Bumetanide improves social behaviour in the BTBR mouse model of Autism

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Background

- Autism Spectrum Disorder (ASD) is a heterogeneous neurodevelopmental disorder characterized primarily by two prominent (core) behavioural symptoms including impaired social communication and restricted or repetitive behaviours.
- Preclinical ASD studies have indicated altered neuronal chloride homeostasis affecting the polarity of y-aminobutyric acid (GABA) neurotransmission as a potential treatment target [1,2].
- Bumetanide is a sodium-potassium-chloride cotransporter isoform 1 (NKCC1) inhibitor in the CNS regulating intracellular chloride levels and consequently GABA-related actions.
- Previous studies in two animal models of ASD (fmr1 KO mice and pregnant mice treated with valproic acid) have shown the ability of a prenatal treatment with bumetanide to attenuate autism-relevant behaviors in the offspring [3,4].

ASD: Autism Spectrum Disorder
CNS: central nervous system

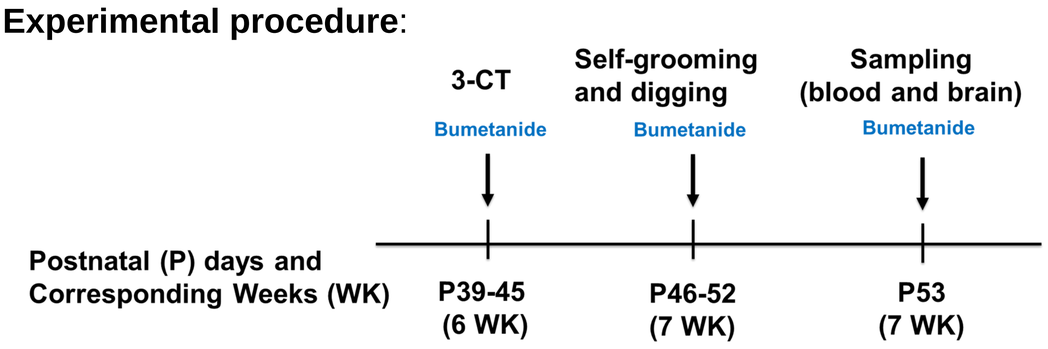
Aim of the study

The present study investigated the effect of bumetanide (3 doses; acute) on social and repetitive behaviors in young-adult BTBR T+ Itpr3tf/J (BTBR) mice, an inbred strain with high face validity for autistic-like symptoms.

Methods

Juvenile (6 week-old) male C57BL/6J (B6) mice as control animals or BTBR mice received an acute intraperitoneal (i.p.) injection of vehicle (VEH) or bumetanide at three doses (0.3, 3 or 30 mg/kg; 8 groups; N=15 per group).

- **Experimental procedure**:



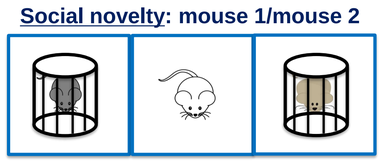
- **Behavioural studies**:

- 3-Chamber test (3-CT) was used to evaluate social behaviour of mice. After an habituation period (5 min in the center + 10 min in entire apparatus), the test mouse had the choice to explore a new congener or a novel object during 10 min (sociability; test 1). Immediately after, the test mouse had the choice to explore the now familiar congener or a new one for additional 10 min (social novelty preference; test 2). Main evaluation criteria: exploration (sniffing) time.

- Self-grooming and digging test was used to evaluate repetitive behaviour. After a 5 min habituation period, self-grooming was recorded for 10 min. Immediately after, the mouse was transferred to another cylinder filed with sawdust bedding and digging recorded for 5 min. Main evaluation criteria: time spent self-grooming and digging.

