A Ca^{2+}-activated potassium channel (BK_{Ca}) in Leydig cells is involved in testosterone production

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BACKGROUND & OBJECTIVE

Previously, we found that human steroid-producing ovarian granulosa cells express all major types of Ca^{2+}-activated potassium channels (K_{Ca}), including BK_{Ca}, IK and SKs (Traut *et al.*, RB&E, 2009), and that modulation of the activity of these channels resulted in alteration of steroid production. In the male gonad Leydig cells produce androgens, but whether these cells are endowed with K_{Ca}s is not known. We addressed these points and focussed on BK_{Ca}, which is Ca^{2+}-activated and the underlying channel for a prominent current. It can be manipulated, e.g. by a specific blocker, the red scorpion toxin iberiotoxin (IbTx), which binds to the outer face with high affinity and selectively inhibits the current by decreasing both the probability of opening and the open time of the channel.

RESULTS

Immunohistochemistry (Fig. 1) and RT-PCR (Fig. 2) of human and hamster testicular tissue showed the presence of BK_{Ca} in Leydig cells. The role of this channel in testosterone production was analyzed in hamster Leydig cells. During a 3h stimulation period, human chorionic gonadotropin (hCG, 100 mIU/ml) led to a significantly increased release of testosterone over basal levels (Fig. 3). Importantly, the presence of IbTx (100nM, 3h) further significantly increased the stimulated testosterone production. This appeared not to be associated with significant changes in the levels of StAR or the steroidogenic enzyme 17βHSD RNAs, shown by real time PCR (Fig. 4). However, the membrane potential changed. Measurements showed hyperpolarization of Leydig cells after addition of hCG (Fig. 5 A, Table 1). When BK_{Ca} was blocked with IbTx (100nM, 60min), hyperpolarization could not be detected (Fig. 5 B, Table 1).

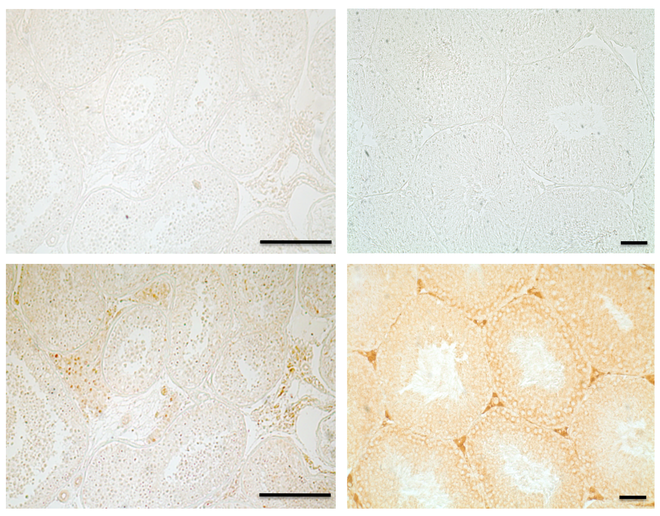


Fig. 1 Immunohistochemistry of human (left) and hamster testes (right) stained with antibody to BK_{Ca} . Both, human and hamster show distinct staining of Leydig cells. Negative control above. Bars = 100µm.

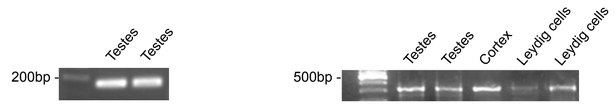


Fig. 2 Expression of BK_{Ca} in human testes (left) and hamster testes, hamster cortex and hamster Leydig cells (right) shown by RT-PCR.

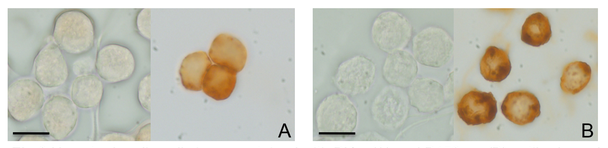


Fig.3 Hamster Leydig cells immunostained with BK_{Ca} (A) and P450scc (B) antibody each with negative control (without first antibody) on the left. Bars = 5 µm.

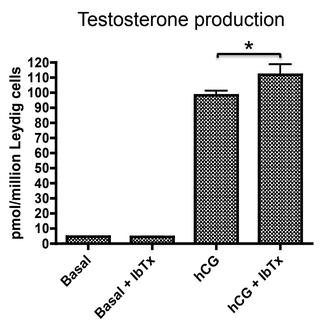


Fig. 4 Testosterone production of Leydig cells: unstimulated (Basal), stimulated with IbTx, hCG and hCG plus IbTx.

