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Aldosterone, STX and amyloid-$\beta_{1\text{-}42}$ peptides modulate GPER (GPR30) signalling in an embryonic mouse hippocampal cell line (mHippoE-18)

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ABSTRACT

The GPCR, GPER, mediates many of the rapid, non-genomic actions of 17β-estradiol in multiple tissues, including the nervous system. Controversially, it has also been suggested to be activated by aldosterone, and by the non-steroidal diphenylacrylamide compound, STX, in some preparations. Here, the ability of the GPER agonist, G-1, and aldosterone in the presence of the mineralocorticoid receptor antagonist, eplerenone, to potentiate forskolin-stimulated cyclic AMP levels in the hippocampal clonal cell line, mHippoE-18, are compared. Both stimulatory effects are blocked by the GPER antagonist G36, by PTX, (suggesting the involvement of Gi/o G proteins), by BAPTA-AM, (suggesting they are calcium sensitive), by wortmannin (suggesting an involvement of PI3Kinase) and by soluble amyloid-β peptides. STX also stimulates cyclic AMP levels in mHippoE-18 cells and these effects are blocked by G36 and PTX, as well as by amyloid-β peptides. This suggests that both aldosterone and STX may modulate GPER signalling in mHippoE-18 cells.

1. Introduction

Evidence is accumulating that many of the rapid, non-genomic actions of steroids may be mediated by specific membrane located receptors (Wehling, 2017). However, the specific identity of the receptors for individual steroids is still highly controversial. One G-protein-coupled receptor (GPCR), GPER (GPR30) has been extensively characterized as a receptor for the rapid, non-genomic actions of estrogen in a wide variety of cancer cell lines and in tissues, such as the brain (Prossnitz and Arterburn, 2015; Barton et al., 2018; Hadjimarkou and Vasudevan, 2018). In many cases, it has been shown to signal from the plasma membrane (Cheng et al., 2011) but has also been suggested to be able to signal from endoplasmic reticular membranes (Revankar et al., 2005). The latter suggestion would not be problematic for lipid soluble molecules, such as steroids. In addition, it should be noted that a considerable number of non-steroid activated GPCRs are now thought to be capable of activating a range of second messenger pathways from endosomal locations (Irannejad et al., 2013; West and Hanyaloglu, 2015). A range of other steroids has been shown to signal to intracellular second messenger pathways via plasma membrane located receptors. These include the mineralocorticoid hormone, aldosterone (see Wehling, 2017). However, there have been suggestions that the plasma membrane located receptor for aldosterone may actually be GPER in some tissues (Gross et al., 2011, 2013; Batenburg et al., 2012; Ashton et al., 2015) and renal cells (Ren et al., 2014; Feldman et al., 2016). However, the suggestion has been questioned in the absence of detailed pharmacological signalling studies and in the absence of convincing binding studies (Wendler and Wehling, 2011; Barton and Meyer, 2015; Wehling, 2017).

GPER is able to activate a wide range of second messenger pathways in different cell types (see Srivastava and Evans, 2013; Prossnitz and Arterburn, 2015; Alexander et al., 2017; Barton et al., 2018). It appears to be able to regulate intracellular cyclic AMP levels in hippocampal and other neuronal cells. In previous studies, we have characterized the signalling properties of GPER in an immortalized embryonic hippocampal cell line, mHippoE-18 (Gingerich et al., 2010; Evans et al., 2016). This preparation overcomes many of the difficulties associated with the identification of the molecular pathways activated by GPER in intact brain tissue, in primary cultures of isolated hippocampal neurons or in clonal cell lines over expressing the receptor (Gingerich et al., 2010). GPER has a high expression level in mHippoE18-cells combined with a moderate expression level of the classical estrogen receptors, ERα and ERβ. We have shown that GPER activation in this preparation, by either 17β-estradiol or the GPER agonist, G-1, can lead to a dose-dependent increase in forskolin-stimulated cyclic AMP levels (Evans et al., 2016). However, it does not seem to change the levels of activation of the mitogen-activated protein kinase (MAPK) pathway as measured by the level of phosphorylation of ERK1/2 in these cells. The GPER antagonists, G15 and G36, block these increases in forskolin-stimulated cyclic AMP levels. They are also mimicked by the actions of

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tamoxifen and ICI 182,780, compounds which have been shown to activate GPER in other tissues (Evans et al., 2016). In contrast, aldosterone produced a decrease in forskolin-stimulated cyclic AMP levels in these cells at concentrations between 10^{-10} M and 10^{-9} M (Evans et al., 2016).

The present study reports on a comparison of the molecular basis of the effects of G-1 on GPER in mHippoE-18 cells, with those of aldosterone. It also explores the question of whether the diphenylacrylamide ligand, STX, can also activate GPER in these cells. In addition, it also explores the modulation of GPER induced second messenger effects by various agonists in this preparation after exposure to soluble amyloid-β peptides.

2. Materials and methods

2.1. Culture of mHippoE-18 cells

mHippoE-18 cells were obtained from VH Bio Ltd and maintained in culture as recommended by CELLutions Biosystems Inc., Burlington, Ontario, Canada. Briefly, cells were grown in 1x Dulbecco's Modified Eagle's medium (DMEM) with 10% fetal bovine serum (FBS – Hyclone Fetal Bovine Serum Charcoal/Dextran Treated. Fischer Scientific 10611235), 25 mM glucose and 1% penicillin/streptomycin and maintained at 37 °C with 5% CO_2 in 12 well plates. Prior to incubation cells were serum starved for 16 h overnight in DMEM minus phenol red (21063-045 InVitrogen, Fischer Scientific).

2.2. Preparation of soluble oligomers of amyloid-β peptides

Conditioned medium was obtained from Chinese Hamster Ovary cells stably expressing human APP_{751} Alzheimer disease associated V751 mutant (7PA2 cells) (Koo and Squazzo, 1994) using the method of Walsh et al. (2000, 2005). Before use medium was concentrated 10-fold using YM-3 Centriprep filters (Amicon). 50 μl of concentrated conditioned medium was added to each well of mHippoE-18 cells containing 1 ml of DMEM minus phenol red for a 1 h pre-incubation.

Synthetic amyloid $\beta_{1\text{-}42}$ was prepared using the protocol of Origlia et al. (2009). Briefly, 1 mg of peptide was suspended in 100% 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP, Sigma-Aldrich) and the HFIP allowed to evaporate overnight in a fume hood. Twenty-four hours prior to use, the aliquot was taken up into 50 μl of DMSO. This was dispersed into 1 ml of DMEM minus neutral red to give a 100 μM stock and left at 4 °C for 24 h to allow formation of soluble amyloid $\beta_{1\text{-}42}$ oligomers. This synthetic amyloid $\beta_{1\text{-}42}$ preparation has been characterized both biochemically and electrophysiologically, and shows similar biological effects as naturally secreted soluble amyloid $\beta_{1\text{-}42}$ oligomers (Haass and Selkoe, 2007).

2.3. Cyclic AMP determination

Cyclic AMP levels in mHippoE-18 cells were determined as described previously in detail (Burman et al., 2009; Burman and Evans, 2010; Srivastava et al., 2005; Bayliss et al., 2013), except 100 μM isobutylmethylxanthine (IBMX) was used. Briefly, cells were pre-incubated with 100 μM IBMX for 20 min, followed by incubation with 10 μM forskolin and 100 μM IBMX in the presence of increasing concentrations of the various agonists for a further 20 min. In experiments where antagonists and inhibitors were used, mHippoE-18 cells were pre-incubated with 100 μM IBMX and various concentrations of antagonists or inhibitors for 20 min. This was followed by incubation with varying concentrations of agonist, antagonists or inhibitors, plus 10 μM forskolin and 100 μM IBMX for a further 20 min. Cyclic AMP levels were measured using a [^{3}H]-cyclic AMP (NET275, PerkinElmer) protein kinase A radiometric binding assay (Munirathinam and Yoburn, 1994).

Forskolin was used both to increase basal cyclic AMP levels to make it easier to detect increases and decreases in cyclic AMP levels in the same experiments and also to potentiate responses to agonists to more accurately determine their threshold effects (Insel and Ostrom, 2003). A non-saturating 10 μM concentration of forskolin was used. Basal levels of mHippoE-18 cell cyclic AMP were 5.1 ± 0.42 pmoles/mg protein (n = 12) and these were raised to 160.9 ± 7.2 pmoles/mg protein (n = 123) after exposure to 10 μM forskolin. Protein levels were determined using a Bradford assay.

2.4. Statistics

Cyclic AMP levels are represented as a percentage of basal levels determined from three appropriate control wells on each twelve well dish. One-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison correction was used to test for significance in GraphPad Prism, with $p < 0.05$ considered significant. All data are shown as mean ± SEM. Each data point plotted was the mean of data obtained from six to eight different experiments for conditions where cyclic AMP levels were increased and from at least three experiments for conditions where increases in cyclic AMP levels were blocked, unless stated otherwise. Within each experiment, three separate replicate wells were analysed for each condition and the cyclic AMP assays on each of the wells was carried out in duplicate.

