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[^{3}H]Dofetilide binding to HERG transfected membranes: a potential high throughput preclinical screen

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Abstract

The pharmacological characteristics of [^{3}H]dofetilide binding were examined in membranes prepared from human embryonic kidney (HEK293) cells stably expressing human ether-á-go-go related gene (HERG) K^+ channels. The classIII antiarrhythmic compounds dofetilide, clofilium, 4′-[[1-[2-(6-methyl-2-pyridyl)ethyl]-4-piperidyl]carbonyl]methanesulfonanilide (E-4031), *N*-methyl-*N*-[2-[methyl-(1-methyl-1*H*-benzimidazol-2-yl)amino]ethyl]-4-[(methylsulfonyl)amino]benzene-sulfonamide (WAY-123,398) and *d*-sotalol all inhibited [^{3}H]dofetilide binding. In addition, the structurally unrelated compounds pimozide, terfenadine and haloperidol, all of which prolong the QT interval in man, also inhibited binding. These data indicate that a [^{3}H]dofetilide binding assay using HERG membranes may help identify compounds that prolong the QT interval. © 2001 Published by Elsevier Science B.V.

Keywords: [^{3}H]dofetilide; HERG; QT-interval

Long QT syndrome is a potentially lethal cardiac condition associated with mutations in genes that encode for ion channel proteins. Of these, the best recognised is the K^+ channel I_{Kr}, encoded by the human ether-á-go-go related gene (HERG; De Ponti et al., 2000). An interaction with HERG has been implicated in the cardiotoxic effects of second-generation anti-histamines and other drugs that have been withdrawn from the market in recent years. At present, screening for long QT potential in industry is being evaluated using animals (electrocardiogram), cardiac tissue (microelectrode), isolated myocytes and HERG transfected cells (patch clamp). However, these approaches are time-consuming and expensive and the need for a simple high throughput assay has been highlighted (Netzer et al., 2001). In this study, we show that [^{3}H]dofetilide binding to HERG transfected membranes is inhibited by antiarrhythmics and structurally unrelated compounds known to prolong the QT interval in man.

Human embryonic kidney (HEK293) cells stably expressing HERG (Zhou et al., 1998) were maintained in culture and membranes prepared as described previously (Finlayson et al., 2001a). [^{3}H]dofetilide binding to HERG transfected membranes was performed in a sodium buffer without bovine serum albumin, as for whole cell studies (Finlayson et al., 2001b). Assay buffer or test drug (30 μl) was incubated with 20 μl of [^{3}H]dofetilide (final concentration 10 nM) and 150 μl of membranes at 37°C for 60 min. Binding was terminated by filtration onto glass filters (GF/C in 0.25% polyethylenimine), followed by three rapid washes (2 ml) with Tris–HCl buffer, radioactivity was determined by scintillation counting and data were analysed as described before (Finlayson et al., 2001b).

[^{3}H]dofetilide binds to an unidentified endogenous protein in whole HEK293 cells, but not to membranes prepared from these cells. By examining [^{3}H]dofetilide binding to membranes from HERG transfected HEK293 cells, we avoided any contribution from the endogenous binding site (Finlayson et al., 2001b). The affinity ($K_d = 58.6 \pm 13.5$ nM; $n = 5$) and Hill slope ($n_H = 0.82 \pm 0.04$) of [^{3}H]dofetilide were similar to that described previously (Finlayson et al., 2001b). However, the rank order of potency for antiarrhythmic compounds was different from

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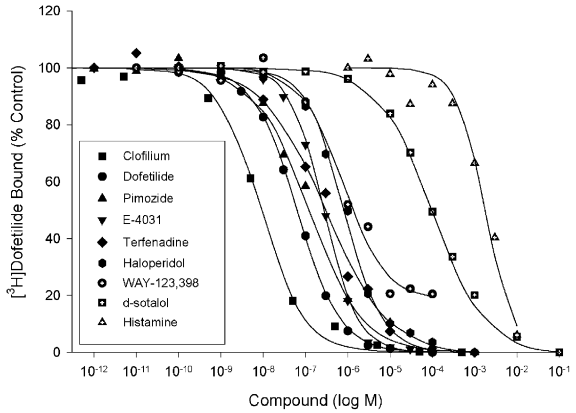

Fig. 1. Inhibition of [^{3}H]dofetilide binding in HERG transfected membranes. Membranes were spun, resuspended in assay buffer and [^{3}H] dofetilide binding was performed as described. Data shown are representative competition curves from single experiments; K_d / K_i values were determined from independent experiments and are shown in the text.

previous studies in whole cells: clofilium ($K_i = 8.12 \pm 0.37$ nM, $n_H = 1.10 \pm 0.19$, n = 4), 4′-[[1-[2-(6-methyl-2-pyridyl)ethyl]-4-piperidyl]carbonyl]methanesulfonanilide (E-4031; $K_i = 241 \pm 29.0$ nM, $n_H = 1.13 \pm 0.16$, $n = 4$), *N*-methyl-*N*-[2-[methyl-(1-methyl-1*H*-benzimidazol-2-yl)-amino]ethyl]-4-[(methylsulfonyl)amino]benzene-sulfonamide (WAY-123,398; $K_i = 687 \pm 77.5$ nM, $n_H = 1.18 \pm 0.15$, $n = 4$) and *d*-sotalol ($K_i = 134 \pm 22.5$ μM, $n_H = 0.72 \pm 0.06$, $n = 5$) (Fig. 1). The high affinity of clofilium in this assay suggests that it may be a useful ligand for HERG. Furthermore, LY97241 a tertiary analogue of clofilium, with 10-fold higher affinity for HERG, may be better still (Suessbrich et al., 1997). Interestingly, WAY-123,398, which did not inhibit [^{3}H]dofetilide binding to whole HEK293 cells (Finlayson et al., 2001b), reduced [^{3}H]dofetilide binding to HERG membranes, although not completely (Fig. 1). In addition, [^{3}H]dofetilide binding to HERG membranes was also reduced in a concentration dependent manner by the antipsychotics pimozide ($K_i = 70.6 \pm 11.8$ nM, $n_H = 0.89 \pm 0.12$, $n = 4$) and haloperidol ($K_i = 549 \pm 83.6$ nM, $n_H = 0.75 \pm 0.07$, $n = 4$), and by the antihistamine terfenadine ($K_i = 381 \pm 60.0$ nM, $n_H = 0.76 \pm 0.14$, $n = 4$), all of which cause QT prolongation (Kang et al., 2000; Vandenberg et al., 2001). The affinities of pimozide, terfenadine and haloperidol are consistent with those in electrophysiological studies (Suessbrich et al., 1997; Kang et al., 2000). Intriguingly, high concentrations of histamine also inhibited binding ($K_i = 1.58 \pm 0.13$ mM, $n_H = 1.16 \pm 0.19$, $n = 4$, Fig. 1).

It has been questioned whether a nonfunctional assay such as [^{3}H]dofetilide binding could be used as a preclinical screen for HERG channel blockers, as compounds chemically unrelated to dofetilide may act at different sites on the channel (Netzer et al., 2001). However, we have shown that unrelated molecules known to cause long QT prolongation such as pimozide, terfenadine and haloperidol also inhibit [^{3}H]dofetilide binding, perhaps due to the unique properties and pore size of this channel (Vandenberg et al., 2001). Therefore, this assay could provide a useful preclinical screen to detect compounds that may prolong the QT interval in man.

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