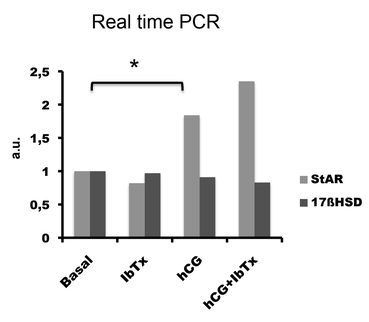
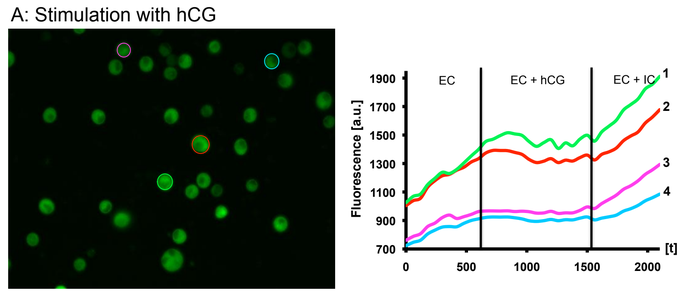


Fig. 5 Changes in StAR and 17βHSD to IbTx, hCG and hCG plus IbTx stimulation shown by real time PCR.

Table 1 Slopes of calculated for eight cells, four without and four with 60min pre incubation with IbTx.

	Zelle 1	Zelle 2	Zelle 3	Zelle 4	Zelle 5	Zelle 6	Zelle 7	Zelle 8
Stimulanz	**ohne IbTx**				**mit IbTx**			
EC	m = 0,61	m = 0,55	m = 0,31	m = 0,30	m = 0,58	m = 0,40	m = 0,47	m = 0,19
EC+hCG (100mIU/ml)	m = -0,04	m = -0,07	m = 0,01	m = -0,02	m = 0,64	m = 0,49	m = 0,44	m = 0,39
EC+IC (positiv control)	m = 0,83	m = 0,65	m = 0,57	m = 0,35	m = 0,94	m = 0,67	m = 0,60	m = 0,43

A: Stimulation with hCG



B: Stimulation with hCG after 60 min incubation with IbTx

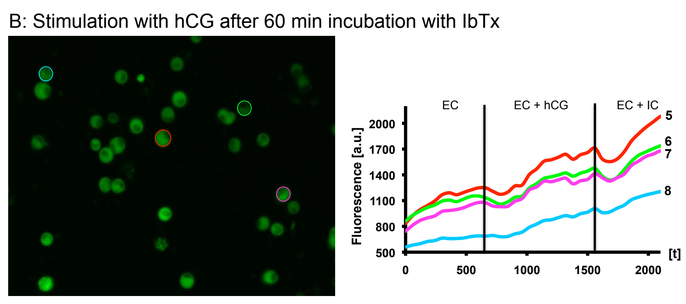


Fig. 6 Membrane potential during stimulation with hCG. Hamster Leydig cells were loaded with $DiBAC_4(3)$ dye and increase and decrease respectivly of fluorescence was measured. Comparison of calculated slopes of one cell for different time periods (during application of diefferent stimuli) indicates depolarization and hyperpolarization respectivly.
A: Stimulation with hCG. Leydig cells show hyperpolarization.
B: Stimulation with hCG after 60 min incubation with 100nM IbTx. Leydig cells show depolarization.

SUMMARY & CONCLUSIONS

BK_{Ca} is present in human and hamster Leydig cells and is involved in hCG stimulated testosterone production, at least in hamster. Thus, inhibition of BK_{Ca} by IbTx during hCG stimulation further increased testosterone production, an event not associated with changes in StAR or 17βHSD levels. Rather hCG activates BK_{Ca} possibly via an initial increase of the intracellular Ca^{2+} level, which results in hyperpolarization of the membrane. Thus we speculate that the observed increase in steroid production is linked to membrane potential changes.

METHODS

Human: Paraffin embedded sections of human testicular biopsies were studied by immunohistochemistry. RT-PCR was performed by using human testes samples of biopsies and samples from Leydig cells obtained by laser microdissection (LMD).
Hamster: RT-PCR was performed from testes and isolated Leydig cells, and PCR products were sequenced. The release of testosterone was examined in supernatant of isolated and cultured Leydig cells by radioimmunoassay (RIA). Paraffin embedded testes sections were subjected to immunohistochemistry for BK_{Ca}. Real time PCR was performed to evaluate levels of StAR and of 17βHSD. For normalization housekeeping gene 18S was used.

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