

Report for the Small Grant Program 2007 awarded to Dr E. Karteris

Progress to date and future experiments:

The primary objective of this preliminary project was to investigate the role of membrane progesterone receptors (mPRs) in adrenal steroidogenesis using the human adrenocortical cell-line H295R.

More specifically, we sought to investigate the following:

- 1) Expression and G-protein coupling characteristics of mPRs in H295R cells.
- 2) Effects of progesterone on corticosteroid secretion in H295R cells.

With regards to the first aim, we have used specific primers for the three different mPRs (mPR α , mPR β , and mPR γ) and assessed the gene expression of these genes in human fetal and adult adrenal and H295R cells. During the preparation of this report, a study has indicated the existence of two additional human mPRs named mPR δ , and mPR ϵ ¹. We are currently designing primers for these newly discovered genes in order to complete our detailed mapping of mPRs in our preparations. So far we can confirm the following:

	mPR α	mPR β	mPR γ	mPR δ	mPR ϵ
Adult Adrenal	+	+	--	?	?
Fetal Adrenal	+	+	--	?	?
H295R cells	+	+	--	?	?

Table 1. Summary of gene expression of mPRs in adrenals and H295R cells.

Expression of mPR α , and mPR β was further assessed at protein using commercially available antibodies (Santa Cruz, USA). Western blotting revealed that both mPRs are expressed at protein level primarily as 40kDa peptides in H295R cells. This is in agreement with their predicted molecular weight. Previous studies have indicated that mPRs might have the potential to form homodimers²⁻³. However in our protein preparations from H295R cells, we were unable to detect any high molecular weight (80kDa) immunoreactive bands.

Immunofluorescent analysis revealed that mPRs are primarily localized on the cell surface of H295R cells, although cytoplasmic localization is also evident. In collaboration with Professor Robert Jaffe (University of California San Francisco, USA) we plan to extend our observations in human adrenals. Moreover, we have

established another fruitful collaboration with Dr Alan Reynolds (Experimental Techniques Centre, Brunel University) and currently we are using the same antibodies for immunogold electron microscopy in order to elucidate further the cellular distribution of mPRs.

Functionality of mPRs in H295R cells was confirmed by binding studies in collaboration with Professor Peter Thomas (University of Texas at Austin, USA). One set of tubes (total binding) contained 2.0 nM [³H]P4 (specific activity, 102.1 Ci/mmol), and another set also contained 100-fold excess non-radiolabeled progesterone (nonspecific binding). After a 30-min incubation at 4°C with the membrane fractions, the reaction was stopped by filtration, the filters were washed and bound radioactivity measured by scintillation counting.

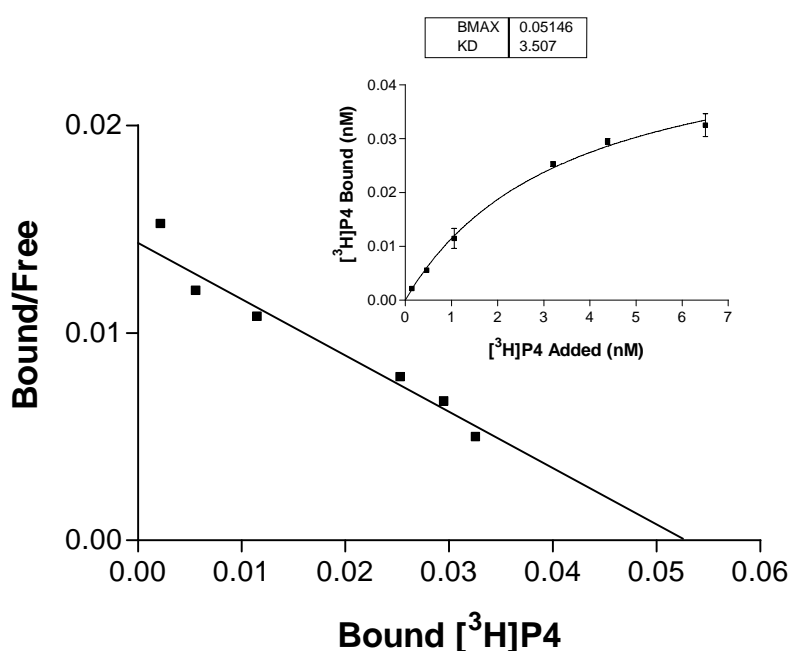


Figure 1. Saturation and Scatchard analysis of P4 binding in H295R cell membranes

