THE OXYTOCIN REGULATES KIDNEY FUNCTION THROUGH V_2 RECEPTOR

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Abstract

BACKGROUND: During maturation of oxytocin (OT) prohormone, several bioactive intermediate molecules are formed. The plasma concentrations of OT and these forms (OT-G; OT-GK and OT-GKR) increase markedly in rat circulation at the end of gestation. At low concentration in the circulation OT stimulates while OT-GKR inhibits diuresis. Since OT and OT-GKR show different effects on the urine flow, we hypothesized that OT-GKR modulates renal action by targeting the V_2 receptor.

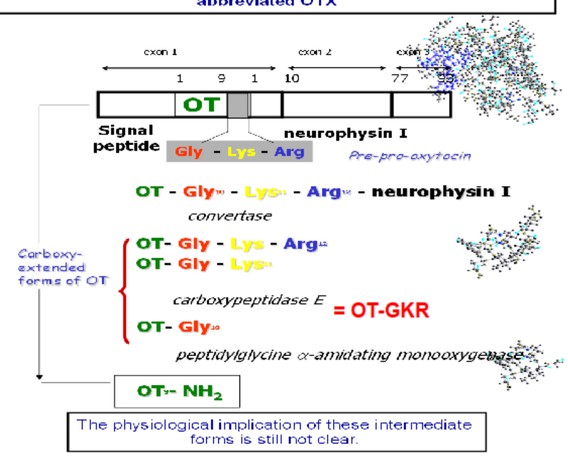
METHODOLOGY/PRINCIPAL FINDINGS: The 8-weeks-old Wistar rats were injected (i.e.) with vehicle, OT and OT-GKR or in combinations. OT (10 µmol/kg) increased urine outflow by 40% ($p<0.01$) and the sodium excretion by 47% ($p<0.01$). The treatment with 10 µmol/kg of OT-GKR decreased diuresis by 50% ($p<0.001$), decreased sodium by 50% ($p<0.05$) and lowered potassium by 42% ($p<0.05$). OT antagonist (OTA) reduced diuresis and natriuresis exerted by OT administration, whereas the anti-diuretic effect of OT-GKR was unaffected by OTA. The treatment with V_2 receptor antagonist (V_2A) in the presence and absence of OT induced significant increase in urine, sodium and potassium outflow.
The V_2A in the presence of OT-GKR only partially increased diuresis and natriuresis.
Molecular docking showed potent binding energies of OT-GKR to V_2R as well as to OTR, which are unique for each molecule. Moreover, the cAMP release from CHO cells overexpressing V_2 receptor has been induced by low concentration of AVP (EC50:4.2e-011), the higher concentration of OT (EC50:3.2e-010) and by very high concentration of OT-GKR (EC50:1.1e-006). The OT-GKR potentiated cAMP release when combined with AVP, but blocked cAMP release when combined with OT.

CONCLUSIONS/SIGNIFICANCE: These results indicate that the OT-GKR inhibits diuresis and natriuresis exerted by OT. The effects of OT-GKR oppose those evoked by OT, suggesting an auto-regulation of the renal function by the OT/OT-GKR system. This led us to the conclusion that OT-GKR regulates the kidney effects by specific interactions with V_2 receptor.

Background

- Oxytocin (OT) is a nonapeptide mainly produced by the hypothalamus and stored in the neurohypophysis.
- The plasma concentrations of OT and its carboxy-extended precursors (OT-G; OT-GK and OT-GKR) increase markedly in rat circulation during development and at the end of gestation.
- OT acts via its receptor both centrally as a neuromodulator and peripherally as a hormone, released by the neurohypophysis into the circulation.
- OT stimulates biological effects by binding to oxytocin receptor (OTR) and arginine-vasopressin (AVP) receptors.
- OT is involved in the regulation of salt and water homeostasis.
- In the kidney, OT activates OTR and AVP receptor V2.
- During volume expansion OT released from the neurohypophysis contributes to enhanced diuresis and natriuresis by stimulation of atrial natriuretic peptide from the heart.
- We hypothesized that OT carboxy-extended precursors influence on renal effects exerted by OT treatment in the rat.

The initial gene product from translation of OT mRNA is a peptide containing both OT and neurophysin I.
The tripeptide Gly-Lys-Arg separates the nonapeptide OT from neurophysin I by different enzymes, to produce, biologically active amidated OT. The intermediate forms containing 10, 11, or 12 amino acids are collectively referred to as carboxy-extended forms of OT, **abbreviated OTX**



The physiological implication of these intermediate forms is still not clear.

