

FlashPlate® File #15

Use of Novel FlashPlate Technology
to Measure cAMP Accumulation
in Chinese Hamster Ovary Cells
Expressing Human β -2
Adrenoreceptors

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Introduction

Due to recent technologies in the pharmaceutical industry that have resulted in an increase in the production of potential drug candidates, it has become vital that screening assays are high throughput. FlashPlate technology from PerkinElmer Life Sciences has provided a platform for the adaptation of a variety of assays which can be automated. One such application is monitoring the activity of the enzyme adenylyl cyclase by measuring cAMP accumulation.

Until recently, cAMP has been generated in a biological system (e.g., receptor expressing cells), extracted and then measured by FlashPlate (SMP001) (FlashPlate File #7, J. Watson). However the recent introduction of a novel adenylyl cyclase FlashPlate assay (SMP004, SMP004A) has provided a more efficient method for measuring levels of cAMP from whole cells which eliminates the extraction step (FlashPlate File #1, P. Kasila).

We have investigated the use of this novel FlashPlate technology to measure the activation of adenylyl cyclase in Chinese Hamster Ovary (CHO) cells expressing human β -2 adrenoreceptors (h β -2 receptors).

Materials

Adenylyl Cyclase Activation FlashPlate Assays (96-well format) were supplied by PerkinElmer Life Sciences. Isoprenaline was obtained from Sigma-Aldrich, and pindolol from RBI chemicals. Ham's F-12 media, foetal bovine serum and Geneticin 418 were obtained from Gibco BRL.

Method

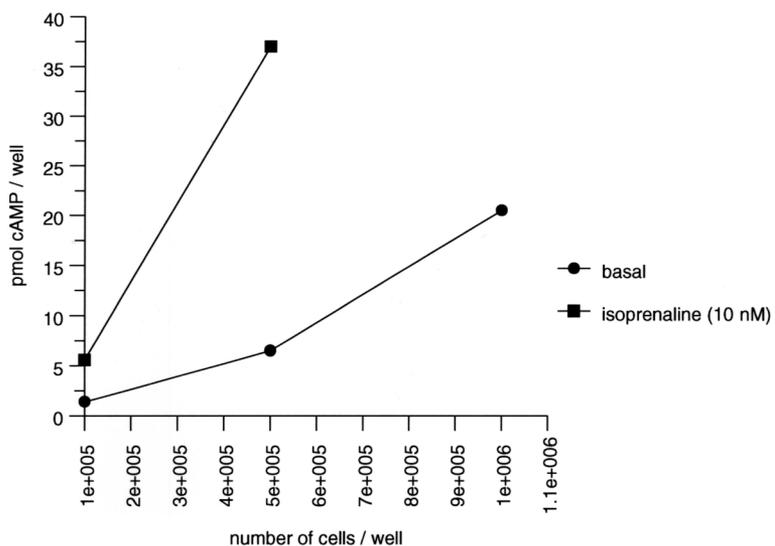
CHO cells expressing h β -2 receptors were grown to confluence in Ham's F-12 media containing 10% foetal bovine serum and 500 μ g/ml Geneticin 418, harvested and resuspended in stimulation buffer, containing a phosphodiesterase inhibitor, provided with the FlashPlate kit. The cells were then spun at 200 g for 5 mins. at room temperature, the supernatant removed and the pellet resuspended in stimulation buffer. A standard curve of known cAMP concentrations was constructed and pipetted into the designated wells. Cells (50 μ l) were added to the remaining wells containing either water or test drug to give a final volume of 100 μ l, and the plate incubated at 37°C for 15 mins. The reaction was then terminated by addition of 100 μ l of detection buffer containing 0.018 μ Ci of 125 I-cAMP and membrane permeabilising reagent (provided with the FlashPlate kit). After 2 hrs. at room temperature, the FlashPlate was placed onto a Packard TopCount® Microplate Scintillation Counter and the level of bound radioactivity measured.

Results

β -2 adrenoreceptors are positively coupled to adenylyl cyclase and so stimulate the production of cAMP. It was important to establish the number of cells per well that would produce measurable basal and stimulated levels of cAMP, i.e., that would lie on the standard curve. Figure 1 shows a linear relationship between the number of cells added per well and basal levels of cAMP produced. The β receptor agonist, isoprenaline (10 nM), stimulated cAMP levels approximately 5 fold for 1×10^5 and 5×10^5 cells per well, but could not be measured from the standard curve for 1×10^6 cells per well. These results suggest that a range from 1×10^5 to 5×10^5 cells per well could be used in this assay system. Because this assay is homogeneous, i.e., a uniform cell suspension is added to each well, we would expect very little intra-assay variation, as shown in Figure 2. Mean basal cAMP levels over 10 wells = 1.34 ± 0.04 and isoprenaline-stimulated levels = 5.93 ± 0.12 , thus showing approximately 2% error between wells.

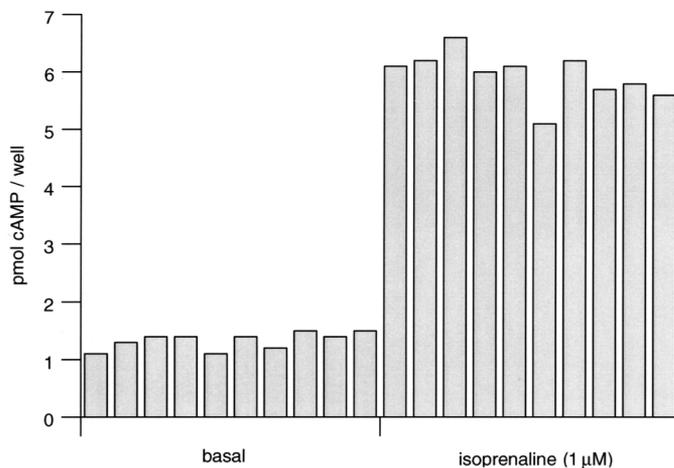
Isoprenaline stimulated cAMP accumulation (Fig. 3) with a $pEC_{50} = 7.8 \pm 0.1$ and could be dose-dependently blocked by the β receptor antagonist pindolol. Schild analysis of this data gave a pK_b for pindolol = 9.1 ± 0.1 (Fig. 4) which is consistent with its affinity for native β receptors (Tsuchihashi *et al.* 1989). Pindolol also appeared to depress basal levels of cAMP (Fig. 3) which could be due to the presence of endogenous agonist or that pindolol has negative intrinsic activity.

