**Bryophyllum pinnatum** Inhibits Oxytocin and Vasopressin Signaling in Myometrial Cells

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*Bryophyllum pinnatum*, Crassulaceae, oxytocin, vasopressin, V₁A receptor, OT receptor, myometrium

**ABSTRACT**
The medicinal plant *Bryophyllum pinnatum* was previously shown to block oxytocin (OT)-induced signals in myometrial cells, consistent with its tocolytic effect observed in patients. OT activates not only OT receptors but also V₁A receptors, two receptors with high receptor homology that are both expressed in the myometrium and play a crucial role in myometrial contraction signaling. We aimed to study the molecular pharmacology of *B. pinnatum* herbal preparations using specific receptor ligands, the human myometrial cell line hTERT-C3, and cell lines expressing recombinant human OT and V₁A receptors.

We found that press juice from *B. pinnatum* (BPJ) inhibits both OT- and vasopressin (AVP)-induced intracellular calcium increases in hTERT-C3 myometrial cells. In additional assays performed with cells expressing recombinant receptors, BPJ also inhibited OT and V₁A receptor-mediated signals with a similar potency (IC₅₀ about 0.5 mg/mL). We further studied endogenous OT- and AVP-sensitive receptors in hTERT-C3 cells and found that OT and AVP stimulated those receptors with similar potency (EC₅₀ of ~ 1 nM), suggesting expression of both receptor subtypes. This interpretation was corroborated by the antagonist potencies of atosiban and relcovaptan that we found. However, using qPCR, we almost exclusively found expression of OT receptors suggesting a pharmacological difference between recombinant OT receptors and native receptors expressed in hTERT-C3 cells.

In conclusion, we show that *B. pinnatum* inhibits both OT and AVP signaling, which may point beyond its tocolytic effects to other indications involving a disbalance in the vasopressinergic system.

**Introduction**
*Bryophyllum pinnatum* (Lam.) Oken, (syn. *Kalanchoe pinnata* (Lam.) Pers.; family Crassulaceae) is a perennial succulent plant that is found in tropical and subtropical regions [1]. The plant species is a rich source of secondary plant metabolites including flavonoids, triterpenes, and bufadienolides [2]. In its countries of origin, the plant is employed in a wide range of indications, e.g., treatment of wounds, inflammatory diseases, and gastrointestinal diseases [3, 4]. Since the 1970s, *B. pinnatum* has been used in obstetrics as an alternative tocolytic for the treatment of preterm labor [5, 6]. Over the past 20 years, researchers aimed to gain more information about the potential mode of action of *B. pinnatum* to explain its observed tocolytic potential.

Preterm labor—triggered to a considerable part by preterm myometrial contractions—constitutes one of the leading causes for mortality and morbidity in newborns [7]. The main therapeutic approach for the prevention of preterm birth is the inhibition of preterm myometrial contractions by tocolytics (such as calcium channel blockers, beta-adrenergic receptor agonists, or oxytocin (OT) receptor antagonists). The most important purpose of acute tocolysis is to prolong pregnancy by 48 hours and gain time for...
the administration of glucocorticoids for fetal lung maturation and in utero transfer to a perinatal center with neonatal intensive care unit [8]. Although a meta-analysis from 2016 showed that there was no evidence for currently used tocolytics of prolonging pregnancy or reducing the rate of perinatal deaths [9], a more recent meta-analysis suggests that the assessed tocolytics were probably all effective in delaying birth for 48 hours [10]. When looking at clinical evidence for the effectiveness of tocolytic treatment, a distinction between acute tocolysis and long-term or maintenance tocolysis (continuation of therapy beyond the initial 48 hours) must be made [11]. Despite there being insufficient evidence for the benefit of maintenance tocolysis (resulting of omission of long-term treatment in current guidelines), it is frequently performed in clinical practice [12]. The lack of evidence for better maternal and fetal outcomes after long-term tocolysis and the frequent occurrence of fetal, neonatal, and maternal side effects due to currently used tocolytics that are not utero-specific demonstrate the need for new tocolytics.