2.5. Drugs

The drugs used in these experiments were obtained from the following sources:

17β-Estradiol, aldosterone, eplerenone, pertussis toxin (PTX), wortmannin, BAPTA-AM and IBMX were purchased from Sigma-Aldrich (Poole, Dorset, UK); G-1, G15, G36, were purchased from Tocris Bioscience (Bristol, UK). Forskolin was obtained from Abcam Biochemicals (Cambridge, UK). Amyloid-$\beta_{1\text{-}42}$ HCL was obtained from rPeptide. We thank Professor Jeffrey Arterburn, New Mexico State University, Las Cruces, New Mexico, USA for initial samples of G36. We also thank Professors Martin Kelly and Philip Copenhaver, Oregon Health and Science University, Portland, Oregon, USA for the sample of STX.

All drugs were dissolved to give a stock solution of either 10^{-2} or 10^{-3} M in 100% ethanol, except for STX and BAPTA which were dissolved in DMSO to give stocks of 10^{-3} M. All experimental solutions were obtained by appropriate dilution of stocks in phenol red free DMEM containing 100 μM IBMX. Appropriate vehicle controls were added to all basal wells on each 12 well plate. In no case did the final experimental solutions contain more than 1% DMSO. The final concentrations of ethanol in the experimental solutions was from 1% to 1.3% depending upon the number of experimental drugs used in each experiment.

3. Results

3.1. G-1 and aldosterone activation of adenylyl cyclase activity in mHippoE-18 cells

In the present study, the dose-dependent increases in forskolin-stimulated cyclic AMP levels produced by G-1 in mHippoE-18 cells had a threshold for a significant effect between 10^{-10} M and 3×10^{-10} M (Fig. 1A). The G-1 stimulatory effects were blocked in the presence of 10^{-6} M of the GPER antagonist, G36 (Fig. 1A). However, 10^{-6} M eplerenone, a specific mineralocorticoid receptor antagonist, did not significantly affect the dose-dependent stimulatory effects of G-1 alone, or the blocking of the effects of G-1 in the presence of 10^{-6} M G36 (Fig. 1A).

In contrast, the slight inhibitory effects of aldosterone alone on forskolin-stimulated cyclic AMP levels were converted into a dose-dependent stimulation in the presence of 10^{-6} M eplerenone, with a threshold for a significant response between 10^{-8} M and 3×10^{-8} M

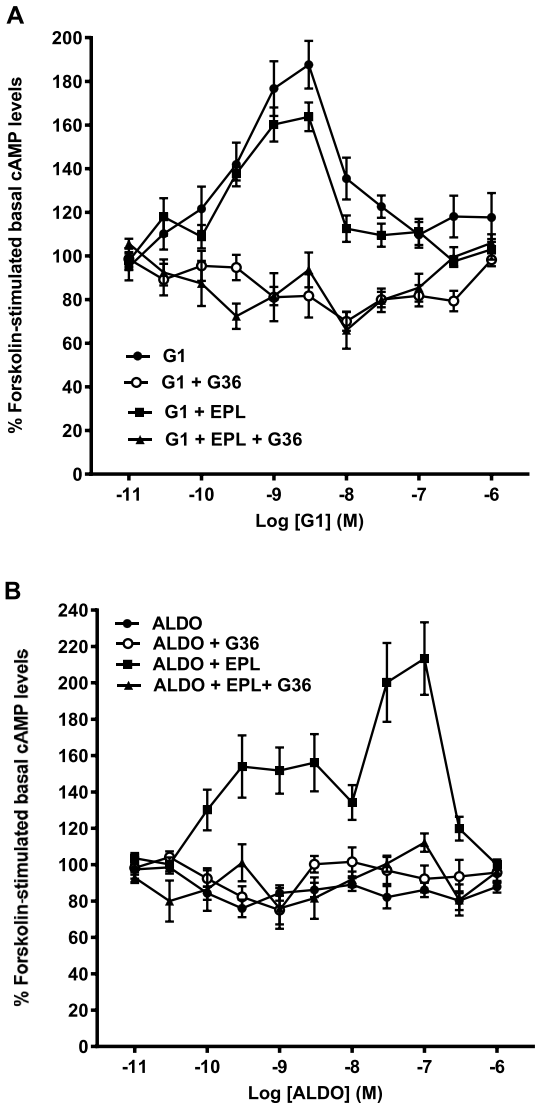

Fig. 1. Effects of the GPER antagonist, G36, and the mineralocorticoid receptor antagonist, eplerenone (EPL), on (A) G-1 and (B) aldosterone (ALDO) forskolin-stimulated cyclic AMP levels in mHippoE-18 cells. mHippoE-18 cells were pre-incubated with 100 μM IBMX, plus or minus 1 μM of either antagonist, for 20 min, followed by incubation with varying concentrations of either agonist, plus or minus 1 μM of either or both antagonists, 10 μM forskolin and 100 μM IBMX for a further 20 min. The basal values in the absence of agonist and antagonist are shown as 100%. Values are significantly different from basal as follows, with experiment numbers (n) in brackets: G-1 alone (n = 8), 0.3 nM, p = 0.0495; 1 nM, p = 0.0278; 3 nM, p = 0.0028 and 10 nM, p = 0.0462. G-1 + EPL (n = 3), 0.3 nM, p = 0.05; 1 nM, p = 0.0240; 3 nM, p = 0.0248. G-1 + G36 (n = 5). G-1 + G36 + EPL (n = 3). ALDO, (n = 7). ALDO + EPL, (n = 8) 30 nM, p = 0.0352; 100 nM, p = 0.0112. ALDO + G36, (n = 3). ALDO + EPL + G36, (n = 3).

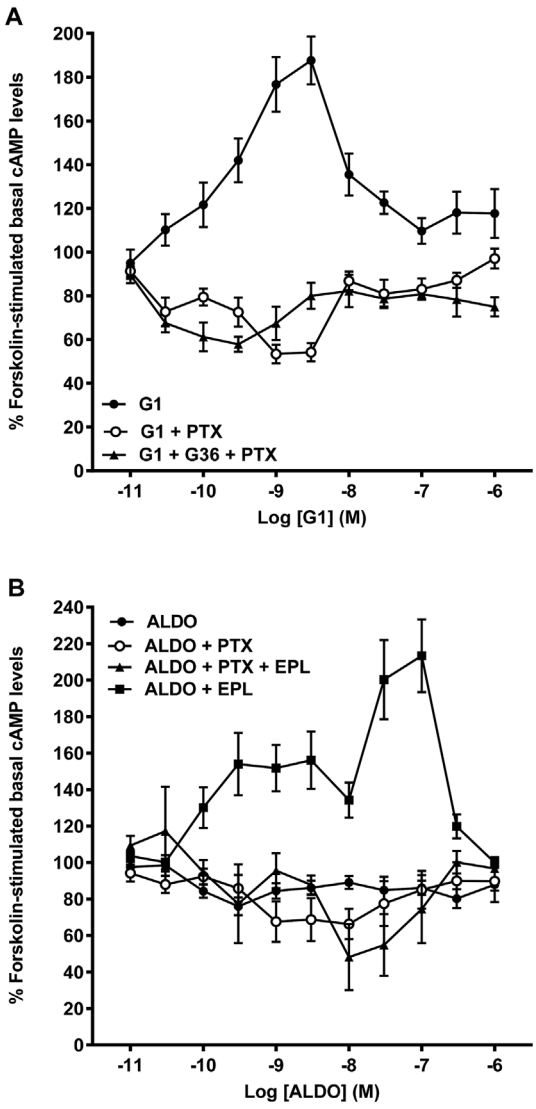

Fig. 2. Effects of PTX on (A) G-1 or G-1 plus G36 and (B) aldosterone alone (ALDO), or in the presence of eplerenone (ALDO + EPL), forskolin-stimulated cyclic AMP levels in mHippoE-18 cells. mHippoE-18 cells were pre-incubated with PTX (100 ng/ml) overnight before exposure to 100 μM IBMX, plus or minus 1 μM of either G36 or eplerenone as appropriate, for 20 min, followed by incubation with varying concentrations of either agonist, plus or minus 1 μM antagonist as appropriate, 10 μM forskolin and 100 μM IBMX for a further 20 min. The basal values in the absence of agonist and antagonist are shown as 100%. The dose-dependent agonist responses to G-1, aldosterone and aldosterone plus eplerenone, are shown for comparison. Values are significantly different from basal as follows with experiment numbers in brackets: G-1, ALDO and ALDO + EPL, see Fig. 1. G-1 + PTX, (n = 3), 1 nM p = 0.0496; 3 nM p = 0.0316. (N = 3 for all other traces).

(Fig. 1B). The apparent shoulder on the dose-response curve at lower aldosterone concentrations, between 10^{-10} M and 10^{-8} M, appears due to a small, consistent, higher affinity component of the curve with maximal values at different aldosterone concentrations in different experiments, which did not quite reach the significance threshold (10^{-9} M, p = 0.0714; 3×10^{-9} M, p = 0.0746; 10^{-8} M, p = 0.0672). In addition, both components of the stimulatory effects of aldosterone on forskolin-stimulated cyclic AMP levels in the presence of 10^{-6} M eplerenone were blocked in the presence of 10^{-6} M G36 (Fig. 1B), suggesting that aldosterone may be producing these stimulatory effects via an action on GPER. The inhibitory effects of aldosterone alone were not blocked by 10^{-6} M G36 and were not significantly different from the inhibitory effects produced by aldosterone in the presence of 10^{-6} M eplerenone plus 10^{-6} M G36 (Fig. 1B).

