Freedman, N. J., Koch, W. J. and Lefkowitz, R. J. (1993) *J. Biol. Chem.* **268**, 23735–23738
- 30 Majid, M. A. and Okajima, F. K. Y. (1993) *Biochem. Biophys. Res. Commun.* **195**, 415–421
- 31 Conigrave, A. D. and Jiang, L. (1995) *Cell Calcium* **17**, 111–119
- 32 Jenkins, A. L., Bootman, M. D., Berridge, M. J. and Stone, S. R. (1994) *J. Biol. Chem.* **269**, 17104–17110