The Development and Utility of Rodent Paradigms to Assess the Activity of the Vasopressin V1a Receptor Antagonists, Balovaptan and JNJ-17308616, *In Vivo*

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1 BACKGROUND

The activity of vasopressin at the V1a receptor has been implicated in psychiatric indications and V1a receptor antagonists are being evaluated as novel therapeutics. Two small molecule V1a antagonists, balovaptan and JNJ-17308616, have been reported previously to be efficacious in animal models (Bleickerd *et al.,* 2009, . Previously it has been reported that central administration of vasopressin (AVP) produces a characteristic scratching behavior in mice that can be used as a surrogate behavioral measure of V1a receptor target engagement (Meisenberg *et al*., 1988). In addition AVP plays a role in regulating anxiety, that is partially mediated via V1a receptor (Bielsky *et al*. 2004, Egashira *et al.,* 2007).

The objective of these studies was to evaluate the effects of V1a receptor antagonists balovaptan and JNJ-17308616 in animal paradigms associated with vasopressin activity.

2 METHODS

Presented data is a result of three independent experiments. In Study 1 different doses of vasopressin (AVP: 1 ng, 10 ng, 25 ng) were tested in order to identified optimal AVP concentration. In Study 2 we tested efficacy of balovaptan and JNJ-17308616 to reverse AVP-triggered scratching behavior. In Study 3 balovaptan and JNJ-17308616 were tested in the elevated plus maze test of anxiety in rats.

Animals. In total of 72 Wistar rats, and 35 CD1 mice (Charles River, Germany) were used in the studies.

Efficacy of balovaptan and JNJ-17308616 in AVP-inducerd scratch model in mice:

Scratch Model Induction. Mice were anesthetized with 5% isoflurane (in 70% N_2O and 30% O_2) and placed in a stereotactic frame. During the injection, the concentration of the anesthetic was reduced to 1–1.5%. The rectal temperature was maintained at 37.0 ± 1.0 °C with a homeothermic blanket system. Single dose vasopressin (AVP, [Arg8]-Vasopressin acetate salt, Sigma-Aldrich) was administered as bolus injection (total volume 2.5 µl) using 10 µl Hamilton syringe and 28 G needle intracerebroventricularly (i.c.v.) at the following coordinates: AP = +0.5 mm; ML = +1.0 mm; DV = -2.5 mm relative to bregma from the brain surface. Injection has been made without skin incision through the scull and coordinates were achieved by calculation of the AP and ML distance from the point in midline between right and left eye and placing a stopper on the needle. After AVP was delivered the needle was left in place for 3 minutes before being withdrawn.

Scratching behavior monitoring. Immediately after vasopressin infusion mice were placed in a single cages and total time of scratching behavior was assessed and quantified by a trained observer for 15 minutes following AVP delivery. In addition video recording was performed and analyzed by second trained observer to confirm in-life assessments. Scratching behavior was defied as hind limb scratching, forelimb scratching, digging, face washing body grooming. The concentration of AVP and the pre-treatment time were selected following parametric studies.

V1a antagonists treatment. Once the optimal AVP concentration was established (10 ng) mice were pretreated with Balovaptan (100 or 300 mg/kg, p.o., PPT 30 min before AVP delivery), JNJ-17308616 (30 or 100 mg/kg, p.o., PPT 15 min before AVP delivery) or vehicle (p.o., PPT 15 min n=4 and 30 min n=3 before AVP delivery) and dosed with AVP to assess the effect on scratching behavior.

Efficacy of Balovaptan and JNJ-17308616 in elevated plus maze (EMP) test of anxiaty like behavior in rats:

Elevated Plus Maze (EPM) test in rats. Rats were administered with Balovaptan (100 mg/kg or 300 mg/kg, p.o. PPT 1h), JNJ-17308616 (100 mg/kg, p.o., PPT 1h), diazepam (5 mg/kg, i.p., PPT 30 min). Following administration of compounds rats were placed at the center of a black plastic EPM constructed in house. The arms of the maze measured 50 cm in length, 10 cm in width, and were elevated 50 cm above the floor. The closed arms were enclosed with plastic on three sides. Activity on the maze was monitored for 5 min by a camera mounted directly above the maze. The room was illuminated with red light, which measured 40 lx in the open arms and 13.5 lx in the closed arms. Analysis of videos was perform by observer blinded to the treatment using in house designed exel macro. Position of the rat was defined when all 4 paws were located in the same area (open arm, close arm, center). Data analysis: % of visit in the open arms = open arm visits *100/total number of visits in open and closed arms; % of time in open arms = time spent in open arm *100/time spent in open and closed arms

Pharmacokinetic (PK) analysis of Balovaptan and JNJ-17308616 exposure levels in plasma and brain.