Figure 1



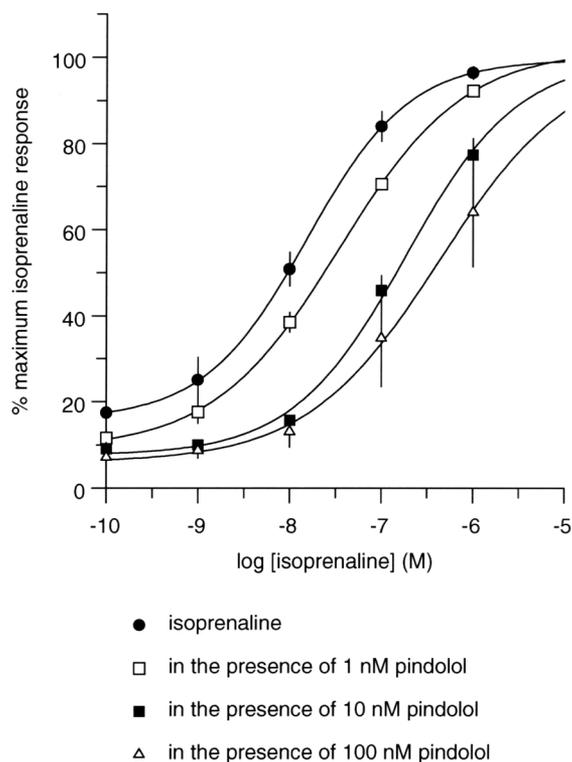
Effect of increasing number of CHO cells expressing hβ-2 receptors per well on basal and isoprenaline-stimulated levels of cAMP. Results are shown as pmol cAMP produced in each well.

Figure 2



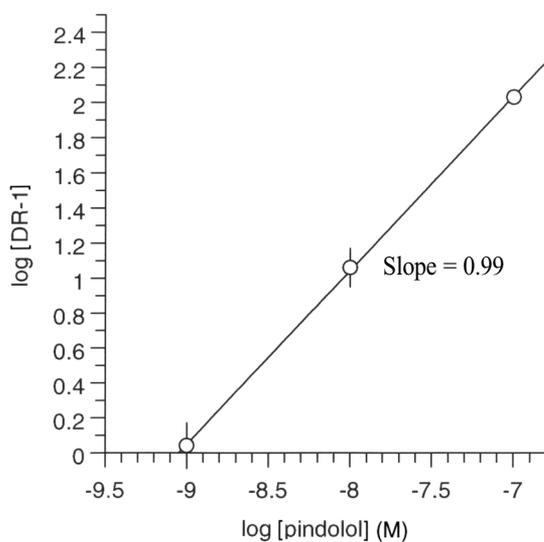
Intra-assay variation within a single FlashPlate. Each well contains 1×10^5 cells expressing hβ-2 receptors. Results show cAMP levels produced in individual wells in the absence of (basal) and presence of $1 \mu\text{M}$ isoprenaline.

Figure 3



Concentration-related response to isoprenaline in CHO cells expressing hβ-2 receptors. Cells (1×10^6 per well) were exposed to increasing concentrations of isoprenaline in the absence or presence of 1, 10 or 100 nM pindolol. Results are shown as % maximum isoprenaline response and are mean \pm s.e.m. of 3 experiments.

Figure 4



Schild analysis of potency of pindolol at antagonizing isoprenaline-induced stimulation of cAMP levels. Results show the mean \pm s.e.m. of 3 experiments.

Discussion

We have investigated the use of novel FlashPlate technology to directly measure cAMP accumulation in CHO cells expressing h β -2 receptors. Previous assay methods involve a 2-step process: 1) generation of cAMP and 2) measurement of cAMP levels, whereas this new method is comprised of a single step process that can be carried out in either 96-well or 384-well FlashPlates.

Our studies revealed that 1×10^5 cells per well produced a suitable signal, although this may change depending on the cell line, receptor type and/or G protein-coupling involved. It is therefore essential to validate each new system that is to be used. Studies on assay variability showed the production of cAMP to be robust within plates, and measurement of pharmacological parameters of standard compounds (pEC₅₀ for isoprenaline and pK_b for pindolol) showed robust responses between plates.

In conclusion, Adenylyl Cyclase Activation FlashPlate Assay can be used to directly measure the production of cAMP from CHO cells expressing human β -2 adrenoreceptors. This provides a simple method that can be easily automated and hence be used as a high-throughput assay.

References

P.A. Kasila. A Novel Adenylyl Cyclase Activation Assay on FlashPlate, *FlashPlate File #1*, PerkinElmer Life Sciences (1998).

Tsuchihashi *et al.* Binding Characteristics of ³H-CGP12177 to β -adrenoreceptors in Rat Myocardial Membranes. *Japan. J. Pharmacol.* 49, 11-19 (1989).

J. Watson. *In Vitro* Measurement of the Second Messenger cAMP: RIA vs. FlashPlate, *FlashPlate File #7*, PerkinElmer Life Sciences (1998).



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