Objective

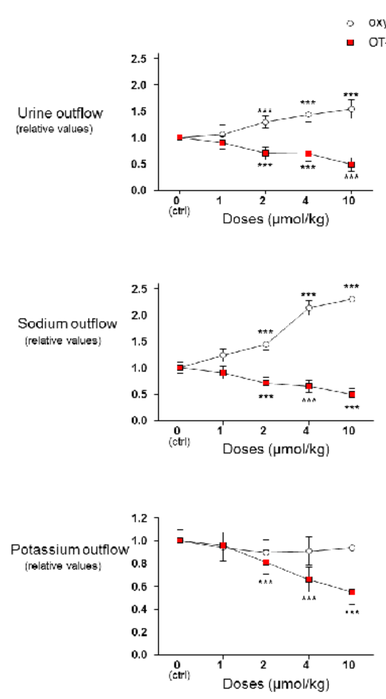
TO DEMONSTRATE THAT OT-GKR FORM OF OXYTOCIN IS INVOLVED IN REGULATION OF DIURESIS BY EFFECTS ON OXYTOCIN RECEPTOR (OTR) AND/OR V2 RECEPTOR OF VASOPRESSIN

Materials & Methods

- 8-weeks-old Wistar rats (225-250g) were housed in a standard temperature and light condition with water and standard rodent diet ad libitum.
- To determine the effect of circulating OT and OT-GKR on urine water and electrolytes excretion, the rats (n=24) received intravenous (i.v.) injections of OT and OT-GKR.
- After treatments rats were placed in metabolic cages for a 5-hour to collect and analyse urines. The concentrations of excreted sodium and potassium were determined by flame spectrometry.
- Virtual interaction of OT-GKR with OTR and V_2R were analysed with MolDock software. 3-D models of OT-GKR and OTR and V_2R were constructed with the Biopolymer module of the SYBYL molecular modeling package (Tripos Associates, St. Louis, MO).
- cAMP release from CHO cells transfected with human V2 receptor construct served for testing of peptides signalling .

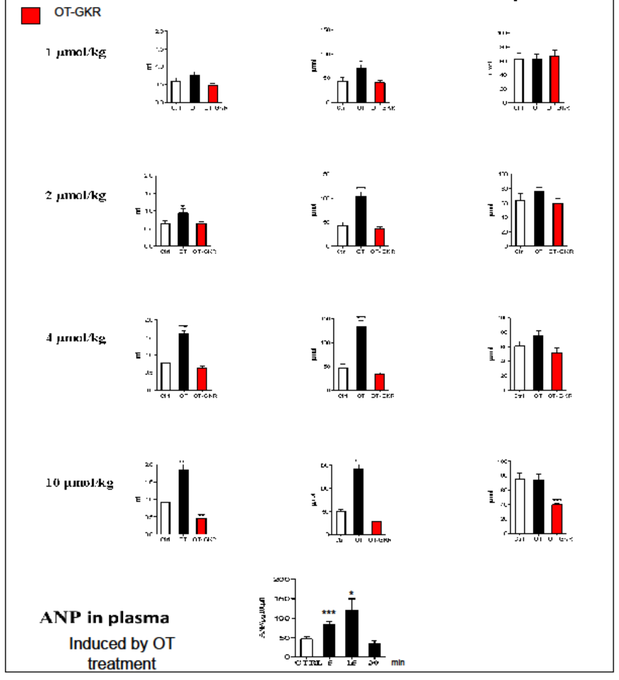
Results

Dose-related hydromineral effects of oxytocin (OT) and its extended form - OT-GKR