The hormone OT is known to play a leading role in triggering myometrial contractions (term and preterm) via its receptor and has been the topic of intense research in the past, as well as a main target for the development of tocolytics [13, 14]. The OT receptor is a G-protein-coupled receptor (GPCR) signaling via heterotrimeric Gq/11 proteins to PLC-β and IP3, leading to an increase in intracellular calcium concentration ([Ca2+]i). Ca2+ then forms a complex with calmodulin, leading to the activation of myosin light chain kinase (MLCK), resulting in the phosphorylation of MLC, initiating the actin-myosin cross-bridge cycle and myometrial contraction (Fig. 1a) [15]. OT and the structurally related arginine-vasopressin (AVP) are pseudo-cyclic nonapeptides, which only differ by two amino acids. AVP also signals via GPCRs, namely V1A, V1B, and V2 receptors. Due to the high degree of ligand similarity and receptor homology, there is commonly observed cross-talk (Fig. 1a) between the agonist-receptor systems since both ligands are agonists at all four receptors [16]. Therefore, the importance of AVP in the onset of labor has become apparent and is being recognized [14]. The OT receptor antagonist atosiban, for instance, which is the only tocolytic specifically developed for this purpose, is actually a more potent V1A receptor antagonist [16] but may also act as an agonist at the V1B receptor [17]. It antago-
nizes the OT receptor with an IC$_{50}$ of 400 nM and the V$_{1A}$ receptor with an IC$_{50}$ of 5 nM [16].

Previous research has shown that *B. pinnatum* preparations (including press juice; BPJ) reduce the strength of spontaneous contractions of myometrial biopsies gained during C-sections in the *ex vivo* organ bath model [18–20]. *In vitro* experiments showed that BPJ inhibits an OT-induced [Ca$^{2+}$]$_i$ increase in human myometrial cell lines [21, 22]. These results support the clinically observed tocolytic effect of *B. pinnatum* preparations and make this herbal medicine an additional treatment option for preterm labor. It is currently unclear whether *B. pinnatum* exerts its contraction-reducing effect via OT receptors or another signaling pathway (e.g., the V$_{1A}$ receptor) or a combination thereof. Here, we aimed to decipher the molecular signaling signature of *B. pinnatum* focusing on OT and V$_{1A}$ receptors in myometrial and recombinant cell lines.

**Results and Discussion**

As recently shown, BPJ inhibits an OT-induced [Ca$^{2+}$]$_i$ increase in the human myometrial hTERT-C3 cell line in a concentration-dependent manner [21,22]. We now continued this study using a Ca$^{2+}$-sensitive fluorescent dye and a higher throughput format to quantify the pharmacological effect. For consistency with our earlier studies, we used a saturating OT concentration of 100 nM. As shown in ▶ Fig. 1b, BPJ inhibited the transient fluorescent signals induced by OT in a concentration-dependent manner, resulting in an IC$_{50}$ for BPJ of 0.42 mg/mL (pIC$_{50}$ = 3.38 ± 0.04, n = 3). While the antagonist potency of BPJ is weak, the slope of the inhibition curve represented by the Hill coefficient of approximately 1.7, determined by fitting our data with a 4-parametric Hill equation, was high compared to synthetic antagonists like relcovaptan or atosiban.

Knowing that OT receptors are also activated by AVP, we also tested whether BPJ is able to block AVP-induced signals in this myometrial cell line, as this has not yet been tested. As shown in ▶ Fig. 2c, BPJ is also blocking signals in hTERT-C3 cells induced by 10 nM AVP with an IC$_{50}$ of 0.26 mg/mL (pIC$_{50}$ = 3.38 ± 0.04; n = 3). There is no statistically significant difference between the pIC$_{50}$ values of BPJ against OT or AVP (p > 0.05).

This data would be consistent with two interpretations: 1. OT and AVP both stimulate OT receptors in hTERT-C3 cells and BPJ is an OT receptor antagonist, or 2. hTERT-C3 cells express both OT and V$_{1A}$ receptors.
receptors and AVP receptors, and BPJ is an antagonist at both receptors.

As it was already shown that human myometrium expresses both OT receptors and V1A receptors [23], we aimed to pharmacologically dissect those receptors and study the effect of BPJ. Relcovaptan is reportedly a specific V1A receptor antagonist with an IC50 of around 10 nM [16]. We confirmed this in CHO-K1 cells stably expressing recombinant human V1A receptors using the same assay conditions as above. In line with published data, we determined an IC50 for relcovaptan at hV1A receptors of 14.7 nM against 100 nM OT (pIC50 = 7.83 ± 0.07, n = 3) and 3.0 nM against 10 nM AVP as agonist (pIC50 = 8.53 ± 0.12, n = 3; ▶ Fig. 2a). Of note, relcovaptan was also antagonizing OT receptors but with about a 100-fold lower potency: at human OT receptors stably expressed in CHO-K1 cells, we found an IC50 for relcovaptan of 1 µM (pIC50 = 5.94 ± 0.08, n = 3; ▶ Fig. 2b) when using 100 nM OT as agonist. Thus, we pre-incubated hTERT-C3 cells with 1 µM relcovaptan to fully block their V1A receptors while leaving a significant part of the OT receptors unaffected. Under those conditions, OT-induced signals decreased by about 20%, and BPJ was able to block this remaining signal with similar IC50 values as without relcovaptan (▶ Fig. 2d; pIC50 = 3.4 ± 0.2, n = 3).

