Proinflammatory role of vasopressin through V1b receptors in hapten-induced experimental colitis in rodents: implication in IBD

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Vasopressin and its receptors modulate several gut functions, but their role in intestinal inflammation is unknown. Our aims were to determine 1) the localization of V1b receptors in human and rodent colon, 2) the role of vasopressin and V1b receptors in experimental colitis using two approaches: V1b+/− mice and a selective V1b receptor antagonist, SSR149415, and 3) the mechanisms involved. V1b receptors were localized in normal and inflamed colon from humans and rats. Experimental colitis was induced in rats and mice and some tissues with SSR149415 abolished the vasopressin effect. Similarly, vasopressin had no effect in colonic preparations from WT mice pretreated with mast cell stabilizers. Vasopressin, through V1b receptor interaction, has proinflammatory properties linked to mast cell activation and downstream alterations of the colonic epithelial barrier. These findings underline the potential interest of V1b receptor blockers in gut inflammatory diseases.

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ground, no study has investigated so far the involvement of vasopressin and vasopressin receptors in gut inflammatory states as IBD or in animal experimental models.

Therefore, the aims of our study were 1) to compare the expression of V1b receptors in human and rat colonic tissues under normal and inflammatory conditions, 2) to determine a possible local involvement of vasopressin and V1b receptors in experimental colitis in V1b-deficient mice, 3) to evaluate the anti-inflammatory properties of a selective nonpeptide V1b receptor antagonist, SSR149415 (37), and finally 4) to explore the mechanisms through which vasopressin may participate in the inflammatory response.

MATERIAL AND METHODS

Human Samples

Human colonic biospecimens were obtained from several commercial providers (Asterand, Royston, UK; Indivumed, Hamburg, Germany; Cytomyx, Cambridge, UK; BioCat, Heidelberg, Germany), and represent samples from patients (male and female) diagnosed with IBD [ulcerative colitis, n = 11; Crohn’s disease, n = 8] as well as samples without pathological finding from gastrointestinal tract tumor patients (control tissue n = 10). The surgical biospecimens were granted for research by informed consent.

Chemicals

The V1b receptor antagonist SSR149415 (37) was synthesized at Sanofi-Aventis R&D, France. It displays nanomolar affinity for the V1b receptor in human and murine tissues and is 800 and 2,000 times more potent on V1b than on murine V1a and V2 receptor, respectively (37). It was dissolved in 0.6% methylcellulose in water and administered to rats or mice by oral gavage. Vasopressin was from Bachem (Weil am Rhein, Germany). Doxantrazole and sodium cromoglycate were purchased from Sigma-Aldrich (Saint Quentin Fallavier, France).

Animals

All protocols have been approved by the Animal Care and Use Committee of the different institutions. Male Wistar rats (200–250 g) were purchased from Elevage Janvier (Le Genest-St-Ise, France). V1b knockout (KO) mice (Avpr1b<sup>tm1Dgen</sup>) were purchased from Deltagen (San Mateo, CA). In this model, avpr1b gene was disturbed by insertion of the bacterial lacZ vector such that the endogenous gene promoter drives expression of β-galactosidase. Mice were backcrossed to C57Bl/6J genetic background and were bred at Sanofi-Aventis transgenic service by He × He mating. KO mice were compared with their wild-type (WT) littermates. Genomic DNA for PCR genotyping analysis was isolated from tail biopsies for all mice with DNasey 96 tissue kit (Macherey-Nagel, Düren, Germany) by use of a 96-well plate format on a Hamilton Microlab Start automated robotic system.

Experimental Colitis in Rats and Mice

DNBS-induced colitis in rats. Male Wistar rats weighing 200–250 g were used in this study. They were provided free access to food and water. Under light anesthesia, a rubber catheter was inserted by the rectal route into the colon so that the tip was ~8 cm proximal to the anus (approximately at the level of the splenic flexure). DNBS (2,4-dinitrobenzene sulfonic acid, ICN Biomedicals, Aurora, OH, 60 mg/kg in 50% ethanol) was then instilled into the lumen of the colon through the rubber catheter (total volume = 0.25 ml). Animals were used for subsequent experimental procedures on day 6 after DNBS treatment, at a time when the intestinal inflammatory process is maximal. In control experiments, the animals received 0.25 ml 0.9% NaCl alone. Body weight was observed over this 8-day period. Each group of rats consisted of 8–11 animals. SSR149415 was administered daily, at the same time, for 7 days, starting the day before the induction of colitis.

