ATP as a Regulator of Anxiety-like Behaviour in the Paraventricular Nucleus of Rats

Stefanie Martinetz, Erwin H. van den Burg, Inga D. Neumann, David A. Slattery

Department of Behavioural and Molecular Neurobiology, University of Regensburg, Germany
Stefanie.Martinetz@ur.de

INTRODUCTION

- Selective breeding (i.e. for anxiety extremes) is an approach to discover novel gene candidates for the phenotype

- A microarray of PVN tissue of high- (HAB) and low- (LAB) anxiety-related behaviour rats [1] revealed *p2x4r* as such a potential target.

- P2X receptors are ATP-activated, ligand-gated trimeric cation channels, which display the highest permeability to calcium.

- Seven P2X subunits (P2X1-P2X7) have been characterized with the P2X4 receptor showing the most widespread distribution in the brain.

AIMS OF THE STUDY

- To verify the microarray data.

- To verify the existence of P2X4R within the PVN and its cellular distribution.

- To assess whether pharmacological activation of P2X4R alters anxiety-like behaviour in out-bred and HAB or LAB rodents using CTP [2] and 5-BDBD [3] (P2X4R agonist and antagonist, respectively).

CONCLUSION

- Quantitative-RT-PCR and Western Blot confirmed high P2X4 receptor expression in LAB rats - independent of sex.

- Immunohistochemistry revealed that the P2X4 receptor is strongly, but not exclusively, expressed on oxytocin-positive neurons within the PVN.

- Local P2X4R stimulation with CTP results in a dose-dependent anxiolytic effect in the PVN of male Wistar rats.

- Inborn anxiety levels of HAB and LAB rats can be altered by modulation of the P2X4R system.

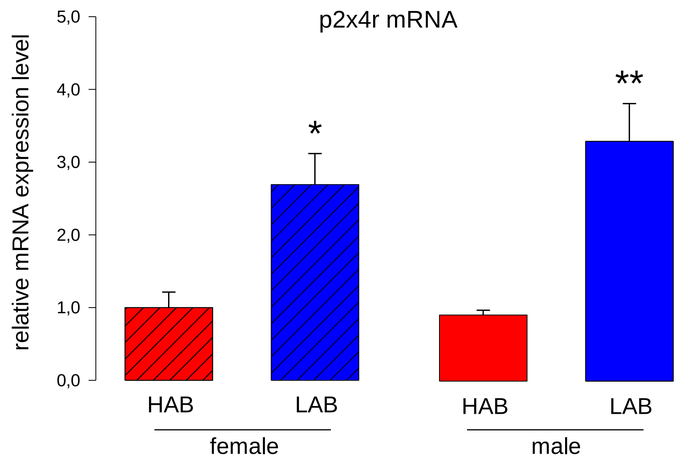
- **These findings suggest that purinergic neurotransmission within the PVN is involved in anxiety-like behaviour.**

1) Neumann ID, et al. Prog Neuropsychopharm Biol Psych 2001; 35 (6): 1357-75
2) Soto F, et al. Proc Natl Acad Sci 1996; 93: 3684-88
3) Fisher R, et al. 2005 Patent Number: EP1608659A1

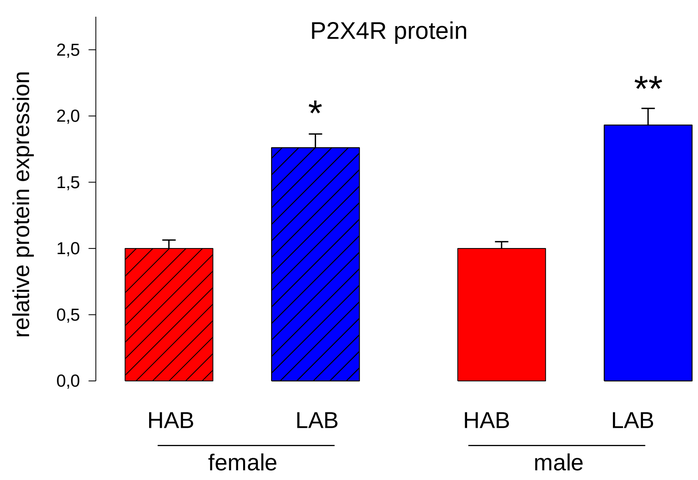
Disclosure: None of the authors has a conflict of interest to declare.