Those pharmacological results suggest that most of the OT- and AVP-sensitive receptors in the hTERT-C3 cells are OT receptors, supporting that BPJ is an OT receptor antagonist. Indeed, BPJ antagonized OT-induced signals in CHO-K1 cells expressing hOT receptors with an IC50 of 0.66 mg/mL (pIC50 = 3.01 ± 0.04, n = 3; ▶ Fig. 3a). Surprisingly, BPJ was similarly inhibiting OT- and AVP-induced signals in CHO-K1 cells expressing hV1A receptors with very similar IC50 values of 0.99 mg/mL and 1.18 mg/mL, respectively (pIC50 = 2.91 ± 0.05; n = 3 for OT as agonist; pIC50 = 2.93 ± 0.04, n = 3 for AVP as agonist; ▶ Fig. 3c), showing that BPJ is an equipotent antagonist at both recombinantly expressed human receptors, equipotently inhibiting OT- and AVP-induced signals in the myometrial cell line.

We observed in a few experiments small fluorescent signals upon application of the two highest concentrations of BPJ (▶ Fig. 3b). The kinetics of the signal was slow and clearly different to OT- or AVP-induced signals, and the signals were not significantly reduced by pre-application of a concentration of relcovaptan.
tan that blocks >90% of both OT and V1A receptors, suggesting BPJ is not a partial agonist of OT or V1A receptors in those cells (▶Fig. 3d).

To further investigate the receptor subtypes expressed in myometrial hTERT-C3 cells, we studied the agonist pharmacology in our functional cellular assay. Based on literature data, we expected that AVP is about a 10-fold less potent agonist at OT receptors than OT, and vice versa: OT is about a 10-fold less potent agonist than AVP at hV1A receptors [16]. In our recombinant cell lines, we basically confirmed this agonist pharmacology as shown in ▶Fig. 4a, b and Table 1. However, when testing both agonists at the native OT- and AVP-sensitive receptors in hTERT-C3 cells, they showed statistically not different EC50 values slightly below 1 nM (▶Fig. 4c and Table 1). The observed agonist potencies indicate that the previously used OT concentrations of 100 nM have been rather high in relation to the OT’s EC50 value. Usually, it is advised in antagonist experiments to use agonist concentrations that range in their EC80-90 value to remain sensitive to competitive antagonists that show less inhibition at higher agonist concentrations. Therefore, we also reduced the OT concentrations in the following antagonist experiments to 10 nM, like the AVP concentration. Interestingly, the IC50 for relcovaptan in hTERT-C3 cells is between the values found using the recombinantly expressed human receptors: IC50(relcovaptan) = 467 nM (pIC50 = 6.33 ± 0.09, n = 4) when stimulated with 10 nM OT and 110 nM (pIC50 = 6.96 ± 0.09, n = 4) when stimulated with 10 nM

▶Fig. 4 Agonist and relcovaptan pharmacology suggests that oxytocin-sensitive receptors in hTERT-C3 cells are a mixed population of hOT receptors and hV1A receptors. a, b CRC of vasopressin (AVP; filled symbol, solid line) and oxytocin (OT; open symbol, dotted line) on CHO-K1 cells expressing human V1A receptors (a, blue markers and lines) and human OT receptors (b, green markers and lines). Markers show peak ΔF/F fluorescent values; error bars SEM (n = 4). Sigmoidal curves are 4-parametric Hill curve fits. EC50 values determined for those curves are 0.32 nM for AVP and 3.40 nM for OT at V1A receptors (a) and 9.81 nM for AVP and 1.33 nM for OT at OT receptors (b). c CRC of AVP (filled symbol, solid line) and OT (open symbol, dotted line) on myometrial hTERT-C3 cells. EC50 values determined for those curves are 0.76 nM for AVP and 0.88 nM for OT. d CRC of relcovaptan at hTERT-C3 cells inhibiting OT- (10 nM, open diamonds) or AVP- (10 nM, closed diamonds) induced signals. ΔF/F values were normalized to the agonist responses in the presence of vehicle (0.1% DMSO).