TNBS-induced colitis in mice. Male WT and V1b<sup>−/−</sup> mice weighing 20–25 g were used. Animals received water and food ad libitum. Under general anesthesia (Imalgène-Rompun 1:5 vol/vol, 0.05 ml/mouse sc) colitis was induced by intracolonic instillation of TNBS/ethanol solution (2,4,6-trinitrobenzene sulfonic acid, Sigma, 50 mg/kg, 50% ethanol, in 40 μl) via a catheter introduced by rectal route at 4 cm from the anus. Animals were euthanized 2 days after TNBS instillation. Three protocols were carried out. In a first series of experiments, SSR149415 was given daily from 1 day before to 1 day after TNBS intracolonic instillation. In a second set, SSR149415 was injected in a curative manner, i.e., from 1 day after colitis induction by TNBS. Finally, other animals were submitted to water deprivation for 24 h, and colitis was induced the next day. Drugs (SSR149415 30 mg/kg po, doxantrazole 10 mg/kg ip, or their respective vehicles) were administered in a preventive manner, i.e., from 1 day before to 1 day after colitis induction.

DSS-induced colitis in mice. Other WT and V1b<sup>−/−</sup> mice were treated orally with dextran sodium sulfate (4% wt/vol in drinking water) for 6 days and were euthanized at day 7. One group of animals was treated with SSR149415 from day 1 prior to DSS administration to day 6 (preventive treatment). Another group was treated from day 1 after colitis induction (curative treatment).

Genotyping

Genotyping assay was developed and executed using Taq Qiang Hot mix and the following primers for WT: V1361, 5’ TCT GGC CAC AGG AGG CAA CCT 3’; V1900c, 5’ ATC TCG TGG CAG ATG AGG CCA 3’; for KO animals: NEO S, 5’ GAT GGA TTG CAC GCA GGT TCT 3’; NEO AS: 5’ AGG TAG CCG GAT CAA GCG TAT 3’. PCR was performed using the following conditions: an initial step at 95°C for 15 min, then 34 cycles consisting of 45 s at 94°C, 45 s at 61°C, and 45 s at 72°C; and a final elongation step for 10 min at 72°C. Under these conditions, the common WT forward and WT reverse primers yield a 539-pb band for the WT allele, vs. a 378-pb band when utilizing the WT forward/Neo reverse primer for the targeted allele.

DIG-Labeled Riboprobe Generation

The DIG-labeled riboprobe was generated by in vitro transcription (Roche) of a PCR product generated with RNA polymerase binding site extended ratAVPR1b specific primers (T3 linked sense primer 5’-AAATACCCCTACTAAAGGCTACTCT-GAATTCTGAGCCTTC-3’ and T7 linked antisense primer 5’-TAATACGACT-CTACTATAGGGCTGGAAGCGTGAGTGT-GATGTC-3’) or rat PECAM-specific primers (T3 linked sense primer 5’-GAAGGACAACGGCTGCAAAGCAGCCAGT-GATGTC-3’ and T7 linked antisense primer 5’-AGGGTGAGTCTGAGGCTGGAAGCGTGAGTGT-GATGTC-3’).
primer 5'-AATTAACCCTCACTAAAGGGTGAGCGACAGTGGGACAC and T7 linked antisense primer 5'-TAATAGCAGCTC-ATATAGGGAGACATTAGCACACCTG from a rat AVPR1b or rat PECAM cDNA clone as the substrate and Photor Turbo DNA-polymerase (Stratagene, Cedar Creek, TX), respectively. After column-mediated purification (GE Healthcare), the DIG-labeled riboprobes were evaluated for integrity and quantity by gel electrophoresis (Invitrogen, Karlsruhe, Germany) and tested for their specificity and condition setup on transient transfected, ratAVPR1b overexpressing Chinese hamster ovary cells or control tissue.

**Immunohistochemistry and In Situ Hybridization**

Tissue sections (5 μm on SuperFrost/Plus) were generated (Microme HM340E), hematoxylin and eosin stained, and utilized in immunohistochemistry (IHC) or digoxigenin-mediated RNA in situ hybridization (ISH) experiments. IHC experiments were performed using the Ventana Discovery system. Briefly, tissue sections were deparaffinized by heat, pretreated according to the need of the individual primary antibody applied, and incubated with various primary antibodies, i.e., rabbit polyclonal anti-human AVPR1b antibody [LifeSpan LS-A3739, 10 ng/μl with low heat (950–124)/protease (760–2020) combination treatment at 37°C for 1 h] or with custom-made, rabbit polyclonal anti-rat AVPR1b antibody directed against the epitope amino acid 308–319 of accession number NP_05891 (Cambridge Research Biochemicals, Cleveland, UK). Secondary incubation was then performed with matching biotinylated goat anti-rabbit secondary antibody (Vector, Wertheim-Bettingen, Germany) for 16 min. For visualization, sections were incubated with the biotin-free, multimer technology detection kit UltraMap DAB, counterstained, and mounted afterward (Sakura, Staufen, Germany). High-resolution images were taken at the Aperio Scanscope Digital slide scanner (×20 objective).