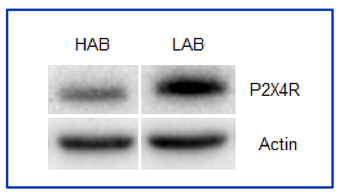
RESULTS

Basal P2X4 receptor expression is increased in LAB rats compared to HAB rats at mRNA and protein level within the PVN:



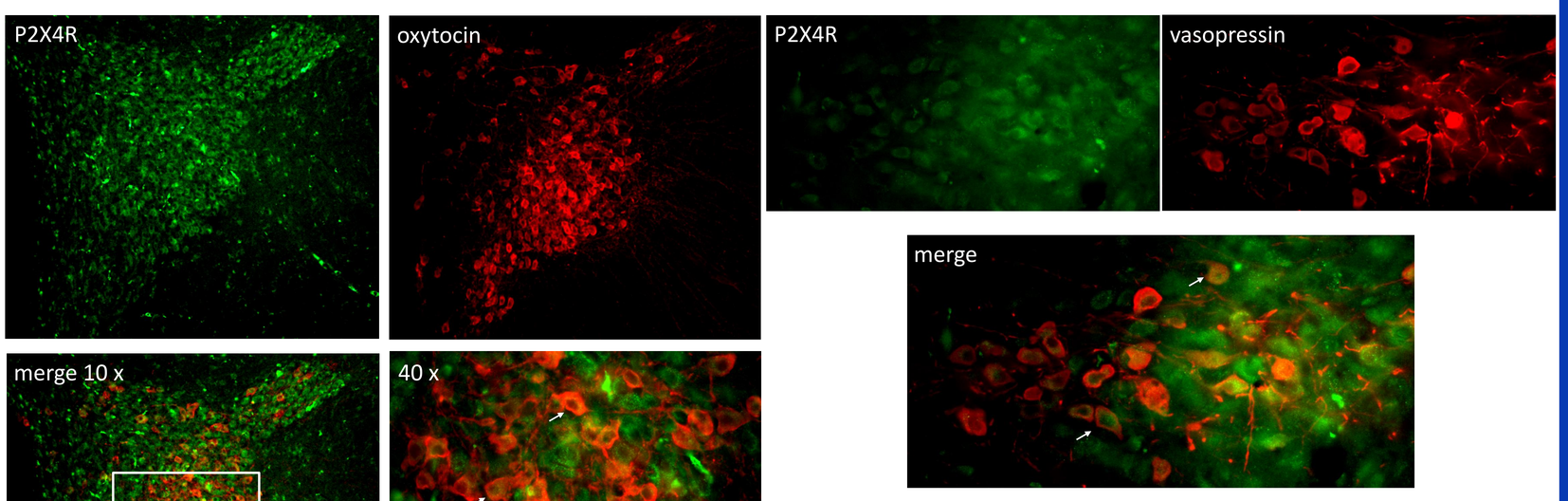
Basal levels of p2x4 receptor mRNA in the PVN of female and male HAB and LAB rats. P2x4 receptor mRNA levels were normalised to Gapdh mRNA levels as reference. Plot shows means + SEM; * $p < 0.05$, ** $p < 0.001$ vs HAB





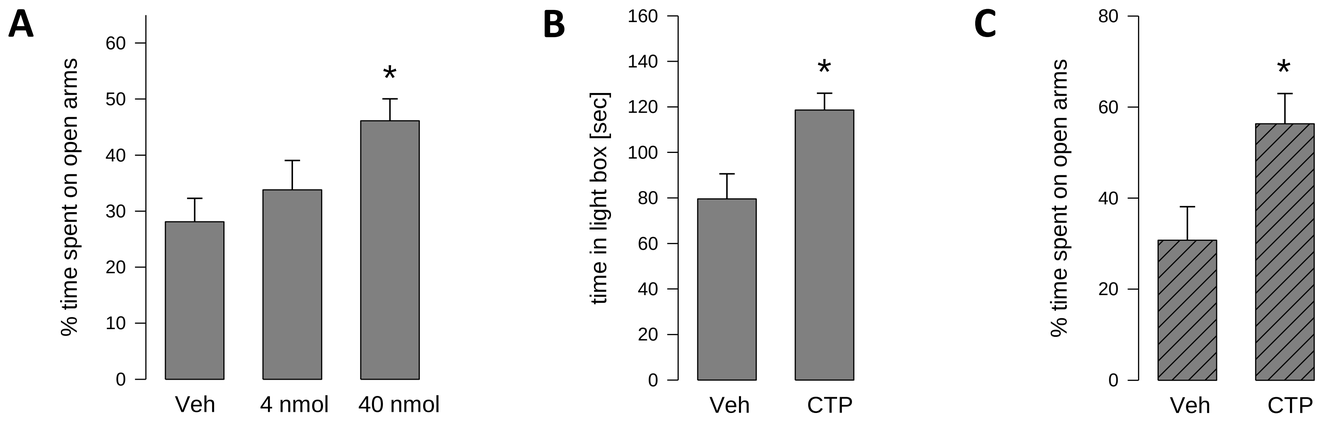
Basal levels of P2X4 receptor in the PVN of male HAB and LAB rats, normalised to actin levels. Plot shows means + SEM; * $p < 0.05$ vs HAB

