Metformin treatment affects ACTH receptor activation and downstream signaling: a potential treatment for ACTH Excess disorders and management of hyperandrogenic states

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Introduction

The peptide hormone adrenocorticotropin (ACTH or Corticotropin) is a major component of the stress response system in the Hypothalamus-Pituitary-Adrenal (HPA) axis. Under stress, it is secreted from the anterior pituitary and stimulates cortisol production from the adrenal cortex. Changes in ACTH production or action are associated with multiple disease conditions. In clinical situations like Cushing's disease, ectopic ACTH syndrome and congenital adrenal hyperplasia, there is excess ACTH production and blocking the interaction of ACTH at its site of action would be a therapeutic option. Currently, effective therapy to block the action of ACTH is unavailable. Insulin-sensitizing treatment, such as metformin, has been used to ameliorate a few reported cases of adrenal disorders. However, the exact mechanism of how these insulin-sensitizing drugs affect the HPA axis is not known. To test whether insulin-sensitizing drugs such as metformin have a direct effect on the activity of ACTH.

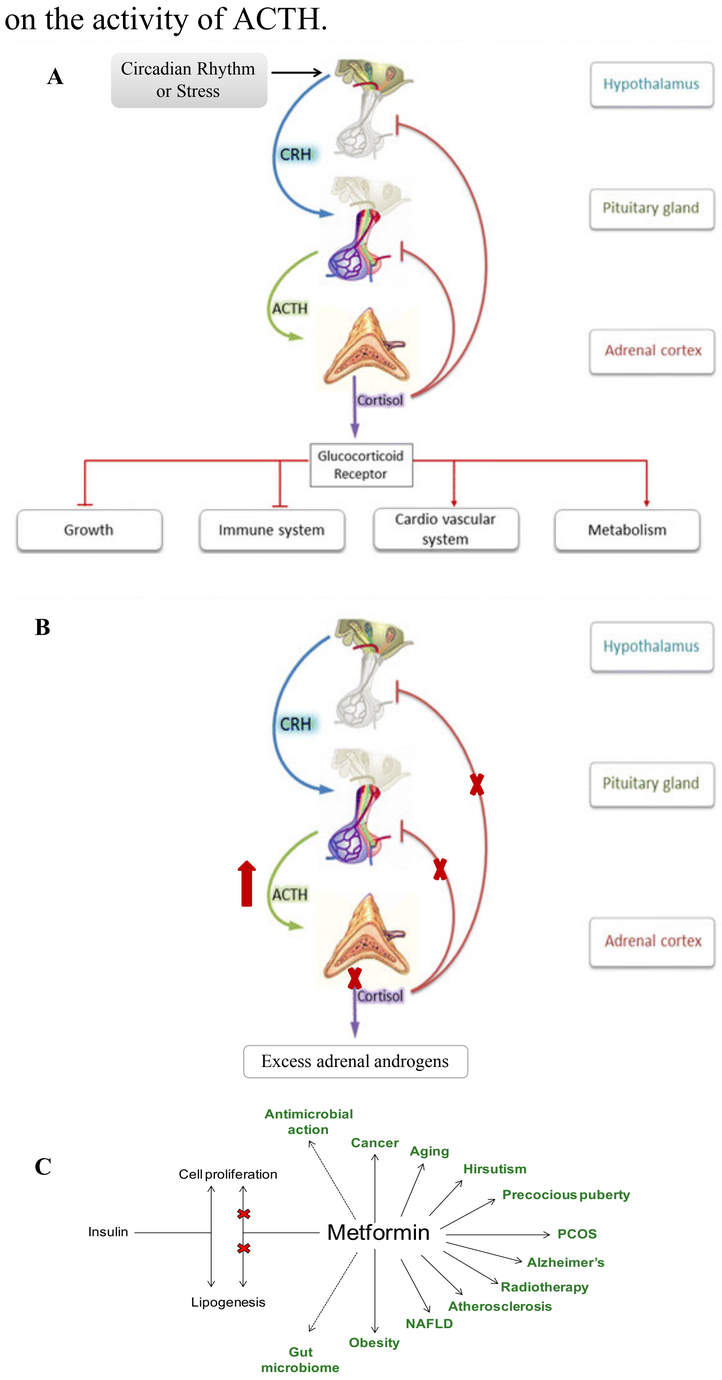