▶Table 1 EC50/pEC50 values of OT and AVP in different cell lines.

<table>
<thead>
<tr>
<th>Cells</th>
<th>Agonist</th>
<th>pEC50 [M]</th>
<th>EC50 [nM]</th>
</tr>
</thead>
<tbody>
<tr>
<td>CHO-K1 OT R</td>
<td>OT</td>
<td>8.88 ± 0.06</td>
<td>1.33</td>
</tr>
<tr>
<td></td>
<td>AVP</td>
<td>8.01 ± 0.02</td>
<td>9.81</td>
</tr>
<tr>
<td>CHO-K1 V1A R</td>
<td>OT</td>
<td>8.47 ± 0.17</td>
<td>3.40</td>
</tr>
<tr>
<td></td>
<td>AVP</td>
<td>9.50 ± 0.12</td>
<td>0.32</td>
</tr>
<tr>
<td>hTERT-C3 OT</td>
<td>OT</td>
<td>9.06 ± 0.11</td>
<td>0.88</td>
</tr>
<tr>
<td></td>
<td>AVP</td>
<td>9.12 ± 0.08</td>
<td>0.76</td>
</tr>
</tbody>
</table>
AVP (▶ Fig. 4d). This observation led us to study receptor expression in hTERT-C3 cells at the RNA level using qPCR for hOT receptors and hV1A receptor-coding mRNA. As shown in ▶ Fig. 5a, we found in this cell line almost exclusively mRNA coding for hOT receptors and 6000-fold less mRNA coding for hV1A receptors.

We went on and studied the pharmacology of those native hOT receptor populations in hTERT-C3 cells using atosiban, a competitive OT receptor antagonist with reported IC50 values at hV1A receptors in the single-digit nM range and at hOT receptors in the range of several hundred nM [16]. When we stimulated hTERT-C3 cells with 10 nM OT, atosiban concentration dependently inhibited the agonist-induced signals with an IC50 of 180 nM. ▶ Fig. 5b shows that atosiban was about 20-fold more potent in inhibiting signals induced by 10 nM AVP (IC50 = 7 nM). Thus, atosiban seems preferentially to inhibit the signaling induced by AVP rather than by OT in cells that apparently only express OT receptors. This difference cannot be simply explained by a differential competition of atosiban with two agonists with different potencies because we used both almost equipotent agonists (see ▶ Fig. 4c) at the same concentration of 10 nM. It is well known that atosiban is a potent V1A receptor antagonist [16], which we confirmed with an IC50 of 10 nM in our assay (▶ Table 2). However, it is new that atosiban inhibits the apparently pure OT receptor population in hTERT-C3 cells with different potencies dependent on whether they are activated by OT or by AVP. The preferential inhibition of AVP-mediated signals of atosiban might explain its frequently observed side effects like nausea and vomiting, hypotension, tachycardia, and headaches [24].

Taken together, we could confirm the previously described inhibitory effect of BPJ on an OT-stimulated [Ca2+]i increase in myometrial cells and show for the first time that BPJ additionally inhibits AVP-induced Ca2+ signaling in an equipotent manner. At least in the often-used human myometrial cell line hTERT-C3, the inhibitory effect seems to be primarily mediated through the OT receptor as shown by qPCR. However, BPJ also inhibited AVP-induced signals at recombinant human V1A receptors expressed in CHO-K1 cells. Notably, the fitted Hill coefficients for BPJ are about twofold higher than those fitted for relcovaptan and atosiban (1.5–1.7 vs. 0.7–0.85). Higher Hill coefficients indicate that there seems to be a positive cooperation of B. pinnatum to inhibit receptor signaling, which is in line with earlier results showing that a flavonoid-enriched fraction and a bufadienolide-enriched fraction synergistically inhibited an OT-induced [Ca2+]i increase [22].

It should be mentioned that the observed potency of BPJ is moderate with an IC50 of around 0.4 mg/mL. The values measured in the present work (using a lyophilized press juice with a dry residue of 3.7%) are in line with inhibitory effects previously observed in experiments performed with fresh press juice (20 mg/
than at V1A receptors, and AVP, vice versa, has about a 10-fold higher potency at OT receptors compared to that known to have about a 10-fold higher potency at OT receptors. In these conditions, OT plays an important role in the recovery of the preparations.