Partial co-localization of P2X4R with oxytocin and vasopressin neurons within the PVN:



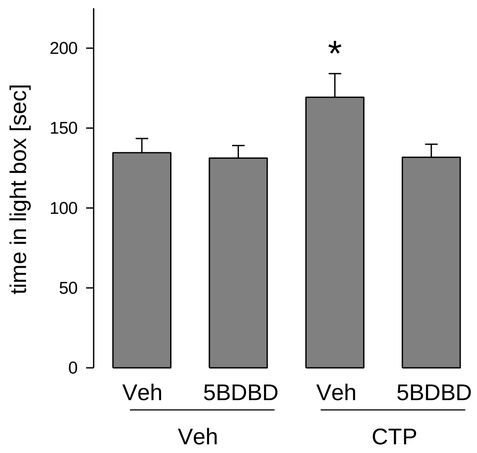
Immunostaining for P2X4R (green) and oxytocin or vasopressin (red) in neurons of the PVN. Overlay shows P2X4R positive cells co-expressing oxytocin or vasopressin (arrows).

Intra-PVN infusion of the P2X4R agonist CTP results in anxiolysis in Wistar rats:



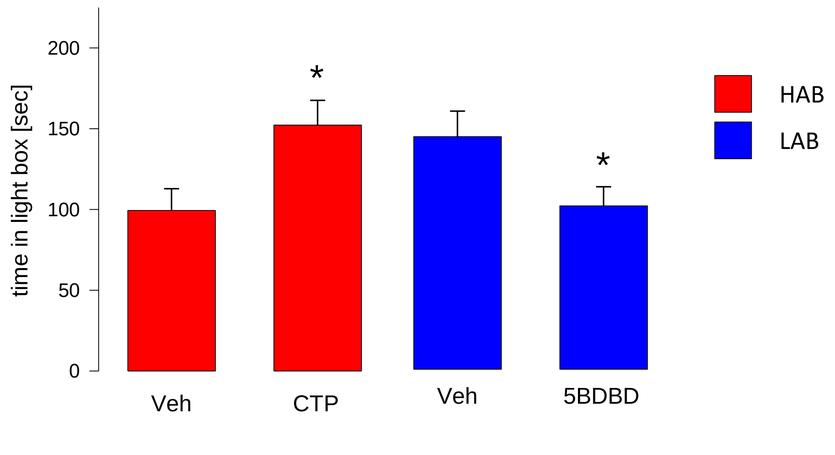
Local infusion of 4 nmol and 40 nmol CTP into the PVN of male (A + B) or female (C) Wistar rats 10 min prior to the EPM or the LDB. Rats treated with 40 nmol CTP show less anxiety-related behaviour mirrored by an increased amount of time spent on the open arms of the EPM (A + C) and in the light compartment of the LDB (B). Plots show means + SEM; * $p < 0.05$ vs Veh

Pre-infusion of the P2X4R antagonist blocks the effect of CTP:



Pre-treatment with 500 µM of the P2X4R inhibitor 5BDBD abolishes the anxiolytic effect of 40 nmol CTP in male Wistar rats.

Inborn anxiety levels can be altered by modulation of the P2X4R system:



Anxiolytic effect of a local infusion of 40 nmol CTP within the PVN of male HAB rats 10 min prior to the testing in the LDB. Anxiogenic effect of a local 5BDBD infusion in male LAB rats in the LDB. Plots show means + SEM; * $p < 0.05$ vs respective Veh