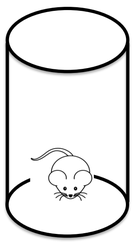
Sociability: novel object/mouse 1

Social novelty: mouse 1/mouse 2



Stimulus mice: C57BL6J, same age and sex

Self-grooming and digging
10 + 5 min



- **Tissue sampling:**

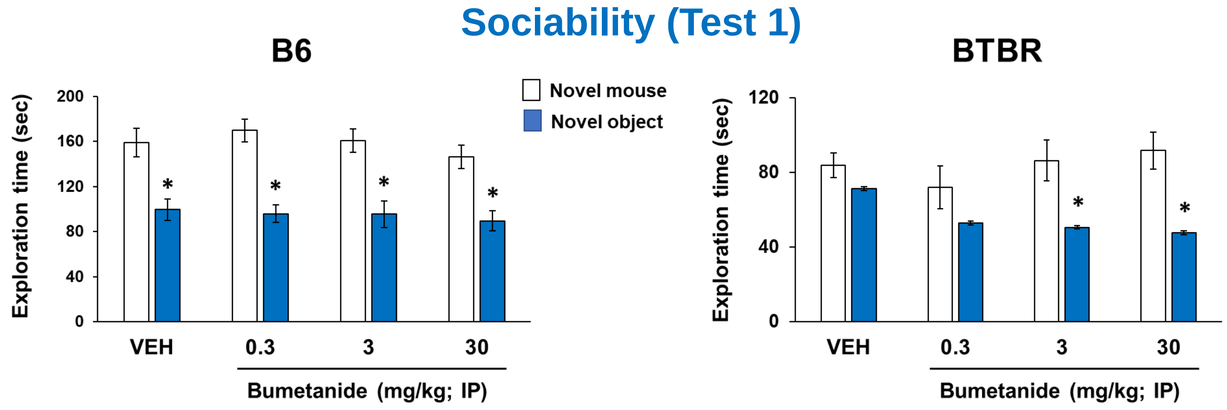
One or two days after behavioural testing, 36 animals of only the BTBR bumetanide-treated group (N=12/group) were administered with bumetanide (0.3, 3, and 30 mg/kg; i.p.) and blood and brain collected for PK and PK-modeling analysis at the following time-point: 5, 15, 30 and 45 minutes after dosing.

References:
1) Cellot G, Cherubini E. (2014) GABAergic signalling as therapeutic target for autism spectrum disorder. *Frontiers in Pediatrics*.2:70.
2) Ben-Ari Y. (2017) NKCC1 chloride importer antagonists attenuates many neurological and psychiatric disorders. *Trends in Neurosciences* 40(9):536-554.
3) Tyzio R et al. (2014) Oxytocin-mediated GABA inhibition during delivery attenuates autism pathogenesis in rodent offspring. *Science* 7;343(6171):675-9.
4) Eftekary S. et al. (2014) Response to comment "Oxytocin-mediated GABA inhibition during delivery attenuates autism pathogenesis in rodent offspring". *Science* 10;346(6206):176
5) Topfer M. et al. (2014) Consequences of inhibition of bumetanide metabolism in rodents on brain penetration and effects of bumetanide in chronic models of epilepsy. *Euro J Neuro* 39:673–687.

Results

Bumetanide dose-dependently increased sociability

Sociability (Test 1)



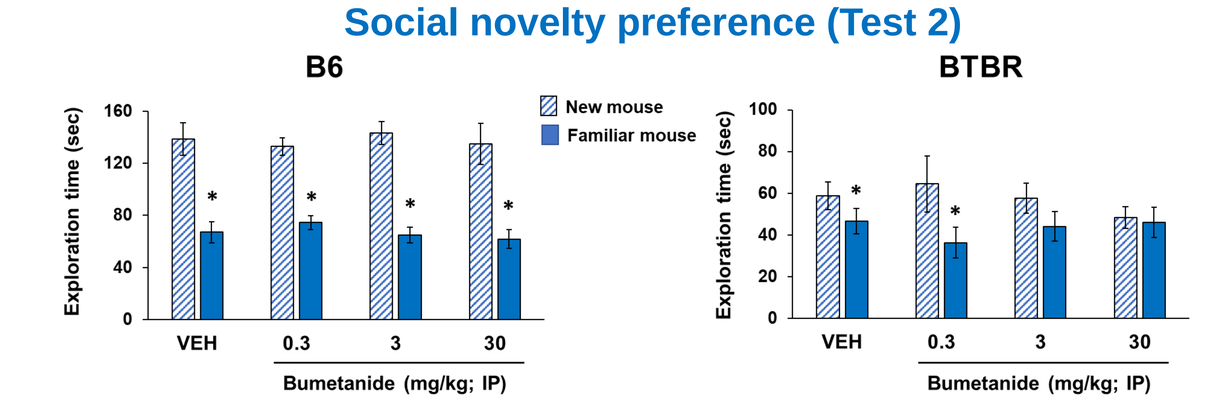
Novel mouse vs novel object (Mean ± SEM, n=15/group); *P<0.01 (Student's t-test)