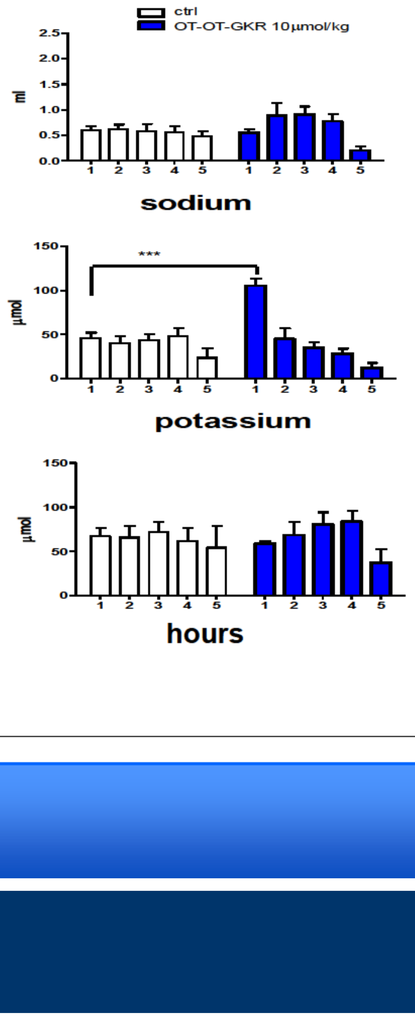


Intravenous equimolar doses of OT and OT-GKR were administrated at different concentrations (1, 2, 4 and 10 µmol/kg) to analyze urine outflow as well as sodium and potassium excretion during 5 hours.

The most significant diuretic and natriuretic effects were observed during first hour after treatment



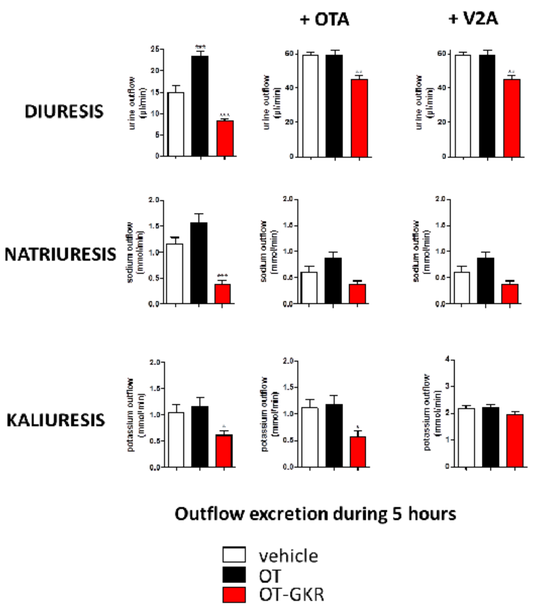
Diuretic effect of OT is inhibited by co-administration with equimolar concentration of OT-GKR



Results

NORMAL CONDITIONS

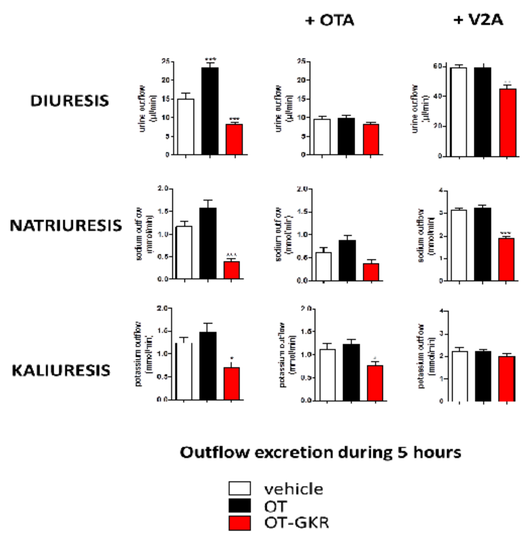
Hydromineral effects of OT and OT-GKR treatments in the presence and absence of oxytocin antagonist (OTA) and arginine-vasopressin antagonist of receptor V2 (V2A)



Intravenous equimolar doses (10 µmol/kg) were administrated to analyze urine outflow, sodium outflow and potassium outflow.

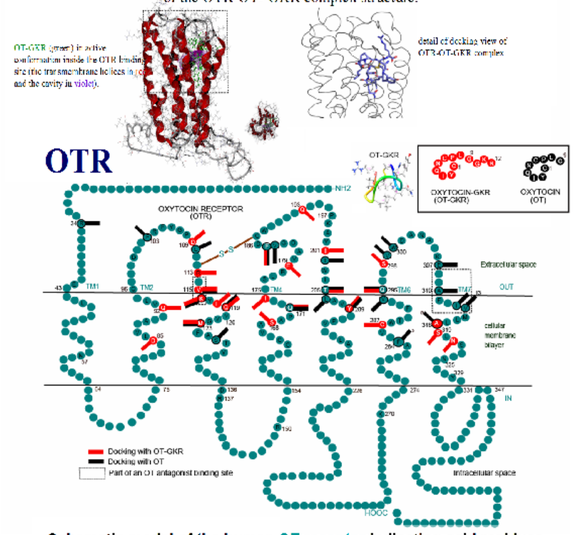
VOLUME EXPANSION

Hydromineral effects of OT and OT-GKR treatments in the presence and absence of oxytocin antagonist (OTA) and arginine-vasopressin antagonist of receptor V2 (V2A)



Intravenous equimolar doses (10 µmol/kg) were administrated to analyze urine outflow, sodium outflow and potassium outflow.