Our pharmacological and molecular investigations suggest that hTERT-C3 cells express OT- and AVP-sensitive receptors that seem to be mostly OT receptors according to expression levels. Still, their pharmacology slightly differs from that found when isolated recombinant human OT receptors expressed in cell lines are used (Fig. 5c): First, the sub-nanomolar equipotency of OT and AVP as receptor agonists at the native receptors is inconsistent with a pure expression of hOT receptors in hTERT-C3 cells: OT is known to have about a 10-fold higher potency at OT receptors than at V1A receptors, and AVP, vice versa, has about a 10-fold higher potency at V1A receptors than at OT receptors [16]. Second, atosiban inhibits OT- and AVP-induced signals in hTERT-C3 cells with different potencies for the two agonists. A possible explanation for our observations is that the expression is associated with protein complexes in the native environment that may change the pharmacology of GPCRs, as it was already shown for several other GPCRs [14, 25]. Also, homo- or hetero-multimerization of the native receptors may change their pharmacology. Third, there are a number of polymorphic OT receptor variants reported [26–28] that may show differential ligand sensitivity. Testing those variants and a comparison of our results with the OT and V1A receptor pharmacology of freshly isolated myometrial cells could help clarify if our results are specific for the hTERT-C3 cell line or a general difference between native and recombinant OT receptor pharmacology. Given those differences, it is important for future drug discovery projects aiming to develop new tocolytics to take the pharmacology of the native OT- or AVP-sensitive receptors in the myometrium into consideration.

The experimental results described in the present work support the use of BPJ-based preparations in the treatment of preterm labor, where both OT and AVP play important roles. Moreover, they suggest that these preparations could be effective in the treatment of other gynecological indications, such as dysmenorrhea, in which AVP plays the major role in contractility regulation [14, 29, 30].

### Materials and Methods

#### Plant material

The leaves of cultivated *B. pinnatum* plants were harvested in São Paulo, Brazil, in March 2021 and were frozen shortly after harvest. Identification of plant material was done by Moacyr Copani Filho and Paulo Copani, supervised by Luzia Natalina Pereira Godinho (all Weleda Brazil). A voucher specimen (ZSS 29 717) was deposited at the Zurich Succulent Plant Collection. The press juice was obtained by the same pressing procedure used for the production of the active ingredient of Weleda Bryophyllum 50% chewable tablets (Weleda AG). The fresh press juice was lyophilized in a three-phase process (freezing at −50 °C; primary drying at −35 °C, 100 mTorr, 168 h; secondary drying at 20 °C, 55 mTorr, 72 h) in vacuum-sealed vials in an FTS Systems Dura-Dry MP Microprocessor Controlled Corrosion Resistant Freeze Dryer. A total of 147.5 g of fresh press juice was lyophilized, leading to 5.51 g lyophilisate (3.7% drying residue). For the experiments, the lyophilized press juice was reconstituted in DMSO (room temperature; stock solution 100 mg/mL) and filtered with a 0.45 µm syringe filter. Aliquots were kept at −20 °C.

#### Compounds and media

**HBSS assay buffer:** For the [Ca2+]i measurements, a buffer solution was used consisting of HBSS, 10 mM HEPES (both Biowest), 1 mM MgCl2, and 2 mM CaCl2 and adapted to pH 7.4 (with NaOH). **Calbryte520 AM working solution:** Calbryte520 AM (AAT bioquest) was used as a fluorescent agent for [Ca2+]i measurement. The stock solution (1 mM in DMSO) was diluted in HBSS, reaching a final concentration of 1 µM working solution on the day of experiment, and all solutions containing Calbryte520 AM were kept in the dark.

**Drugs:** OT, AVP, atosiban, and relcovaptan were obtained from Bachem. DMSO stock solutions were stored at −20 °C and diluted at the day of experiment in HBSS. DMSO and other drugs were obtained from Sigma Aldrich if not stated otherwise.

#### Cell culture

**CHO-K1 expressing hV1A and hOT receptors:** CHO-K1 expressing human V1A and OT receptors were kindly provided by Ch. Grundschober, Roche pRED Basel [31]. Briefly, the cells, which did not show basal expression of those receptors, have been stably trans-

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**Table 2** IC50/pIC50 values of receptor inhibitors atosiban, relcovaptan, and BPJ.

