Biguanides: A new potential therapeutic option for pituitary tumors?

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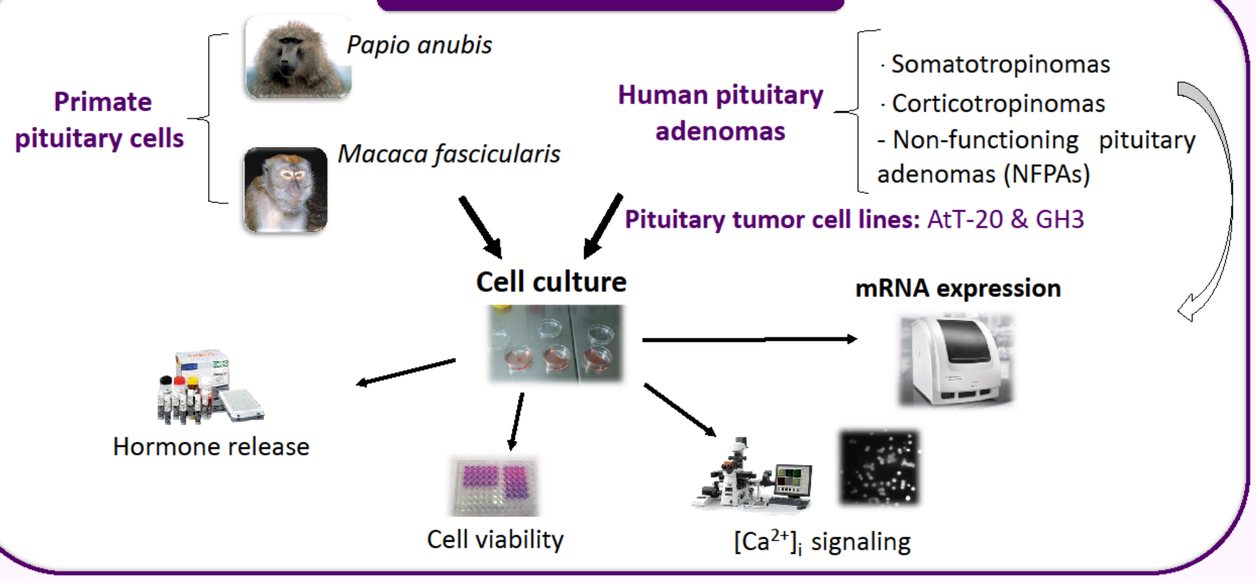
Introduction

Pituitary adenomas (PA) comprise a commonly underestimated pathology in terms of incidence and associated morbimortality. Somatostatin analogs and dopamine antagonists constitute the main medical treatments for PA. However, an appreciable subset of patients are resistant or poorly responsive to these drugs, and hence, the search for new therapies to control tumor growth and/or hormone secretion is crucial. Biguanides such as metformin (MF; commonly used to treat type-2 diabetes), phenformin (PF) and buformin (BF) have been shown to exert antitumoral actions in different tumor types (brain tumors, prostate, breast and lung cancers) but their actions in PA cells have not been reported.