Exposure of mHippoE-18 cells to either 10^{-6} M G36 alone, or to 10^{-6} M eplerenone alone, did not produce any significant changes in cyclic AMP from control levels (G36 alone, 100.7 ± 3.1%; n = 45. Eplerenone alone, 106.2 ± 3.8; n = 36).

3.2. Pathway of GPER activation of adenylyl cyclase activity in mHippoE-18 cells

In the present study, the mechanism of activation of the increase in

forskolin-stimulated cyclic AMP levels after GPER stimulation by different agonists has been investigated. The dose-dependent increases in forskolin-stimulated cyclic AMP levels in mHippoE-18 cells initiated by either G-1, or aldosterone in the presence of 10^{-6} M eplerenone, were inhibited by pre-treatment with 100 ng/ml pertussis toxin (PTX) for 24 h (Fig. 2A and B). This suggests that these effects were mediated by GPER activation of a $G_{i/o}$ pathway. The treatment with PTX did not alter the inhibition of forskolin-stimulated cyclic AMP levels induced after either exposure of the cells to G-1 in the presence of 10^{-6} M G36 or exposure of the cells to aldosterone alone. Exposure of mHippoE-18 cells to 100 ng/ml PTX alone, did not produce any significant changes in cyclic AMP from control levels (PTX alone, 96.2 ± 4.7%; n = 17).

The activation of the $G_{i/o}$ pathway is usually associated with an inhibition of the production of cyclic AMP. Thus, the possibility was investigated that the G-1 mediated dose-dependent increases in forskolin-stimulated cyclic AMP levels in mHippoE-18 cells were the secondary result of increases in intracellular calcium levels. Such increases in calcium have been shown to trigger the activation of one of the forms of calcium/calmodulin-stimulated adenylyl cyclase known to be present in hippocampal neurons (Ferguson and Storm, 2004). Exposure of mHippoE-18 cells to 10^{-5} M BAPTA-AM blocked the dose-dependent increases in forskolin-stimulated cyclic AMP levels initiated by either G-1, or aldosterone in the presence of 10^{-6} M eplerenone, suggesting that these increases were calcium dependent (Fig. 3A and B). This treatment with BAPTA-AM did not alter the modest dose-dependent inhibition of forskolin-stimulated cyclic AMP levels initiated by exposure of the cells to aldosterone alone (Fig. 3B). However, the dose-dependent inhibition of forskolin-stimulated cyclic AMP levels initiated by exposure of the cells to G-1 in the presence of 10^{-5} M BAPTA-AM was increased in the presence of 10^{-6} M G36, at G-1 concentrations between 3×10^{-10} M and 10^{-8} M by an unknown mechanism. Exposure of mHippoE-18 cells to 10^{-5} M BAPTA-AM alone, did not produce any significant changes in cyclic AMP from control levels (BAPTA-AM alone, 106.3 ± 4.5%; n = 12). The reason for the above additivity of the effects of BAPTA-AM and G36 on the G-1 responses is unknown at present.

Many of the actions of GPER activation of the $G_{i/o}$ pathway, in a range of cell types, have also been shown to be linked to the activation of the PI3Kinase/Akt pathway. Thus, the effect of inhibition of the latter pathway by exposure of the cells to 10^{-7} M wortmannin was examined. Fig. 4 A and B show that exposure of mHippoE-18 cells to this concentration of wortmannin blocked the dose-dependent increases in forskolin-stimulated cyclic AMP levels initiated by either G-1, or aldosterone in the presence of 10^{-6} M eplerenone (Fig. 4A and B). This suggests that these increases were dependent also on the PI3Kinase/Akt pathway in mHippoE-18 cells. The dose-dependent inhibitory effects of G-1 in the presence of wortmannin, were also increased in the presence of 10^{-6} M G36 at concentrations between 3×10^{-10} M and 3×10^{-9} M by an unknown mechanism. The modest dose-dependent inhibition of forskolin-stimulated cyclic AMP levels initiated by exposure of the cells to aldosterone alone was not blocked by wortmannin. Exposure of mHippoE-18 cells to 10^{-7} M wortmannin alone, did not produce any significant changes in cyclic AMP from control levels (wortmannin alone, 104.6 ± 3.9%; n = 18).

3.3. The effect of amyloid-β-peptide on GPER activation of adenylyl cyclase activity in mHippoE-18 cells

Evidence is accumulating to suggest that some of the rapid effects of estrogen, on memory and cognition (Alexander et al., 2017) and on neuroprotection (Gingerich et al., 2010; Tang et al., 2014; Alexander et al., 2017) in the hippocampus, may be mediated by the activation of GPER. In addition, several forms of synaptic plasticity, including long-term potentiation in the hippocampus, appear to require calcium-dependent increases in cyclic AMP levels (Xia and Storm, 2012), which can be inhibited by the actions of soluble oligomeric forms of amyloid-

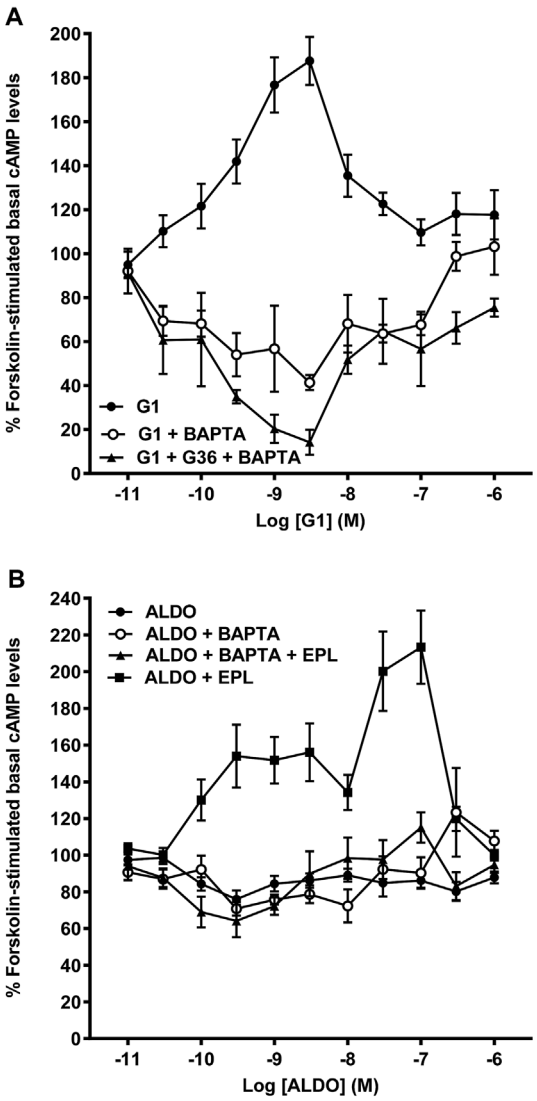

Fig. 3. Effects of BAPTA-AM on (A) G-1 and (B) aldosterone alone (ALDO), or in the presence of eplerenone (ALDO + EPL), forskolin-stimulated cyclic AMP levels in mHippoE-18 cells. mHippoE-18 cells were pre-incubated with 10 μM BAPTA-AM, 100 μM IBMX, plus or minus 1 μM of either antagonist (G36 or eplerenone), for 20 min, followed by incubation with varying concentrations of either agonist, 10 μM BAPTA-AM, plus or minus 1 μM of either antagonist, 10 μM forskolin and 100 μM IBMX for a further 20 min. The basal values in the absence of agonist and antagonist are shown as 100%. The dose-dependent agonist responses to G-1, aldosterone and aldosterone plus eplerenone are shown for comparison. Values are significantly different from basal as follows with experiment numbers in brackets: G-1, ALDO and ALDO + EPL, see Fig. 1. (N = 3 for all other traces). G-1 + BAPTA, 0.3 nM p = 0.050; 3 nM p = 0.0088. G-1 + BAPTA + G36, 0.3 nM, p = 0.0046; 1 nM, p = 0.0034; 3 nM, p = 0.0011; 10 nM, p = 0.0092.