Endpoint sampling and PK analysis. Immediately after EPM test rats were deeply anesthetized with pentobarbital (60 mg/kg, Orion Pharma). Blood sample was collected by cardiac puncture into precooled K_2EDTA tubes, centrifuged at 2 × 1,000 g, 10 min, 4 °C and plasma samples per animal collected, freeze on dry ice and stored at –80 °C. Next whole brain samples were collected. Brain and Plasma levels of Test Compounds were assessed.

3 RESULTS

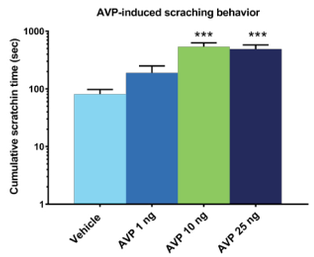


Figure 1. Cumulative time of AVP-induced scratching behaviour. Consistent with previous reports, the AVP-infused mice displayed a characteristic scratching behavior that has been linked to V1a receptor engagement. Intracerebroventricular delivery of AVP resulted in dose dependant effect on total time of scratching behaviour as compared to vehicle group (***p<0.001, 1-way ANOVA followed by Dunnett´s post hoc vs. vehicle group). Data presented as mean ± SEM. n = 7/group.

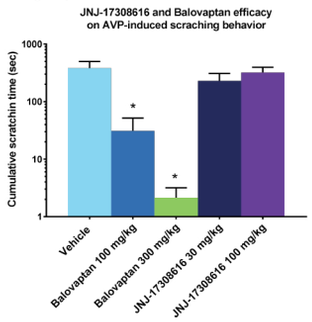


Figure 2. **Balovaptan and JNJ-17308616 efficacy on AVP-induced scratching behavior.** AVP has been administered i.c.v. at the dose of 10 ng to all experimental groups. Balovaptan showed dose –response in decreasing cumulative time of scratching behavior as compared to vehicle group (*p<0.05, Kruscall-Wallis ANOVA). No efficacy has been observed in JNJ-17308616 treated groups. Data is presented as mean ± SEM. n = 7/group.