The three-dimensional models of activated of human OT Receptor with OT-GKR peptide using MolDock Optimizer algorithm from Molegro Virtual Docker.

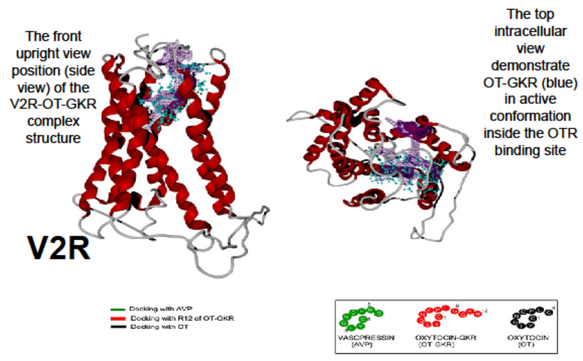


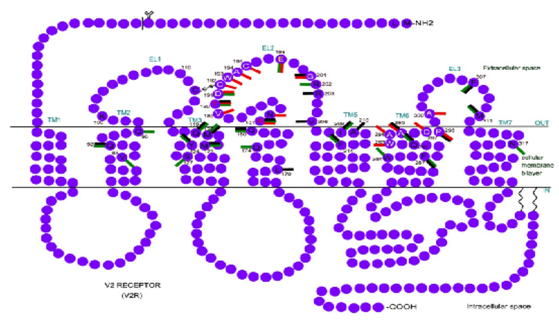
Schematic model of the human OT receptor indicating acid residues that are putatively involved in ligand-binding.The amino acide residues in black circles has been proposed an OT docking site, in red solid circles are residues interacting with the OT-GKR.

Docking to OTR in positions V115, K116, Q119, M123, Q171, F185, T205, Y209 and Q29 was noted for both OT and OT-GKR models. These docking positions constituted 42% of all observed docking sites of OT-GKR. Among OT-GKR interactions, the special notice should be given to binding of the arginine-12 (R12) to OTR.

Results

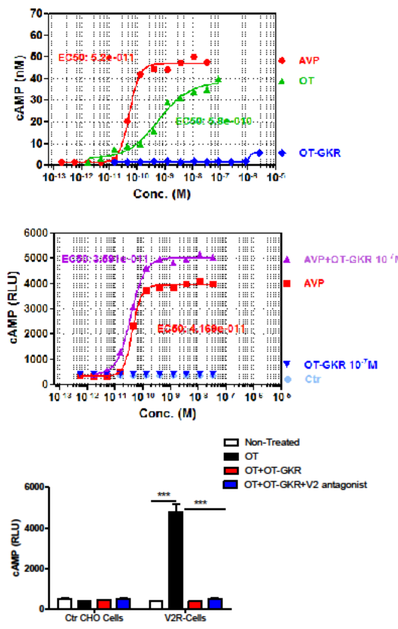
Molecular docking of 3-D model of activated human V_2 receptor with AVP/OT/OT-GKR peptides obtained by the MolDock Optimizer software





Schematic model of human vasopressin V_2R binding with AVP, OT-GKR and OT. OT-GKR binding sites were concentrated in extracellular loop 2 (EL2) of V2R which seems to play the most important part in activation because it is involved in direct binding of the ligand, ligand recognition, and ligand entry.

AVP and OT stimulate cAMP pathway in hamster kidney oocyte cells overexpressing AVP receptor V2. OT-GKR added in combination with AVP potentiate cAMP release but inhibits cAMP when combined with OT.



Highlights

- We have demonstrated that endogenous prohormonal peptide, OT-GKR, induces in the rat renal anti-diuretic, -natriuretic, -kaliuretic effects.
- OT-GKR competes with diuretic and natriuretic effects evoked by low concentration of OT in normal and volume expended conditions.
- The binding sites for OT-GKR were found on the structure of OTR and AVP V2 receptor.
- Although OT-GKR weakly induces cAMP in cells overexpressing V2R, OT-GKR has synergistic effect on cAMP released by AVP and inhibits cAMP induced by OT.

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