$\beta_{1\text{-}42}$ peptide (Vitolo et al., 2002; Puzzo et al., 2005; Rebola et al., 2017). Thus, the ability of soluble oligomeric forms of amyloid-$\beta_{1\text{-}42}$ to modulate the GPER induced increases in calcium-dependent forskolin-stimulated cyclic AMP levels in mHippoE-18 cells was examined. The dose-dependent stimulatory effects of G-1 on forskolin-stimulated cyclic AMP levels in mHippoE-18 cells were reduced by exposure to a 1 h pre-incubation with 2 nM soluble amyloid-$\beta_{1\text{-}42}$ peptide and blocked by exposure to a 1 h pre-incubation of either 20 nM soluble amyloid-$\beta_{1\text{-}42}$ peptide or amyloid-β conditioned medium (Fig. 5A). Further, the two components of the dose-dependent stimulatory effects of aldosterone on forskolin-stimulated cyclic AMP levels in mHippoE-18 cells in the presence of 10^{-6} M eplerenone were also blocked after exposure to 20 nM

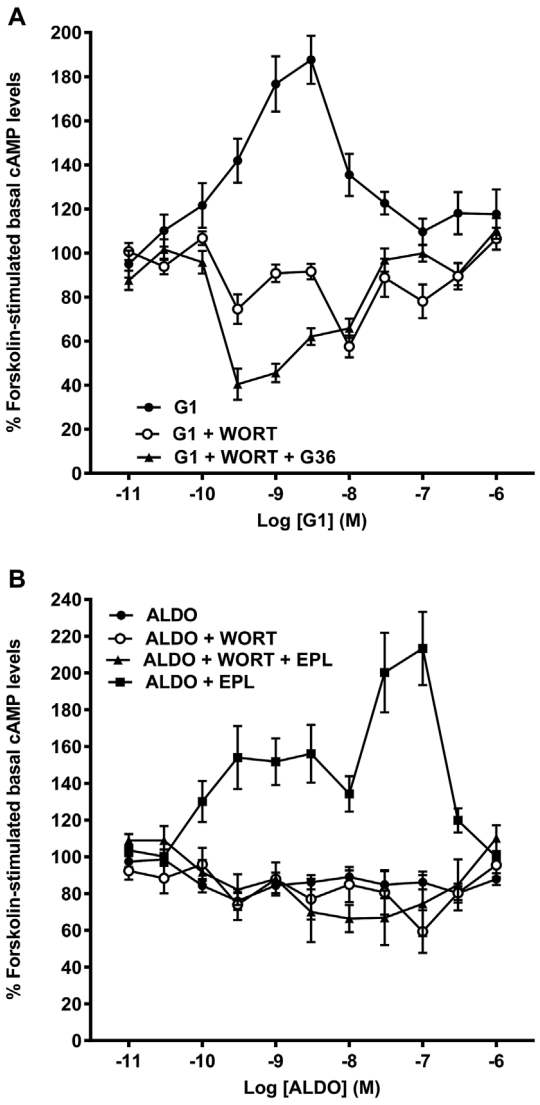

Fig. 4. Effects of wortmannin (WORT) on (A) G-1 or G-1 plus G36 and (B) aldosterone alone (ALDO), or in the presence of eplerenone (ALDO + EPL), forskolin-stimulated cyclic AMP levels in mHippoE-18 cells. mHippoE-18 cells were pre-incubated with 100 nM WORT, 100 μM IBMX, plus or minus 1 μM of either antagonist (G36 or eplerenone), for 20 min, followed by incubation with varying concentrations of either agonist, 100 nM WORT, plus or minus 1 μM of either antagonist, 10 μM forskolin and 100 μM IBMX for a further 20 min. The basal values in the absence of agonist and antagonist are shown as 100%. The dose-dependent agonist responses to G-1, aldosterone and aldosterone plus eplerenone are shown for comparison. Values are significantly different from basal as follows with experiment numbers in brackets: G-1, ALDO and ALDO + EPL, see Fig. 1. (N = 3 for all other traces). G-1 + WORT, 10 nM, p = 0.0312. G-1 + WORT + G36, 0.3 nM p = 0.0017; 1 nM, p = 0.0003; 3 nM, p = 0.0045; 10 nM, p = 0.0303.

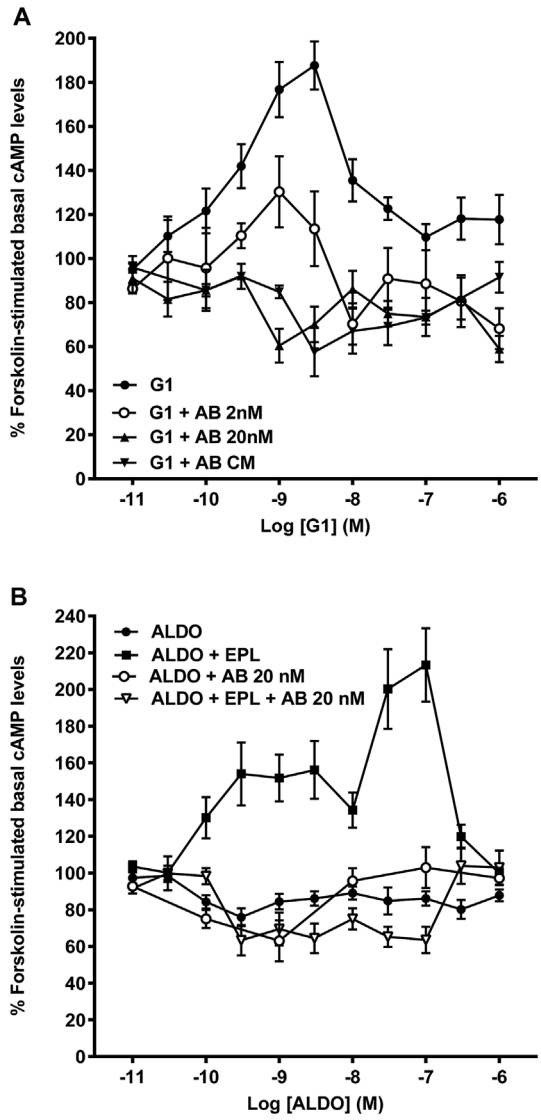

Fig. 5. Effects of Amyloid-β peptides on (A) G-1 and (B) aldosterone alone (ALDO), or in the presence of eplerenone (ALDO + EPL), forskolin-stimulated cyclic AMP levels in mHippoE-18 cells. AB CM represents Amyloid-β peptide conditioned media and AB 2 and 20 nM represents different concentrations of synthetic Amyloid-$\beta_{1\text{-}42}$ peptide. mHippoE-18 cells were pre-incubated for 1 h in the presence of appropriate levels of amyloid-β peptide, prior to addition of 100 μM IBMX for 20 min. They were then incubated with varying concentrations of either agonist, plus appropriate levels of amyloid-β peptide, 10 μM forskolin and 100 μM IBMX for a further 20 min. The basal values in the absence of agonist and amyloid-β peptide are shown as 100%. The dose-dependent agonist responses to G-1, aldosterone and aldosterone plus eplerenone, are shown for comparison. Values are significantly different from basal as follows with experiment numbers in brackets: G-1, ALDO and ALDO + EPL, see Fig. 1. (N = 3 for all other traces). G-1 + (AB 2 nM), 1 nM, p = 0.056; 10 nM, p = 0.0493. G-1 + (AB 20 nM), 1 nM, p = 0.0520. G-1 + (AB CM), 3 nM p = 0.0568; 10 nM, p = 0.0320; 30 nM p = 0.0523; 100 nM p = 0.0392.

amyloid-$\beta_{1\text{-}42}$ peptide (Fig. 5B). In contrast, the slight dose-dependent inhibitory effects of aldosterone alone were not blocked after exposure to 20 nM amyloid-$\beta_{1\text{-}42}$ peptide. Exposure of mHippoE-18 cells to 20 nM amyloid-$\beta_{1\text{-}42}$ peptide alone, did not produce any significant changes in cyclic AMP from control levels (amyloid-$\beta_{1\text{-}42}$ peptide alone, 107.7 ± 4.4%; n = 39).

3.4. STX activation of adenylyl cyclase activity in mHippoE-18 cells

The diphenylacrylamide STX combines structural elements of both 4-hydroxytamoxifen and raloxifene, compounds which have been reported to be potent agonists of GPER (Prossnitz and Arterburn, 2015). STX mimics many of the rapid non-genomic actions of 17β-Estradiol in different tissues, suggesting it might also function as a GPER selective agonist (Lin et al., 2009; Prossnitz and Arterburn, 2015). However, in some tissues STX might also activate additional membrane-based estrogen receptors, such as the postulated, but unidentified receptor, G_q-mER, since it still appears to be active in GPER knockout mice (Qiu et al., 2006; Roepke et al., 2009; Kelly and Rønnekleiv, 2015). Thus, in the present study, the effects of STX on forskolin-stimulated cyclic AMP levels in mHippoE-18 cells have also been assessed.

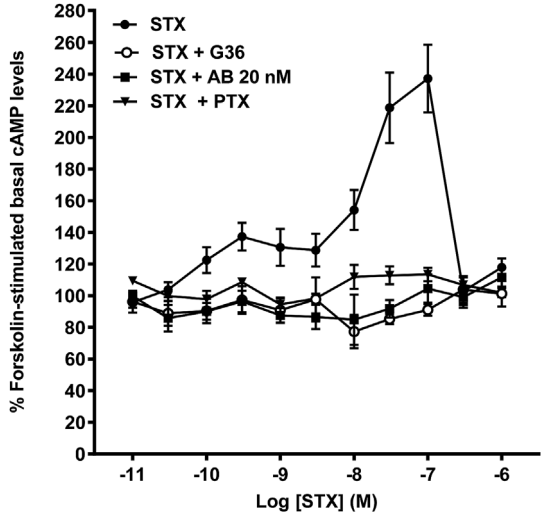

Fig. 6. Effects of the GPER antagonist, G36 (1 μM), PTX (100 ng/ml) or AB 20 nM on STX forskolin-stimulated cyclic AMP levels in mHippoE-18 cells. mHippoE-18 cells were pre-incubated with either PTX overnight, or AB 20 nM for 1 h, prior to addition of 100 μM IBMX 20 min. They were then incubated with varying concentrations of STX, in the presence or absence of 1 μM G36 as appropriate, plus 10 μM forskolin and 100 μM IBMX for a further 20 min. The basal values in the absence of agonist and antagonist are shown as 100%. Values are significantly different from basal as follows with experiment numbers in brackets: STX (n = 6) 10 nM, p = 0.0510; 30 nM, p = 0.0412; 100 nM, p = 0.0324. (N = 3 for all other traces).