Mean ± SEM; n=15/group; *P<0.05 (One-Way Anova, Dunnet's test)

BTBR VEH-treated mice showed an impaired sociability. Bumetanide (3 and 30 mg/kg) improved the sociability deficit displayed by BTBR mice without any detrimental effect on social behaviour as seen in the VEH- and bum-treated B6 control group.

Bumetanide did not affect social novelty preference

Social novelty preference (Test 2)



New mouse vs familiar mouse (Mean ± SEM; n=15/group); *P<0.01 (paired Student's t-test)

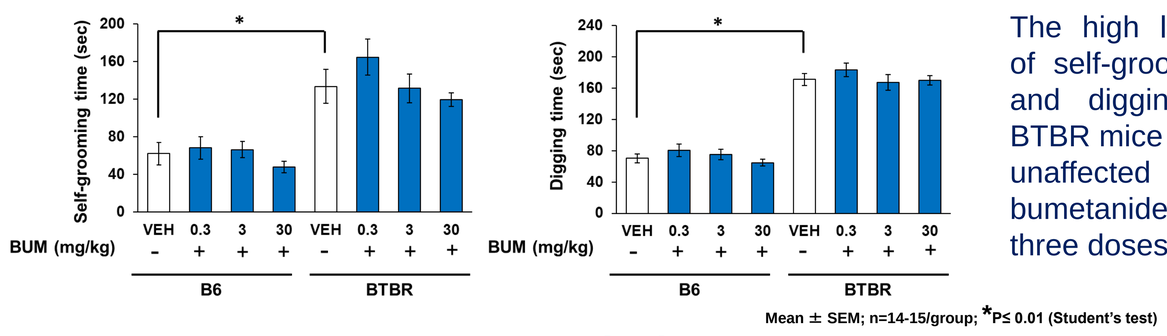
Mean ± SEM; n=15/group; *P<0.01 (One-Way Anova, Dunnet's test)

BTBR VEH-treated mice showed an impaired preference for social novelty compared to VEH-treated B6 mice. Bumetanide, at all tested doses, had no effect on social-novelty preference.

There was no significant difference between VEH- or bumetanide-treated BTBR and B6 mice for the distance travelled in the experimental apparatus during the whole test session.

Bumetanide did not affect repetitive behaviour

Self-grooming and digging

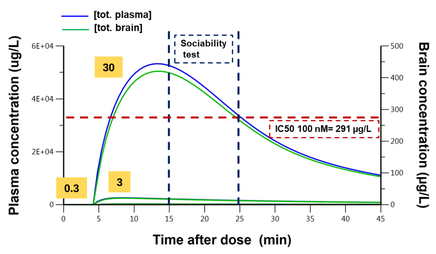


Mean ± SEM; n=14-15/group; *P≤ 0.01 (Student's test)

The high levels of self-grooming and digging in BTBR mice were unaffected by bumetanide (all three doses).

PK analysis

BTBR: exposure 0.3 – 3 – 30 mg/kg



At the highest dose (30 mg/kg; i.p.) the pharmacokinetic-modeling analysis confirmed brain concentrations of bumetanide in the proper range to engage the pharmacological target NKCC1 (IC_{50}= 100-300 nM)[5].

Conclusions

Acute bumetanide administration at the doses of 3 and 30 mg/kg attenuated the deficit in sociability, an autism-relevant symptom, displayed by the adolescent BTBR mice. Bumetanide was however not able to improve social novelty preference and to attenuate repetitive behaviour in this ASD model. Globally, the present study shows for the first time a postnatal effect of bumetanide in a relevant ASD animal model, supporting its use in clinical trials and suggesting bumetanide as a promising drug to enhance sociability deficits in ASD patients.