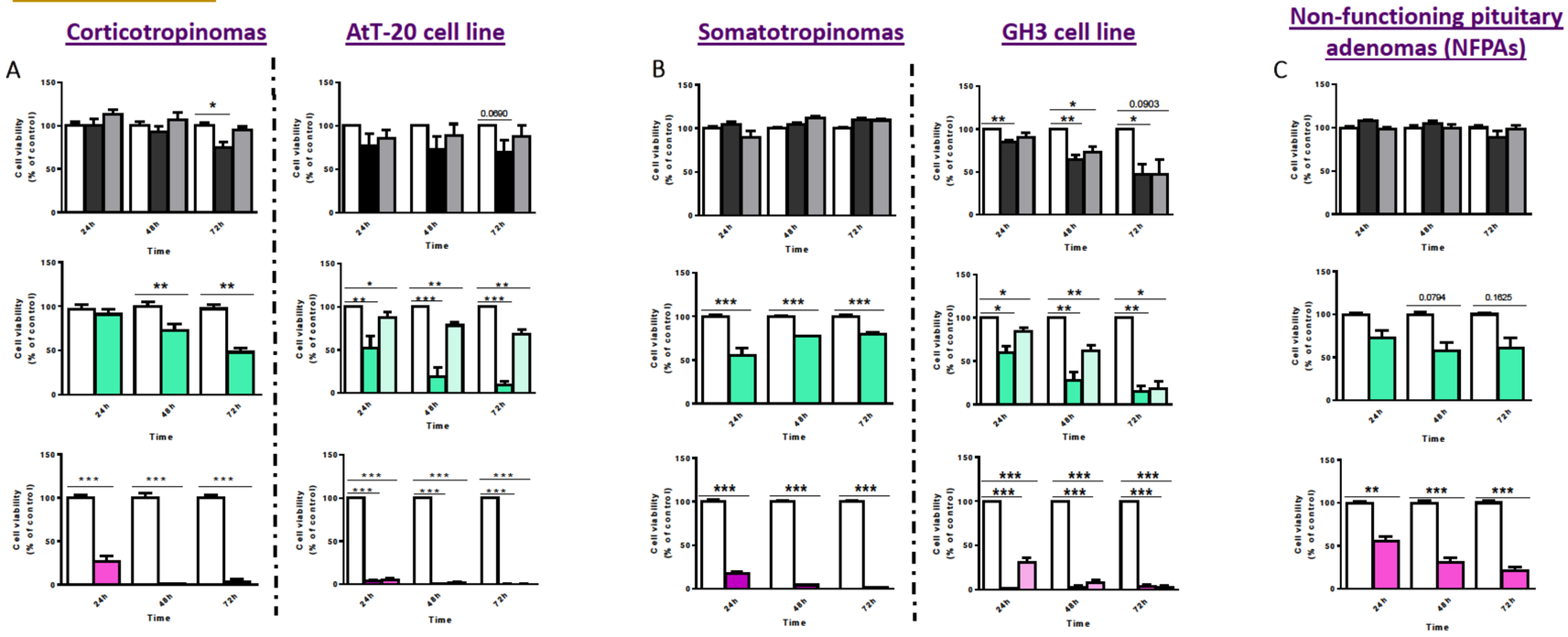
The AIM of this study was to determine the effect of these biguanides on key functional parameters (hormone expression and secretion, gene expression of key modulators of pituitary cell function and signaling pathways) and on cell viability in normal and/or tumoral pituitary cell cultures and cell lines.

Materials & Methods



Results

CELL VIABILITY



Treatment with different biguanides, especially with phenformin and buformin, was able to reduce cell viability in a dose-dependent manner in different pituitary adenomas subtypes, including corticotropinomas, somatotropinomas and non-functioning pituitary adenomas.
Similar results were obtained in pituitary ACTH- and GH-secreting tumors derived cell lines.

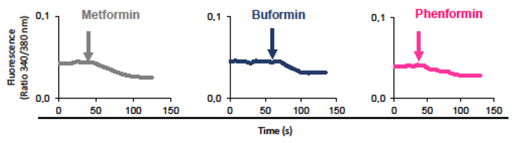
Figure 1 | Cell viability in response to metformin (MF), buformin (BF) and phenformin (PF). (A) Effect of MF (5 and 10 mM), BF and PF (5 and 2 mM) on cell viability (measured by alamar-blue assay) in: **(A)** corticotropinomas (n=5) and AtT-20 (n=7); **(B)** somatotropinomas (n=2) and GH3 cell line (n=4); **(C)** NFPAs (n=4). Data are expressed as percent of vehicle-treated controls (set at 100%) within experiment. Values represent the mean ± SEM.