STX produced a dose-dependent increase in forskolin-stimulated cyclic AMP levels in mHippoE-18 cells, with a threshold for a significant response occurring between 3×10^{-9} M and 10^{-8} M (Fig. 6). There was again an apparent shoulder on the dose-response curve at lower STX concentrations, between 10^{-10} M and 3×10^{-9} M. This also appears to be due to a small consistent higher affinity component of the curve with maximal values at different STX concentrations in different experiments, which did not reach the significance threshold. Both components of the STX response were blocked in the presence of 10^{-6} M of the GPER antagonist, G36, suggesting the involvement of GPER in this response of mHippoE-18 cells to STX. They were also blocked by overnight pre-exposure to PTX (100 ng/ml) suggesting an involvement of Gi/o G proteins. Additionally, they were also both blocked by a 1 h pre-exposure to 20 nM amyloid-$\beta_{1\text{-}42}$ peptide (Fig. 6).

4. Discussion

The G-protein coupled receptor sensitive to estrogen (GPER or GPR30) in the immortalized hippocampal cell line, mHippoE-18, induces a rapid and dose-dependent, increase in forskolin-stimulated cyclic AMP levels when stimulated by 17β-Estradiol or the GPER agonist, G-1 (Evans et al., 2016). In the present study, these effects have been shown to be sensitive to the presence of PTX, suggesting that they are mediated via a Gαi/o pathway. They have also been shown to be calcium-sensitive, since they are blocked after exposure to BAPTA-AM (10^{-5} M) and to involve the PI3Kinase/Akt pathway, since they are blocked by wortmannin (10^{-7} M). GPER is also coupled to a PTX sensitive Gαi/o pathway, which results in the activation of PI3Kinase, in a number of cancer cell lines (Prossnitz and Barton, 2011; Filardo and Thomas, 2012). This leads to the transactivation of the epidermal growth factor receptor, which in turn leads to the stimulation of the ERK signalling pathway. However, in mHippoE-18 cells 17β-Estradiol did not produce consistent activation of the MAPKinase pathway, as assayed by the level of phosphorylation of ERK1/2 (Evans et al., 2016). Nevertheless, GPER has been suggested to increase neuritogenesis in developing mouse hippocampal neurones via the activation of PI3Kinase, since it is inhibited by wortmannin (Ruiz-Palmero et al., 2013). In addition, GPER has been reported to elevate intracellular calcium levels in a number of different preparations (Revankar et al., 2005) by pathways which have variously been described as being completely (Filardo et al., 2000), or partially (Revankar et al., 2005), blocked by PTX, suggesting a role for Gαi/o G-proteins.

GPER has been reported to increase cyclic AMP levels by a Gαs activation of adenylyl cyclase in cancer cells (Thomas et al., 2005; Filardo and Thomas, 2012), in fish oocytes (Pang et al., 2008) and in vascular tissue (Lindsey et al., 2014). In contrast, the GPER mediated cyclic AMP increases in mHippoE-18 cells are calcium sensitive. This is consistent with GPER facilitation of calcium oscillations in GnRH neurons in the hypothalamus (Sun et al., 2010; Alexander et al., 2017) and by the presence of the AC1 and AC8 forms of calcium/calmodulin-stimulated adenylyl cyclase in hippocampal neurones (see Ferguson and Storm, 2004). Indeed, a calcium-dependent synthesis of cyclic AMP is essential for long-term potentiation (LTP) in hippocampal neurones (Chetkovich and Sweatt, 1993; Robertson and Sweatt, 1996; Balakishnan et al., 2016). Thus, GPER might have an important role in the modulation of hippocampal LTP.

The suggestion that the mineralocorticoid, aldosterone, can activate GPER is highly controversial (see Wendler and Wehling, 2011; Barton and Meyer, 2015; Hermidorff et al., 2017; Ruhs et al., 2017; Wehling, 2017). In the present study, it has been shown that the aldosterone-mediated inhibition of forskolin-stimulated cyclic AMP levels in mHippoE-18 cells can be converted into a dose-dependent stimulation in the presence of the specific mineralocorticoid receptor (MR) inhibitor, eplerenone. Further, this stimulation has many parallels with the effects of GPER activation by 17β-Estradiol, or the GPER agonist G-1, in this preparation. They can all be blocked by the presence of the GPER antagonist, G36. They are all sensitive to PTX, suggesting they are mediated by a Gαi/o G-proteins. They are all blocked in the presence of BAPTA-AM, suggestion they are calcium sensitive and they are all blocked in the presence of wortmannin, suggesting an involvement of PI3Kinase.

The above evidence raises the possibility that it might be possible to demonstrate an interaction of aldosterone with GPER in this preparation, when the effects of aldosterone on MR are blocked. In this context, it is interesting to note that aldosterone can also induce a calcium-dependent increase in cyclic AMP levels in primary cultures of cardiomyocytes in the presence of the MR antagonist, spironolactone (Araujo et al., 2016). Further, supporting evidence for an interaction between aldosterone and GPER has been provided in studies from the cardiovascular system (Gross et al., 2011, 2013; Batenburg et al., 2012; Ashton et al., 2015) and renal cells (Ren et al., 2014; Feldman et al., 2016). In addition, cross talk between GPER and MR has been reported in breast cancer cells (Rigiracciolo et al., 2015). Further, aldosterone has also been reported to couple GPER to the activation of the MAP-Kinase pathway when the receptor is heterologously expressed in HEK293 cells (Evans et al., 2014). However, definitive evidence that aldosterone can actually bind to GPER in any preparation is lacking and this represents a severe impediment to the acceptance of this hypothesis. Attempts to demonstrate the binding of aldosterone to GPER in plasma membrane fractions from whole kidney tissue, or from HEK293 cells expressing recombinant GPER, have been unsuccessful (Cheng et al., 2014). However, this study has been criticized for not providing all “the basic characteristics of binding parameters such as Kd and binding capacity” and only providing displacement curves (Wehling, 2017). Equally, the lack of binding of aldosterone to GPER in studies on breast cancer cells (Rigiracciolo et al., 2015) has been criticized because they were only carried out on whole cells (Wehling, 2017). Thus, Wehling (2017) has suggested that the lack of definitive evidence for the binding of aldosterone to GPER represents the strongest argument against GPER as an aldosterone receptor. However, Wehling (2017) points out that it is not unusual that binding studies cannot be reproduced in different systems, or under the different

conditions required to demonstrate specific binding of steroids to membrane fractions (see Wehling et al., 1991; Wehling, 2017). For example, the binding of ^{3}H-ponasterone to a Drosophila GPCR (DmDopEcR) that can be activated by both ecdysteroids and catecholamines, could not be blocked by catecholamines (Srivastava et al., 2005). This was suggested to be due to the much higher affinity of the receptor for ecdysteroids compared with dopamine.

In view of the difficulties in demonstrating definitive binding of aldosterone to GPER, one needs to consider other potential explanations for the results obtained with aldosterone in the present study, and in other studies, where the aldosterone effects have similar pharmacological properties to those of GPER activation. Perhaps GPER and MR can form an obligate heterodimer, where both receptors are required to produce physiological effects. Indeed, the presence of such dimers has been suggested in breast cancer cells (Rigiracciolo et al., 2015). However, in the mHippoE-18 cell preparation the aldosterone signalling in the presence of the MR antagonist, eplerenone, would be difficult to explain on this basis. Alternatively, aldosterone and 17β-Estradiol (or G-1), may bind to different, or overlapping, binding sites on GPER, which interact allosterically to modify receptor signalling. This could account for the lack of mutual displacement activity in binding studies. Further, perhaps aldosterone can only bind specifically to GPER in specific cell types where the correct auxillary receptor binding proteins are present in the cells. This could lead to the receptor displaying some form of “biased agonism” or “agonist-specific coupling” (Evans et al 1995, 2014; Kenakin, 1995; Srivastava and Evans, 2013; Violin et al., 2014) or “biased antagonism” (Azzi et al., 2003; Wisler et al., 2007). A considerable amount of evidence suggests that GPER can interact with other membrane located proteins, including other GPCRs (Akama et al., 2013; Broselid et al., 2014) or auxillary proteins, such as receptor activity modifying proteins (RAMPs) (Lenhart et al., 2013). This would emphasise the importance of carrying out future binding studies in preparations where aldosterone exhibits pharmacological and signalling capabilities compatible with an interaction with GPER.