Fig. 1: A. Schematic overview of the hypothalamic-pituitary-adrenal (HPA) axis. Depicted is the physiological hierarchical pathway to cortisol-secretion from adrenal cortex including negative feed-back loop. Also shown are the most prominent effects of cortisol. **B.** Schematic overview of HPA axis in classic CAH patients. Pathological condition have impaired negative feed-back loop leading to ACTH excess and hyperandrogens. Figure adapted from: Eckstein N, Haas B, Hass MDS, Pfeifer V. *Orphanet Journal of Rare Diseases*. 2014;9:122. **C.** Various pathological conditions claimed to be affected by metformin therapy. Figure adapted from: Manair K, Moideen A, Mittal A, Patil A, Chakrabarti A, Banerjee D. *Pharmacological Research* 2017;117:103

Methods

In-vitro assays were performed to test the effect of metformin on ACTH receptor activation and signaling. The OS3 cells transfected with ACTH receptor (MC2R) and luciferase reporter plasmids were used, and cyclic AMP (cAMP) was measured by luciferase assay. The potential to shift the ACTH concentration-response curve (CRC) was evaluated to characterize the antagonist activity of metformin.

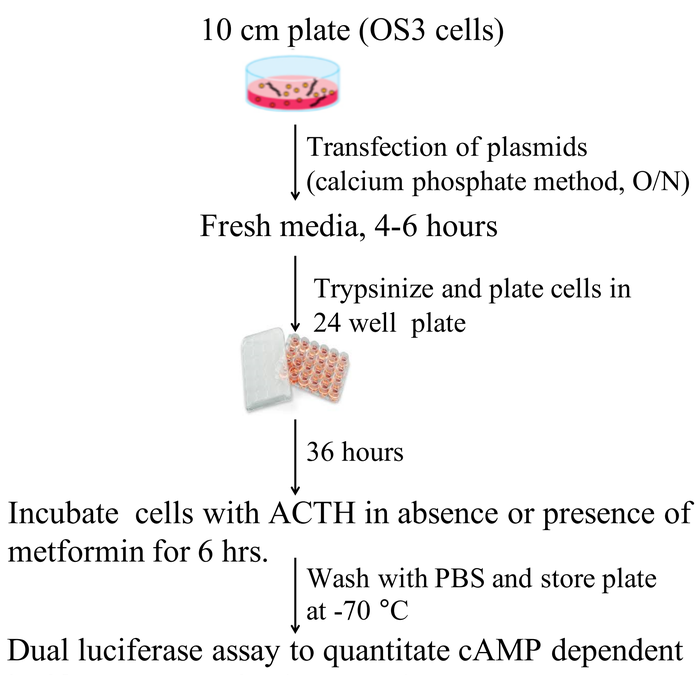


Fig 2: *In-vitro* functional assay

Results

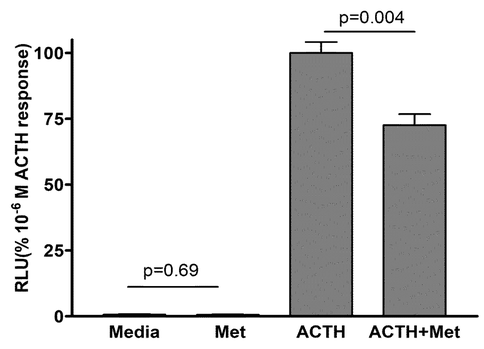
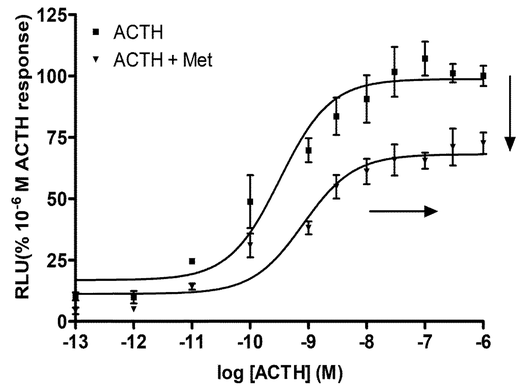