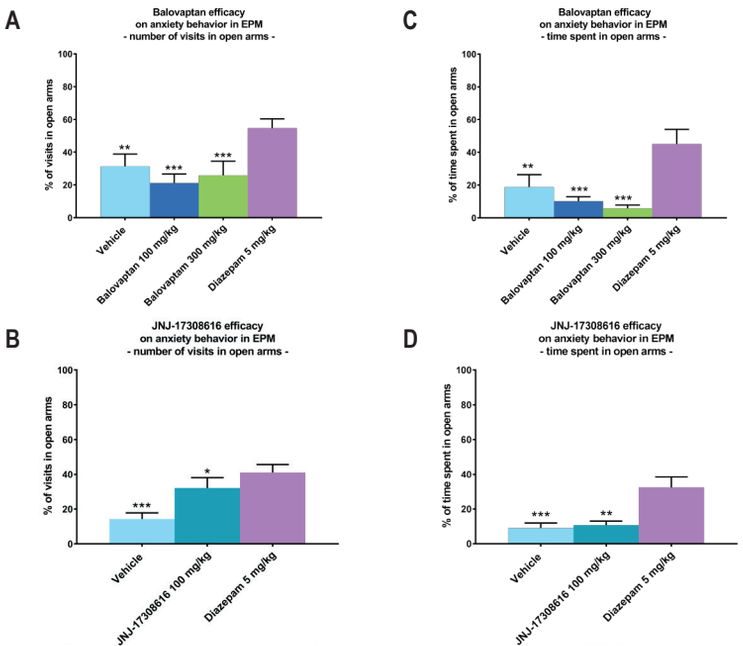
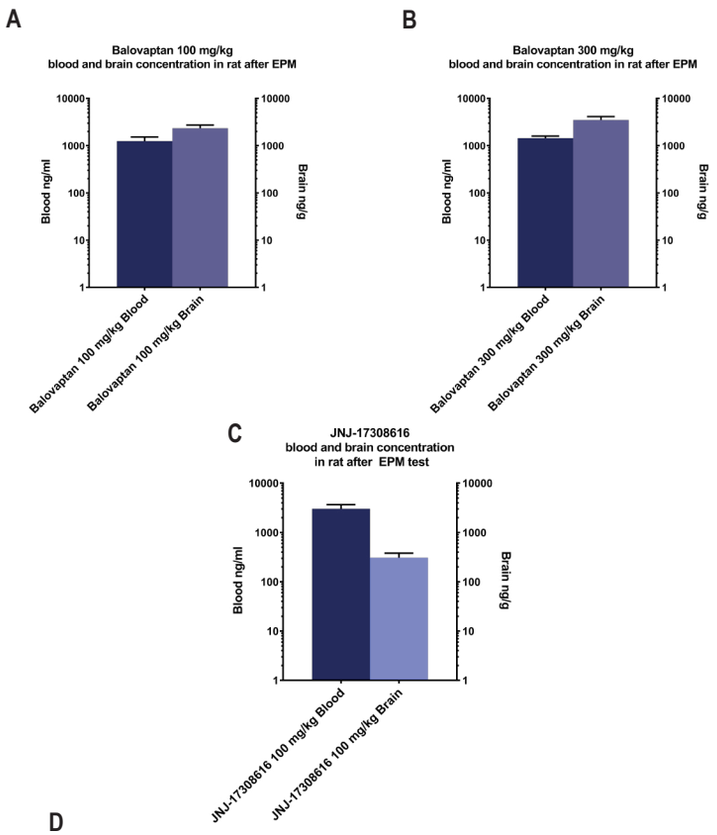


Figure 3. Balovaptan and JNJ-17308616 efficacy in the elevated plus maze (EPM) test of anxiety in rats. Diazepam increased number of visit (**A, B**) and time spent in open arms (**C,D**) as compared to vehicle treated group (**p<0.01, 1-way ANOVA followed by Dunnett´s post hoc). Neither Balovaptan nor JNJ-17308616 was effective in increasing number of visits into open arms (**A, B**) or time animals spent in open arms (**C,D**) as compared to Diazepam treated group (**p<0.01, ***p<0.001, 1-way ANOVA followed by Dunnett´s post hoc vs. Diazepam group). Data is presented as mean ± SEM. n = 11-12/group.

3 RESULTS CONT'D



D

Compound	Mean Brain:Blood ratio Rat p.o.
Balovaptan 100 mg/kg	2.0
Balovaptan 300 mg/kg	2.4
JNJ-17308616 100 mg/kg	0.15

Figure 4. Pharmacokinetic (PK) analysis of Balovaptan and JNJ-17308616 in rats after EPM test. Blood (**A**) and brain (**B**) concentration after single p.o. dosing in rat. Balovaptan had robust mean plasma (100 mg/kg: 1250 ng/ml; 300 mg/kg: 1449 ng/ml) and mean brain (100 mg/kg: 2344 ng/g; 300 mg/kg: 3496 ng/g) exposures. **C.** After p.o. dosing of JNJ-17308616 plasma exposure was 3023 ng/ml (100 mg/kg), but brain exposure was lower as compared to Balovaptan (100 mg/kg: 309 ng/g). **D**. Brain:Blood ratio of Balovaptan and JNJ-17308616 in rat. Data presented as an mean + SEM, n=11-12/group

4 CONCLUSIONS

The data from these studies demonstrate the in vivo activity of small molecule V1a antagonist Balovaptan following systemic administration in AVP-induced scratching behaviors in the mouse. However, additional studies are will need to be conducted to evaluate whether these effects can be extended to animal models of anxiety.

5 REFERENCES

1. Bleickerd *et al*., *Psychopharmacology (Berl). 2009 Mar;202(4):711-8.*
2. Meisenberg *et al*., *Ann N Y Acad Sci 525:257–269*
3. Bielsky *et al.* Neuropsychopharmacology. 2004 Mar;29(3):483-93
4. Egashira *et al.,* Behav Brain Res. 2007 Mar 12;178(1):123-7.