$[Ca^{2+}]_i$ KINETIC

D

Corticotropinomas

Treatment	Responsive cells	Total cells	% PRC	% PRM ± SEM	Time (s) ± SEM
Metformin	15	117	12,8 %	**28,7** ± 1,5	72,3 ± 2,8
Buformin	72	154	46,8 %	**25,5** ± 1,6	61,6 ± 3,1
Phenformin	36	79	45,6 %	**27,1** ± 2,1	66,0 ± 2,8

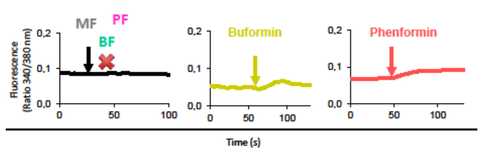


Treatment with different biguanides produced a decrease of $[Ca^{2+}]_i$ levels in primary corticotropinoma cell cultures,

E

Somatotropinomas

Treatment	Responsive cells	Total cells	% PRC	% PRM ± SEM	Time (s) ± SEM
Metformin	0	120	0%	-	-
Buformin	0	53	0%	-	-
Phenformin	0	40	0%	-	-
Buformin	22	40	55%	132,6 ±2,3	51,4 ±2,8
Phenformin	18	40	45%	137,9 ±4,3	51,4 ±4,8

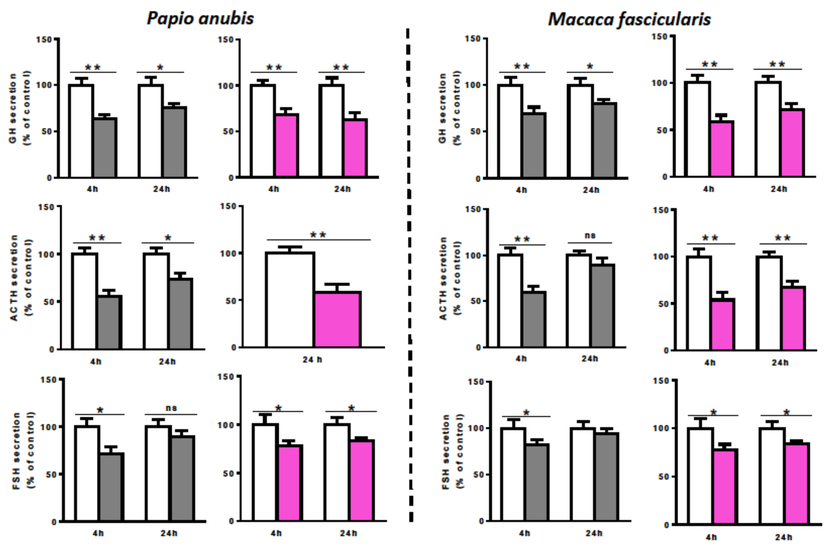


Treatment with MF did not elicit any response in $[Ca^{2+}]_i$ levels but, surprisingly, BF and PF treatment elicited a paradoxical increase on $[Ca^{2+}]_i$ levels in a subset of cultured somatotropinoma cells.

Figure 2 | Results from cytosolic calcium kinetic assays in response to MF, BF and PF (5 mM). PRC (%): percentage of responsive cells in responsive samples. PRM (%): percentage of maximum response. Time: time of maximum response. Results from cytosolic calcium kinetic assays: (D) in primary corticotropinoma cell cultures or, (E) in primary somatotropinoma cell cultures.

PRIMATE PITUITARY CELL CULTURES

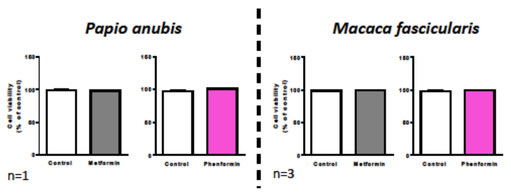
Hormone release



In general, MF and PF decreased GH, ACTH and FSH secretion from normal primate pituitary cultures after 4 and/or 24h of incubation.

Figure 3 | Measurement of hormone release in response to different biguanides in primate pituitary cultures. Effect of MF and PF (5 mM) after 4 and 24h of incubation on GH, ACTH and FSH secretion in two primate models, determined by ELISA. Data are expressed as percent of vehicle-treated controls (set at 100%) within experiment. Values represent the mean ± SEM.