The mode of action of the diphenylacrylamide, STX, in mimicking the different rapid, non-genomic actions of 17β-Estradiol is controversial. In some preparations, STX has been suggested to be a possible activator of GPER (Lin et al., 2009; Prossnitz and Arterburn, 2015). However, GPER knock out studies (Prossnitz and Hathaway, 2015) looking at the effects of STX, have also produced complex results. Lin et al. (2009) found that the effects of STX on endometrial cell proliferation were blocked by GPER knockout. Alternatively, Qiu et al. (2003, 2008) report that the effects of STX on the modulation of hypothalamic activity were not blocked by GPER knockout. They postulate that in this preparation, STX activates an additional Gαq-linked membrane located estrogen receptor, (Gαq-mER). However, this receptor has not yet been identified and sequenced. In addition, there might also be species differences in the involvement of GPER and Gαq-mER in various rapid estrogen responses. Thus, in primates the 17β-Estradiol-mediated rapid release of GnRH may be produced by an action through GPER and/or Gαq-mER (STX-R), whereas in mice it appears to involve ERβ and/or Gαq-mER (Terasawa and Kenealy, 2012). In the present study, the apparent biphasic dose-dependent activation of the calcium-sensitive forskolin-stimulated cyclic AMP levels by STX in mHippoE-18 cells is completely blocked by the GPER antagonists, G36 and G15 (data not shown). This suggests that these actions of STX in this preparation are likely to be mediated by GPER, always presupposing that G15 and G36 do not block Gαq-mER in this preparation. The above conclusion is also supported by the observation that ICI182781 also produces a dose-dependent increase in forskolin-stimulated cyclic AMP levels in mHippoE-18 cells, since this compound is thought to be an antagonist of the effects of STX on Gαq-mER (Qiu et al., 2003, 2008). However, the above observations do not mean that STX cannot modulate other second messenger effects in mHippoE-18 cells by additional membrane located estrogen receptors, such as Gαq-mER or ERX (Toran-Allerand, 2004). Nevertheless, even though STX has been shown to have effects on hippocampal neurons (Lebesgue et al., 2010; Gray et al., 2016), evidence for the presence of Gαq-mER, or ERX, in mHippoE-18 cells is not currently available.

Soluble oligomers of β-amyloid peptides, rather than formation of amyloid-β plaques, appear to be responsible for many of the cognitive defects associated with the early stages of mild cognitive impairment and of Alzheimers's disease (Lue et al., 1999; Li et al., 2009; Harwell and Coleman, 2016; Park et al., 2017). Such peptides are capable of blocking the GPER-mediated rapid increases in forskolin-stimulated cyclic AMP levels produced in mHippoE-18 cells by agonists, such as G-1, aldosterone, STX and also 17β-Estradiol (data not shown). Exposure to soluble oligomers of β-amyloid peptides also reduces the calcium-dependent increase in cyclic AMP levels known to underlie LTP in hippocampal cells (Vitolo et al., 2002; Xia and Storm, 2012). Thus, GPER mediated calcium-dependent increases in cyclic AMP levels may underlie some of the effects of 17β-Estradiol on memory and learning and on neuroprotection in hippocampal cells. The above results suggest that soluble oligomers of β-amyloid peptides may be acting upon specific cell surface receptors in mHippoE-18 cells. The direct binding of β-amyloid peptides to β_2-adrenergic receptors has previously been suggested to produce time-dependent increases and decreases in cyclic AMP levels and to stimulate receptor desensitization and internalization in hippocampal cells (Wang et al., 2010, 2011). The β-amyloid peptides do not bind to the catecholamine-binding site of the β_2-adrenergic receptor but rather bind to an allosteric site involving the N-terminus of the receptor. β-Amyloid oligomeric peptides have also been shown to interact with α-7 nicotinic acetylcholine receptors to induce glutamate release in hippocampal slices resulting in an NMDA receptor-mediated reduction in EPSC frequency (Talantova et al., 2013). Nanomolar concentrations of soluble oligomeric β-amyloid peptides have also been shown to block hippocampal long-term potentiation, cause pyramidal cell dendritic spine retraction and impair rodent spatial memory by direct interactions with cellular prion protein, PrP^{c} (Laurén et al., 2009). However, it is not clear if the soluble oligomeric β-amyloid peptides can interact directly with GPER, or produce their effects on calcium-dependent GPER-mediated stimulation of cyclic AMP levels in mHippoE-18 cells by some modulatory actions on the pathways responsible for this effect or by the induction of oxidative stress effects (Butterfield and Boyd-Kimball, 2018).

Future studies, on computer aided docking on structural models of GPER (Méndez-Luna et al., 2016, 2015), and on ligand binding, will help resolve the controversial problems of whether aldosterone is capable of mediating physiologically relevant effects via GPER. In addition, further studies are required on the second messenger pathways activated by a range of GPER agonists in various tissues, with different levels of expression of different G-proteins and scaffold proteins. These will provide information on the potential role of this receptor for the development of novel therapeutic agents. These will be of use in the control of the defects in synaptic plasticity associated with the actions of soluble oligomeric forms of β-amyloid peptides in mild cognitive impairment and in the early cognitive effects associated with Alzheimer's disease.

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Declaration of interest

None.

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References

Akama, K.T., Thompson, L.I., Milner, T.A., McEwen, B.S., 2013. Post-synaptic density- 95 (PSD-95) binding capacity of G-protein-coupled receptor 30 (GPR30), an estrogen receptor that can be identified in hippocampal dendritic spines. J. Biol. Chem. 288, 6438–6450.

Alexander, A., Irving, A.J., Harvey, J., 2017. Emerging roles for the novel estrogen-sensing receptor GPER1 in the CNS. Neuropharmacology 113, 652–660.

Araujo, C.M., Hermidorff, M.M., de Cassia Sousa Amancio, G., da Silveira Lemos, D., Silva, M.E., de Assis, L.V.M., Isoldi, M.C., 2016. Rapid effects of aldosterone in primary cultures of cardiomyocytes – do they suggest the existence of a membrane-bound receptor? J. Recept. Signal Transduct. 36, 435–444.

Ashton, A.W., Le, T.Y., Gomez-Sanchez, C.E., Morel-Kopp, M.C., McWhinney, B., Hudson, A., Mihailidou, A.S., 2015. Role of nongenomic signaling pathways activated by aldosterone during cardiac reperfusion injury. Mol. Endocrinol. 29, 1144–1155.

Azzi, M., Charest, P.G., Angers, S., Rousseau, G., Kohout, T., Bouvier, M., Piñeyro, G., 2003. β-arrestin-mediated activation of MAPK by inverse agonists reveals distinct active conformations for G protein-coupled receptors. Proc. Natl. Acad. Sci. 100, 11406–11411.

Balakishnan, S., Niebert, M., Richter, D.W., 2016. Rescue of cyclic AMP mediated long term potentiation impairment in the hippocampus of Mecp2 knockout (*Mecp2*$^{-/y}$) mice by rolipram. Front. Cell. Neurosci. https://doi.org/10.3389/fncel.2016.00015.

Barton, M., Meyer, M.R., 2015. Nicolaus Copernicus and the rapid vascular responses to aldosterone. Trends Endocrinol. Metab. 26, 396–398.

Barton, M., Filardo, E.J., Lolait, S.J., Thomas, P., Maggiolini, M., Prossnitz, E.R., 2018. Twenty years of the G protein-coupled estrogen receptor GPER: historical and personal perspectives. J. Steroid Biochem. Mol. Biol. 176, 4–15.

Batenburg, W.W., Jansen, P.M., van den Bogaert, A.J., Danser, J., Danser, A.H., 2012. Angiotensin II-aldosterone interaction in human coronary microarteries involves GPR30, EGFR, and endothelial NO synthase. Cardiovasc. Res. 94, 136–143.

Bayliss, A., Roselli, G., Evans, P.D., 2013. A comparison of the signalling properties of two tyramine receptors from Drosophila. J. Neurochem. 125, 37–48.

Broselid, S., Berg, K.A., Chavera, T.A., Kahn, R., Clarke, W.P., Olde, B., Leeb-Lundberg, L.M., 2014. G protein-coupled receptor 30 (GPR30) forms a plasma membrane complex with membrane-associated guanylate kinases (MAGUKs) and protein kinase A-anchoring protein 5 (AKAP5) that constitutively inhibits cAMP production. J. Biol. Chem. 289, 22117–22127.

Burman, C., Evans, P.D., 2010. Amphioxus expresses both vertebrate-type and invertebrate- type dopamine D1 receptors. Invertebr. Neurosci. 10, 93–105.

Burman, C., Reale, V., Srivastava, D.P., Evans, P.D., 2009. Identification and characterization of a novel Amphioxus dopamine D1-like receptor. J. Neurochem. 111, 26–36.

Butterfield, D.A., Boyd-Kimball, D., 2018. Oxidative stress, amyloid-β peptide, and altered key molecular pathways in the pathogenesis and progression of Alzheimer's disease. J. Alzheimer's Dis. 62, 1345–1367.

Cheng, S.B., Quinn, J.A., Graeber, C.T., Filardo, E.J., 2011. Down-modulation of the G protein-coupled estrogen receptor, GPER, from the cell surface occurs via a trans-Golgi-proteasome pathway. J. Biol. Chem. 286, 22441–22455.

Cheng, S.-B., Dong, J., Pang, Y., LaRocca, J., Hixon, M., Thomas, P., 2014. Anatomical location and redistribution of G protein-coupled estrogen receptor-1 during the estrus cycle in mouse kidney and specific binding to estrogens but not aldosterone. Mol. Cell. Endocrinol. 382, 950–959.

Chetkovich, D.M., Sweatt, J.D., 1993. NMDA receptor activation increases cyclic AMP in area CA1 of the hippocampus via calcium/calmodulin stimulation of adenylyl cyclase. J. Neurochem. 61, 1933–1942.