Fig 3: Effect of 10 mM metformin on cAMP response of MC2R transiently transfected OS3 cells with and without stimulation with ACTH. Data are presented as relative light units (RLU) normalized to Rmax. 10 mM metformin reduced the activation of MC2R by ACTH.



	EC_{50}	Log EC_{50}
ACTH	3.2E-10	-9.5 ± 0.17
ACTH + Met	8.1E-10	-9.1 ± 0.15

Fig 4: Concentration response curve(CRC) of ACTH on MC2R transiently transfected OS3 cells in the absence and presence of 10 mM metformin. The cAMP response of unstimulated cells was used as control. Half log shift in ACTH CRC was observed with 10 mM metformin.

Results

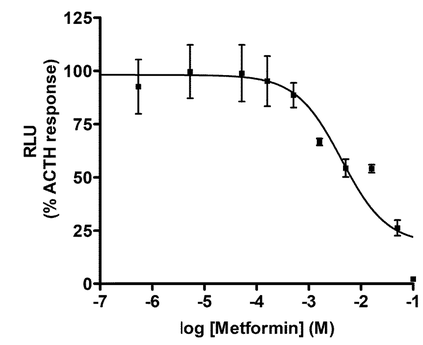


Fig 5: Dose response curve of metformin on MC2R transiently transfected OS3 cells in the presence of EC_{80} concentration of ACTH. The IC_{50} for metformin was 4.2 mM.

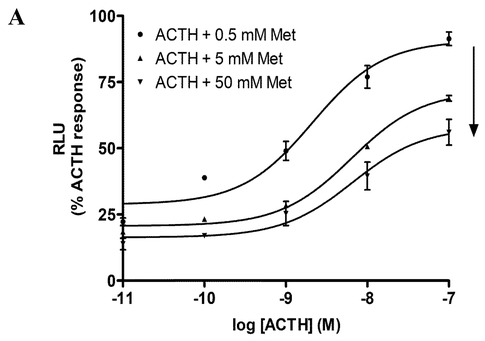


Fig 6: A. Concentration response curves of ACTH on MC2R transiently transfected OS3 cells in the presence of 0.5 mM, 5 mM and 50 mM metformin. **B.** Effect of different concentration of metformin on the Rmax of ACTH on MC2R. Data are presented as % of maximum response to 100 nM ACTH in absence of metformin.

Assays to study the selectivity of metformin for MC2R are ongoing.

Conclusions

Metformin was found to inhibit the activation of the ACTH receptor and downstream signaling associated with ACTH response. Significant inhibition of ACTH induced receptor activation upon treatment with 10 mM metformin was observed. Metformin shifted the ACTH CRC towards the right by half log, indicating antagonism. This study could be useful in developing new strategies for management of hyperandrogenic states.

1. Eckstein N, Haas B, Hass MDS, Pfeifer V. Systemic therapy of Cushing's syndrome. *Orphanet Journal of Rare Diseases*. 2014;9:122. doi:10.1186/s13023-014-0122-8.
2. Manair K, Moideen A, Mittal A, Patil A, Chakrabarti A, Banerjee D. A story of metformin-butyrate synergism to control various pathological conditions as a consequence of gut microbiome modification: Genesis of a wonder drug? *Pharmacological Research* 2017;117:103-128. doi:10.1016/j.phrs.2016.12.003

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The authors declare no conflict of interest.

Acknowledgements

This research is supported by IFCAH-ESPE grant to Christa E Flück and Amit V Pandey. Conference support from Swiss society for Endocrinology(SGED/SSED) to S Parween is acknowledged.