Initial experiments have also shown specific binding of [³⁵S]GTPγS to H295R plasma cell membranes after treatment with 100nM P4. We extended our observations by investigating the coupling of mPRs to G-protein α-subunits in H295R cells. To examine the interaction of mPRs with G_{i/o} α-subunits, M11 cells were treated with P4 (100nM for 3 min) followed by co-immunoprecipitation (using a commercially available kit, SIGMA) with the mPRα/β antibody, and subsequent western blotting with a goat anti G_i alpha subunit antibody. Western blotting analyses revealed that neither of the mPRs appear to couple to G_{i1/2} in our preparations. These

data are not in agreement with previous findings that suggest that mPRs can couple to PTX-sensitive G protein α -subunits³. However, numerous studies have shown that seven transmembrane domain receptors are quite “promiscuous” with relation to their G-protein coupling preferences. Therefore we would like to investigate further their coupling characteristics by assessing other G-protein α -subunits such as: G_{i3} , G_o , $G_{q/11}$, G_s and G_z . Moreover, we also plan (subject to futurefunding) to use photoaffinity labelling (i.e. incorporation of ³²P-GTP-azido-anilide) to study these interactions in a time- and dose-dependent manner.

With regards to the role of mPRs in adrenal steroidogenesis, we have assessed the effect of P4 on cortisol release. Treatment of H295R cells with 100nM P4-BSA (i.e. impermeable P4) induced cortisol release as early as 6 hours (50% above basal). No apparent changes in the release of cortisol were detected at 24 and 48 hours of P4-BSA treatment. Interestingly, this effect was additive to ACTH. Currently we are expanding our knowledge on the involvement of mPRs in adrenal steroidogenesis by assessing using real-time PCR the effect of P4 on the following key steroidal modulators: Steroidogenic Acute Regulatory Protein (*StAR*), Cholesterol Side-Chain Cleavage Enzyme (*CYP11A*); 3 β -hydroxysteroid dehydrogenase, (*3 β HSD1*); 21-hydroxylase, (*CYP21*); 11- β Hydroxylase (*CYP11B1*), Aldosterone Synthase, (*CYP11B2*); and 17-hydroxylase (*CYP17*).

Benefit to applicant

This small grant programme enabled me to establish the “Cellular Endocrinology Laboratory” that I am now the head of. Moreover, I have recruited two PhD students in my laboratory. It is also evident from the progress report, that this project has generated many collaborations with leading authorities in their fields. Their scientific and intellectual contribution towards a research manuscript in the future will be invaluable. Indeed, we anticipate publishing this study by the end of next year in one of the journals of the Society for Endocrinology. Prior to publication, we intend to present some of these data at a national or international endocrine meeting next year.

Benefit to institution and endocrine research

As mentioned already, the establishment of the Cellular Endocrinology Laboratory and the generation of fruitful collaborations will put Brunel University on the map for endocrine research. I am also responsible for organising the departmental seminar series and this academic year I have invited numerous basic and clinical scientists that work in the endocrine field to present their data at Brunel. Moreover, in collaboration with Professors Peter Thomas (University of Texas at Austin, USA) and Ian Mason (University of Edinburgh, UK) I intend to submit a research grant proposal either to Biotechnology and Biological Sciences Research Council (BBRC) or The Wellcome Trust. Although it is too early to predict if our data will be of any pharmaceutical interest, I have been in touch with our commercialisation office and attended a meeting of the London Trading Network, in order to gain a better understanding of these procedures.

Closely Related Studies

Finally, a very exciting collaboration with NASA and NSRL has also been established. A study from NASA revealed that while in space astronauts exhibit a decline of dehydroepiandrosterone, suggesting that there are changes in the function of the adrenal gland during space travel⁴, although the cause of this disruption is not known. To this date, there is little data about the effects of space radiation on the regulation of steroidal secretion. We are in the process of re-submitting a small grant programme in collaboration with Dr Francis Cucinotta (NASA Johnson Space Center), where we propose to investigate the effects of heavy ion radiation (²⁸Si) on the expression of key steroidogenic enzymes and steroid receptors in H295R cells.

References:

1) Smith J.L. et al., *Steroids*. 2008, 73:1160-73; 2) Thomas P. *Front Neuroendocrinol*. 2008, 29:292-312; 3) Karteris E. et al., *Mol Endocrinol*. 2006, 20:1519-34; 4) Kenny A., from "School of Medicine University of Connecticut Health Center Annual Progress Report". pp.162