Evans, P.D., Bayliss, A., Reale, V., 2014. GPCR-mediated rapid, non-genomic actions of steroids: comparisons between DmDopEcR and GPER1 (GPR30). Gen. Comp. Endocrinol. 195, 157–163.

Evans, N.J., Bayliss, A.L., Reale, V., Evans, P.D., 2016. Characterisation of signalling by the endogenous GPER1 (GPR30) receptor in an embryonic mouse hippocampal cell line (mHippoE-18). PLoS One 11, e0152138.

Evans, P.D., Robb, S., Cheek, T.R., Reale, V., Hannan, F.L., Swales, L.S., Hall, L.M., Midgley, J.M., 1995. In: Yu, P.M., Tipton, K.F., Boulton, A.A. (Eds.), Agonist-specific Coupling of G-Protein Coupled Receptors to Second Messenger Systems. Current Neurochemical and Pharmacological Aspects of Biogenic Amines: Their Function, Oxidative Deamination and Inhibition. Prog. In Brain Res., vol. 106. Elsevier Science BV, Amsterdam, pp. 259–268.

Feldman, R.D., Ding, Q., Hussain, Y., Limbird, L.E., Pickering, J.G., Gros, R., 2016. Aldosterone mediates metastatic spread of renal cancer *via* the G protein-coupled estrogen receptor (GPER). FASEB J. 30, 2086–2096.

Ferguson, G.D., Storm, D.R., 2004. Why calcium-stimulated adenylyl cyclases? Physiology 19, 271–276.

Filardo, E.J., Thomas, P., 2012. G-protein-coupled estrogen receptor-1 GPER-1: its mechanism of action and role in female reproductive cancer, renal and vascular physiology. Endocrinology 153, 2952–2962.

Filardo, E.J., Quinn, J.A., Bland, K.I., Frackelton, A.R., 2000. Estrogen-induced activation of Erk-1 and Erk-2 requires the G protein-coupled receptor homolog, GPR30, and occurs via trans-activation of the epidermal growth factor receptor through release of HB-EGF. Mol. Endocrinol. 14, 1649–1660.

Gingerich, S., Kim, G.L., Chalmers, J.A., Koletar, M.M., Wang, X., Wang, Y., Belsham, D.D., 2010. Estrogen receptor alpha and G-protein coupled receptor 30 mediate the neuroprotective effects of 17β-estradiol in novel murine hippocampal cell models. Neuroscience 170, 54–66.

Gray, N.E., Zweig, J.A., Kawamoto, C., Quinn, J.F., Copenhaver, P.F., 2016. STX, a novel membrane estrogen receptor ligand, protects against Aβ toxicity. J. Alzheimer's Dis. 51, 391–403.

Gros, R., Ding, Q., Sklar, L.A., Prossnitz, E.E., Arterburn, J.B., Chorazyczewski, J., Feldman, R.D., 2011. GPR30 expression is required for the mineralocorticoid receptor independent rapid vascular effects of aldosterone. Hypertension 57, 442–451.

Gros, R., Ding, Q., Liu, B., Chorazyczewski, J., Feldman, R.D., 2013. Aldosterone mediates its rapid effects in vascular endothelial cells through GPER activation. Am. J. Physiol. Cell Physiol. 304, C532–C540.

Haass, C., Selkoe, D.J., 2007. Soluble protein oligomers in neurodegeneration: lessons from the Alzheimer's amyloid beta-peptide. Nat. Rev. Mol. Cell Biol. 8, 101–112.

Hadjimarkou, M.M., Vasudevan, N., 2018. GPER1/GPR30 in the brain: crosstalk with classical estrogen receptors and implications for behaviour. J. Steroid Biochem. Mol. Biol. 176, 57–64.

Harwell, C.S., Coleman, M.P., 2016. Synaptophysin depletion and intraneuronal Aβ in organotypic hippocampal slice cultures from huAPP transgenic mice. Mol. Neurodegener. 11, 44. https://doi.org/10.1186/s13024-016-0110-7.

Hermidorff, M.M., de Assis, L.V.M., Isoldi, M.C., 2017. Genomic and rapid effects of aldosterone: what we know and do not know thus far. Heart Fail. Rev. 22, 65–89.

Insel, P., Ostrom, R., 2003. Forskolin as a tool for examining adenylyl cyclase expression, regulation, and G protein signaling. Cell. Mol. Neurobiol. 23, 305–314.

Irannejad, R., Tomshine, J.C., Tomshine, J.R., Chevalier, M., Mahoney, J.P., Steyaert, J., Rasmussen, S.G., Sunahara, R.K., El-Samad, H., Huang, B., von Zastrow, M., 2013. Conformational biosensors reveal GPCR signalling from endosomes. Nature 495, 534–538.

Kelly, M.J., Rønnekleiv, O.K., 2015. Neural signalling of estradiol in the hypothalamus. Mol. Endocrinol. 29, 645–657.

Kenakin, T., 1995. Agonist-receptor efficacy II: agonist-trafficking of receptor signals. Trends Pharmacol. Sci. 16, 232–238.

Koo, E.H., Squazzo, S., 1994. Evidence that production and release of amyloid β-protein involves the endocytic pathway. J. Biol. Chem. 269, 17386–17389.

Laurén, J., Gimbel, D.A., Nygaard, H.B., Gilbert, J.W., Strittmatter, S.M., 2009. Cellular prion protein mediates impairment of synaptic plasticity by amyloid-β oligomers. Nature 457, 1128–1132.

Lebesgue, D., Traub, M., De Butte-Smith, M., Chen, C., Zukin, R.S., Kelly, M.J., Etgen, A.M., 2010. Acute administration of non-classical estrogen receptor agonists attenuates ischemia-induced hippocampal neuron loss in middle-aged female rats. PLoS One 5, e8642.

Lenhart, P.M., Broselid, S., Barrick, C.J., Leeb-Lundberg, L.M., Caron, K.M., 2013. G-protein-coupled receptor 30 interacts with receptor activity-modifying protein 3 and confers sex-dependent cardioprotection. J. Mol. Endocrinol. 51, 191–202.

Li, S., Hong, S., Shepardson, N.E., Walsh, D.M., Shankar, G.M., Selkoe, D., 2009. Soluble oligomers of amyloid beta protein facilitate hippocampal long-term depression by disrupting neuronal glutamate uptake. Neuron 62, 788–801.

Lin, B.C., Suzawa, M., Blind, R.D., Tobias, S.C., Bulun, S.E., Scanlon, T.S., Ingraham, H.A., 2009. Stimulating the GPR30 estrogen receptor with a novel tamoxifen analogue activates SF-1 and promotes endometrial cell proliferation. Cancer Res. 69, 5415–5423.

Lindsey, S.H., Liu, L., Chappell, M.C., 2014. Vasodilation by GPER in mesenteric arteries involves both endothelial nitric oxide and smooth muscle cAMP signaling. Steroids 81, 99–102.

Lue, L.F., Kuo, Y.M., Roher, A.E., Brachova, L., Shen, Y., Sue, L., Beach, T., Kurth, J.H., Rydel, R.E., Rogers, J., 1999. Soluble amyloid beta peptide concentration as a predictor of synaptic change in Alzheimer's disease. Am. J. Pathol. 155, 853–862.

Méndez-Luna, D., Martínez-Archundia, M., Maroun, R.C., Ceballos-Reyes, G., Fragoso-Vázquez, M.J., González-Juárez, D.E., Correa-Basurto, J., 2015. Deciphering the GPER/GPR30-agonist and antagonists interactions using molecular modeling studies, molecular dynamics, and docking simulations. J. Biomol. Struct. Dyn. 33, 2161–2172.

Méndez-Luna, D., Bello, M., Correa-Basurto, J., 2016. Understanding the molecular basis of agonist/antagonist mechanism of GPER1/GPR30 through structural and energetic analyses. J. Steroid Biochem. Mol. Biol. 158, 104–116.

Munirathinam, G., Yoburn, B., 1994. A simple procedure for assaying cAMP. Pharmacol., Biochem. Behav. 48, 813–816.

Origlia, N., Capsoni, S., Cattaneo, A., Fang, F., Arancio, O., Yan, S.D., Domenici, L., 2009. Aβ-dependent inhibition of LTP in different intracortical circuits of the visual cortex: the role of RAGE. J. Alzheimer's Dis. 17, 59–68.

Pang, Y.F., Dong, J., Thomas, P., 2008. Estrogen signaling characteristics of Atlantic croaker GPR30 and evidence it is involved in maintenance of oocyte meiotic arrest. Endocrinology 149, 3410–3426.

Park, D., Na, M., Kim, J.A., Lee, U., Cho, E., Jang, M., Chang, S., 2017. Activation of CaMKIV by soluble amyloid-β_{1-42} impedes trafficking of axonal vesicles and impairs activity-dependent synaptogenesis. Sci. Signal. 10, eaam8661.

Prossnitz, E.R., Arterburn, J.B., 2015. International Union of Basic and Clinical Pharmacology. XCVII. G protein-coupled estrogen receptor and its pharmacological modulators. Pharmacol. Rev. 67, 505–540.