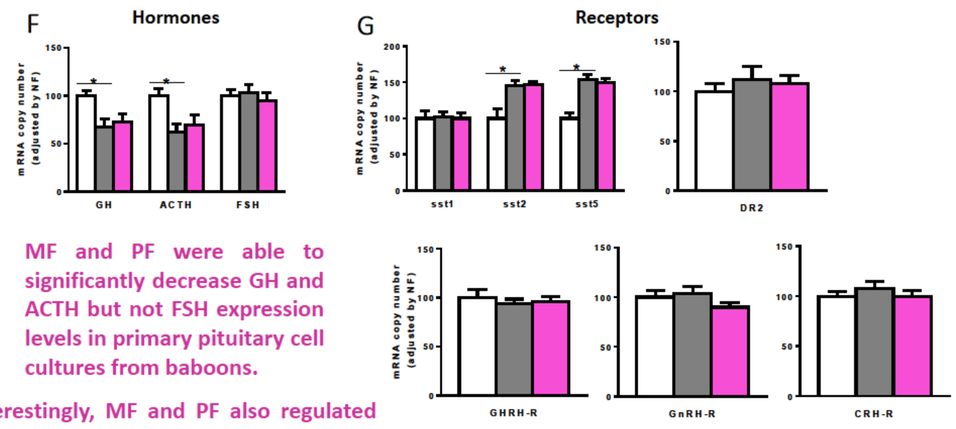
Cell viability



Interestingly, MF and PF treatment did not alter cell viability after 24h in primate pituitary cultures of both primate species.

Figure 4 | Cell viability in response to MF and PF in primate pituitary cultures after 24h using trypan blue assay. Data are expressed as percent of vehicle-treated controls (set at 100%) within experiment. Values represent the mean ± SEM

mRNA expression in *Papio anubis*



MF and PF were able to significantly decrease GH and ACTH but not FSH expression levels in primary pituitary cell cultures from baboons.

Interestingly, MF and PF also regulated the expression of some key receptors important for pituitary function. Specifically, we found that somatostatin receptor types 2 and 5 as well as insulin and IGFI receptors were upregulated in response to MF and PF.

Figure 5| Effects of MF and PF on the level of expression of relevant genes in primate pituitary cultures from *Papio anubis*, measured by qPCR. (F) Expression levels of GH, ACTH and FSH after 24h of incubation with MF (n=3-4) and PF (n=1); (B) Expression profile of relevant receptors in response to 24h-treatment with MF (n=3-4) and PF (n=1). Data represent absolute mRNA copy number adjusted by NF. Values represent the mean ± SEM.

Signalling mechanisms in *Papio anubis*

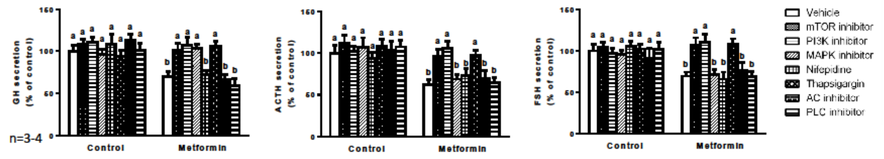


Figure 6| Effects of blockade of different signaling pathways on GH, ATCH and FSH release in response to MF in primary pituitary cell cultures from baboons. Values are expressed as percent of vehicle-treated control without inhibitor (set at 100% within each experiment) and represent the mean ± SEM. Values that do not share a common letter (a and b) are statistically different.

The use of inhibitors of different signalling pathways revealed that the inhibitory effect of metformin on GH, ACTH and FSH release involved the activation of mTOR, PI3K and intracellular calcium signaling. In addition, MAPK pathways were also important for the inhibitory effects of MF on GH release but not on ACTH and FSH release.

Conclusions

1. Different biguanides are able to significantly decrease cell viability in tumoral, but not normal, pituitary cells in a dose- and cell type-dependent manner, through a mechanism that could involve calcium mobilization.

2. Treatment with MF and PF directly decreases GH, ACTH and FSH expression and/or secretion in normal primary pituitary cell cultures from two primate species (*Papio anubis* and *Macaca fascicularis*) through common (mTOR, PI3K and intracellular calcium) and distinct (MAPK) intracellular signaling pathways.

3. The effects of MF and PF in the function of different pituitary cell types from primates also involved the regulation of the expression of key receptors essential in the normal function of these cell types (i.e. sst2, sst5, Ins-R and IGF1-R).

Therefore, given the demonstrated clinical safety of biguanides, our results suggest that these drugs could be used as a potential therapeutic option for the treatment of human pituitary adenomas.

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