<table>
<thead>
<tr>
<th>Cells</th>
<th>Agonist</th>
<th>Atosiban</th>
<th>Relcovaptan</th>
<th>BPJ</th>
</tr>
</thead>
<tbody>
<tr>
<td>CHO-K1-OTR</td>
<td>OT</td>
<td>6.31 ± 0.05</td>
<td>5.94 ± 0.08</td>
<td>1.16</td>
</tr>
<tr>
<td></td>
<td>AVP</td>
<td>7.89 ± 0.08</td>
<td>8.53 ± 0.12</td>
<td>2.96</td>
</tr>
<tr>
<td>hTERT-C3</td>
<td>OT</td>
<td>6.75 ± 0.19</td>
<td>6.33 ± 0.09</td>
<td>3.38</td>
</tr>
<tr>
<td></td>
<td>AVP</td>
<td>8.15 ± 0.21</td>
<td>6.96 ± 0.09</td>
<td>3.58</td>
</tr>
</tbody>
</table>

IC50/pIC50 values of receptor inhibitors atosiban, relcovaptan, and BPJ.
fected with a plasmid containing cDNA coding either for the human V₁A or the human OT receptor under a CMV promotor and a neomycin resistance gene to select positive clones. Single clones have been isolated and selected based on their functional responses to their ligands and kept in liquid nitrogen and thawed for the experiments. After thawing, the cell lines were cultured in F-12 K cell culture medium supplemented with 10% heat-inactivated FBS and antibiotics (100 U/mL penicillin and 100 µg/mL streptomycin) and 800 µg/mL G418. In the culture, cells were split approximately two times a week. CHO-K1 transfected with the hOT receptor showed smaller functional signals and a significant signal run-down after passaging. Therefore, fresh batches of cells were thawed every two weeks.

**Intracellular calcium assay**

One day before the experiment, cells were seeded into 96-well flat bottom microplates (Greiner) at densities of 40 000 (CHO-K1) and 16 000 (hTERT-C3) cells/well, respectively. On the day of the experiment, cells were loaded with 1 µM Calbryte520 AM diluted in HBSS assay buffer at 37 °C for 1 h. Cells were washed twice with 80 µL HBSS assay buffer and let to rest for 5 min before placing the plate in the reading chamber of the FlexStation3 (Molecular Devices). Test substances were pipetted to the cell plate after baseline recording –20 s. For agonist measurements, this was performed once. Relative changes in peak fluorescence over baseline (ΔF/F) values were calculated for each well, and a 4-parametric Hill equation was fitted to the normalized individual data points using the nonlinear fit algorithm in Excel’s solver. pEC₅₀ and pIC₅₀ values are shown as means ± SEM, and a Student’s t-test was used to test for statistical differences. pIC₅₀ values for BPJ are calculated taking a pIC₅₀ = 0 for 1.0 g/mL. Agonist concentrations in antagonist experiments have been set between their EC₈₀ and EC₉₀ at the respective receptor, except for OT, which was initially used at 100 nM to keep consistent with data published earlier [22].

**Receptor expression quantification (quantitative rtPCR)**

Total RNA was isolated from hTERT-C3 myometrial cells using an extraction kit (AllPrep DNA/RNA/Protein Mini Kit, Qiagen) according to the manufacturer’s protocol, and its concentration and purity were measured using NanoDrop One (Thermo Fisher Scientific). cDNA was prepared from 1 µg RNA using a high-capacity RNA-to-cDNA kit (Applied Biosystems, Thermofisher Scientific) according to the manufacturer’s protocol. Analysis of the gene expression of the OT receptor (Hs00168573_m1) and V₁A receptor (Hs00176122_m1) and the housekeeping enzyme glyceraldehyde 3-phosphate dehydrogenase (GAPDH; Hs02758991_g1) was undertaken using TaqMan primers and TaqMan Fast Universal PCR Master Mix (all from Applied Biosystems, Thermofisher Scientific). Quantitative rtPCR was done with the Applied Biosystem 7500 Fast Real-Time PCR System and QuantStudio software (both from Thermofisher Scientific). mRNA expression of receptor genes was normalized to GAPDH expression, and relative quantification was calculated using the ΔCₜ method [33].

**Contributors’ Statement**


**Acknowledgements**

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**Conflict of Interest**

APSW has received research funding from Weleda AG over the last 5 years; however, Weleda AG had no influence on the design of experiments nor on the content of the publication. The other authors have no conflicts of interest to declare.

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