Prossnitz, E.R., Barton, M., 2011. The G-protein-coupled estrogen receptor GPER in health

and disease. Nat. Rev. Endocrinol. 7, 715–726.
Prossnitz, E.R., Hathaway, H.J., 2015. What have we learned about GPCR function in physiology and disease from knockout mice? J. Steroid Biochem. Mol. Biol. 153, 114–126.
Puzzo, D., Vitolo, O., Trinchese, F., Jacob, J.P., Palmeri, A., Arancio, O., 2005. Amyloid- β peptide inhibits activation of the nitric oxide/cGMP/cAMP-responsive element-binding protein pathway during hippocampal synaptic plasticity. J. Neurosci. 25, 6887–6897.
Qiu, J., Bosch, M.A., Tobias, S.C., Grandy, D.K., Scanlan, T.S., Rønnekleiv, O.K., Kelly, M.J., 2003. Rapid signaling of estrogen in hypothalamic neurons involves a novel G-protein-coupled estrogen receptor that activates protein kinase C. J. Neurosci. 23, 9529–9540.
Qiu, J., Bosch, M.A., Tobias, S.C., Krust, A., Graham, S.M., Murphy, S.J., Korach, K.S., Chambon, P., Scanlan, T.S., Rønnekleiv, O.K., Kelly, M.J., 2006. A G-protein-coupled estrogen receptor is involved in hypothalamic control of energy homeostasis. J. Neurosci. 26, 5649–5655.
Qiu, J., Rønnekleiv, O.K., Kelly, M.J., 2008. Modulation of hypothalamic neuronal activity through a novel G-protein coupled estrogen membrane receptor. Steroids 73, 985–991.
Rebola, N., Carta, M., Mulle, C., 2017. Operation and plasticity of hippocampal CA3 circuits: implications for memory encoding. Nat. Rev. Neurosci. 18, 209–221.
Ren, Y., D'Ambrosio, M.A., Garvin, J.L., Leung, P., Kutskill, K., Wang, H., Peterson, E.L., Carretero, O.A., 2014. Aldosterone sensitizes connecting tubule glomerular feedback via the aldosterone receptor GPR30. Am. J. Physiol. Renal. Physiol. 307, F427–F434.
Revankar, C.M., Cimino, D.F., Sklar, I.A., Arterburn, J.B., Prossnitz, E.R., 2005. A transmembrane intracellular estrogen receptor mediates rapid cell signaling. Science 307, 1625–1630.
Rigiracciolo, D.C., Scarpelli, A., Lappano, R., Pisano, A., Santolla, M.F., Avino, S., De Marco, P., Bussolati, B., Maggiolini, M., De Francesco, E.M., 2015. GPER is involved in the stimulatory effects of aldosterone in breast cancer cells and breast tumor-derived endothelial cells. Oncotarget 7, 94–111.
Robertson, E.D., Sweatt, J.D., 1996. Transient activation of cyclic AMP-dependent protein kinase during hippocampal long-term potentiation. J. Biol. Chem. 271, 30436–30441.
Roepke, T.R., Qiu, J., Bosch, M.A., Rønnekleiv, O.K., Kelly, M.J., 2009. Cross-talk between membrane-initiated and nuclear-initiated oestrogen signalling in the hypothalamus. J. Neuroendocrinol. 21, 263–270.
Ruhs, S., Nolze, A., Hübschmann, R., Grossmann, C., 2017. Nongenomic effects via the mineralocorticoid receptor. J. Endocrinol. 234, T107–T124.
Ruiz-Palmero, I., Hernando, M., Garcia-Segura, L.M., Arevalo, M.-A., 2013. G protein-coupled estrogen receptor is required for the neuritogenic mechanism of 17β-estradiol in developing hippocampal neurons. Mol. Cell. Endocrinol. 372, 105–115.
Srivastava, D.P., Evans, P.D., 2013. GPER 1 – trials and tribulations of a membrane oestrogen receptor. J. Neuroendocrinol. 25, 1219–1230.
Srivastava, D., Yu, E., Kennedy, K., Chatwin, H., Reale, V., Hamon, M., Smith, T., Evans, P.D., 2005. Rapid, non-genomic responses to ecdysteroids and catecholamines mediated by a novel *Drosophila* G-protein coupled receptor. J. Neurosci. 25, 6145–6155.
Sun, J., Chu, Z., Moenter, S.M., 2010. Diurnal *in vivo* and rapid *in vitro* effects of estradiol on voltage-gated calcium channels in gonadotropin-releasing hormone neurons. J. Neurosci. 30, 3912–3923.
Talantova, M., Sanz-Blasco, S., Zhang, X., Xia, P., Akhtar, M.W., Okamoto, S., Dziewczapolski, G., Nakamura, T., Cao, G., Pratt, A.E., Kang, Y.J., Tu, S., Molokanova, E., McKercher, S.R., Hires, S.A., Sason, H., Stouffer, D.G., Buczynski, M.W., Solomon, J.P., Michael, S., Powers, E.T., Kelly, J.W., Roberts, A., Tong, G., Fang-Newmeyer, T., Parker, J., Holland, E.A., Zhang, D., Nakanishi, N., Chen, H.S., Wolosker, H., Wang, Y., Parsons, L.H., Ambasudhan, R., Masliah, E., Heinemann, S.F., Piña-Crespo, J.C., Lipton, S.A., 2013. Aβ induces astrocytic glutamate release, extrasynaptic NMDA receptor activation, and synaptic loss. Proc. Natl. Acad. Sci. U.S.A. 110, E2518–E2527. https://doi.org/10.1073/pnas.1306832110.
Tang, H., Zhang, Q., Yang, L., Dong, Y., Khan, M., Yang, F., Brann, D.W., Wang, R., 2014. GPR30 mediates estrogen rapid signalling and neuroprotection. Mol. Cell. Endocrinol. 387, 52–58.
Terasawa, E., Kenealy, B.P., 2012. Neuroestrogen, rapid action of estradiol and GnRH neurons. Front. Neuroendocrinol. 33, 364–375.
Thomas, P., Pang, Y., Filardo, E.J., Dong, J., 2005. Identity of an estrogen membrane receptor coupled to a G protein in human breast cancer cells. Endocrinology 146, 624–632.
Toran-Allerand, C.D., 2004. Minireview: a plethora of estrogen receptors in the brain: where will it end? Endocrinology 145, 1069–1074.
Violin, J.D., Crombe, A.L., Soergel, D.G., Lark, M.W., 2014. Biased ligands at G-protein-coupled receptors: promise and progress. Trends Pharmacol. Sci. 35, 308–316.
Vitolo, O.V., Sant'Angelo, A., Costanzo, V., Battaglia, F., Arancio, O., Shelanski, M., 2002. Amyloid β-peptide inhibition of the PKA/CREB pathway and long-term potentiation: reversibility by drugs that enhance cAMP signalling. Proc. Natl. Acad. Sci. 99, 13217–13221.
Walsh, D.M., Tseng, B.P., Rydel, R.E., Podisny, M.B., Selkoe, D.J., 2000. The oligomerization of amyloid β-protein begins intracellularly in cells derived from human brain. Biochemistry 39, 10831–10839.
Walsh, D.M., Townsend, M., Podlisny, M.B., Shankar, G.,M., Fadeeva, J.V., El Agnaf, O., et al., 2005. Certain inhibitors of synthetic amyloid β-peptide (Aβ) fibrillogenesis block oligomerization of natural Aβ and thereby rescue long-term potentiation. J. Neurosci. 25, 2455–2462.
Wang, D., Yuen, E.Y., Zhou, Y., Yan, Z., Xiang, Y.K., 2011. Amyloid β peptide$_{1\text{-}42}$ induces internalization and degradation of β_2 adrenergic receptors in prefrontal cortical neurons. J. Biol. Chem. 286, 31852–31863.
Wang, D., Govindaiah, G., Liu, R., De Arcangelis, V., Cox, C.L., Xiang, Y.K., 2010. Binding of amyloid β peptide to β_2 adrenergic receptor induces PKA-dependent AMPA receptor hyperactivity. FASEB J. 24, 3511–3521.
Wehling, M., 2017. Rapid actions of aldosterone revisited: receptors in the limelight. J. Steroid Biochem. Mol. Biol. 176, 94–98.
Wehling, M., Christ, M., Theisen, K., 1991. High affinity aldosterone binding to plasma membrane rich fractions from mononuclear leucocytes: is there a membrane receptor for mineralocorticoids? Biochem. Biophys. Res. Commun. 181, 1306–1312.
Wendler, A., Wehling, M., 2011. Is GPR30 the membrane aldosterone receptor postulated 20 years ago? Hypertension 57, e16.
West, C., Hanyaloglu, A.C., 2015. Spatial programming of G protein-coupled receptor activity: decoding signaling in health and disease. Mol. Endocrinol. 29, 1095–1106.
Wisler, J.W., De Wire, S.M., Whalen, E.J., Violin, J.D., Drake, M.T., Ahn, S., Shenoy, S.K., Lefkowitz, R.J., 2007. A unique mechanism of β-blocker action: carvedilol stimulates β-arrestin signaling. Proc. Natl. Acad. Sci. 104, 16657–16662.
Xia, Z., Storm, D.R., 2012. Role of signal transduction crosstalk between adenylyl cyclase and MAP kinase in hippocampus-dependent memory. Learn. Mem. 19, 369–374.