ISH experiments were performed using Ventana Discovery system (Ventana, Illkirch, France). Briefly, tissue sections were deparaffinized by heat, pretreated for 20 min with protease, and hybridized with digoxigenin-labeled ISH riboprobe specific for rat AVPR1b [40 ng antisense (as) or sense (se) riboprobe matching at position 161–468 of Acc. no. NM_017205] or rat PECAM (25 ng, 347 nt) for 6 h at 65°C. After being washed three times for 10 min in 0.1× SSC buffer at 75°C, sections were incubated with alkaline phosphatase-linked anti-DIG antibody (Roche, Mannheim, Germany) for 30 min. For visualization, sections were incubated with the BlueMap kit (NBT/BCIP-mediated detection), counterstained with nuclear fast red, and mounted afterward. High-resolution images were taken at the Aperio Scanscope Digital slide scanner.

**Macroscopic and Microscopic Damage Scores**

Macroscopic colonic damage was assessed by a previously described method. Briefly, macroscopic criteria were based on the following (41, 43): presence of adhesions between the colon and other intra-abdominal organs (0 = none, 1 = mild, 2 = major), consistency of colonic fecal material (as an indirect marker of diarrhea) (0 = formed, 1 = loose, 2 = liquid), thickening of the colonic wall, presence and extent of hyperemia and macroscopic mucosal damage (0 = no damage; 1 = hyperemia; 2 = presence of an ulcer; 3 = ulcer + inflammation; 4 = two or more ulcers; 5 = major damage (presence of necrosis < 2 cm); 6 = very severe damage (presence of necrosis > 2 cm)).

Microscopic criteria for damage and inflammation were determined by light microscopy on hematoxylin and eosin-stained tissue sections obtained from whole gut specimens taken from a region of the inflamed colon immediately adjacent to the gross macroscopic damage and consisted to establish loss of mucosal architecture, cellular infiltration, muscle thickening, presence of crypt abscess, and goblet cell depletion (14).

**Measurement of Myeloperoxidase Activity**

MPO activity, a marker of polymorphonuclear neutrophil primary granules, was measured in colonic tissues, according to the method of Bradley et al. (4), slightly modified. After euthanasia, a sample of colon was suspended in potassium phosphate buffer (44 mM KH2PO4, 6 mM K2HPO4·3H2O, pH 6.0) and homogenized on ice by using a Polytron. A cycle of freezing and thawing was repeated three times. Suspensions were then centrifuged at 6,000 g for 15 min at 4°C. Supernatant fractions were discarded and pellets were resuspended in hexadecyl trimethylammonium bromide buffer (HTAB 0.5%, wt/vol, in 50 mM potassium phosphate buffer, pH 6.0), a detergent inducing the release of MPO from neutrophile primary granules. These suspensions were sonicated on ice and then centrifuged at 6,000 g for 15 min at 4°C. Pellets were discarded and supernatants were assayed spectrophotometrically for MPO activity. Supernatant was diluted in potassium phosphate buffer (pH 6.0) containing 0.167 mg/ml of O-dianisidine dihydrochloride and 0.0005% H2O2. Changes in absorbance at 450 nm were recorded every 30 s over 2 min. One unit of MPO was defined as the quantity of MPO degrading 1 μmol H2O2·min⁻¹·ml⁻¹ at 25°C. Finally, MPO activity expressed as units of MPO activity per milligram of wet tissue (U/mg).

**Determination of TNF-α Tissue Levels**

Samples of colon were removed from the area of gross injury, snap frozen, and stored at −80°C. On thawing, samples were weighed, homogenized in 1 ml of protease inhibitors cocktail (aprotinin, leupeptin, and pepstatin, 1 μg/ml), and centrifuged at 4°C. A 100-μl aliquot of supernatant was then added to the EIA 96-well plate in duplicate and assayed by using the manufacturer’s protocol (Rat TNF-α kit, Bender MedSystems, Vienna, Austria). Absorbance was read at 450 nm. TNF-α was expressed as picograms per milligram of wet tissue.

**Measurement of Colonic Paracellular Permeability**

Immediately after euthanasia, portions of WT and V1b−/− mouse proximal colon (exposed area, 0.3 cm²) were mounted into an Ussing chamber (Physiological Instruments, San Diego, CA), each side containing 5 ml of Krebs buffer (118 mM NaCl, 4.7 mM KCl, 1.2 mM KH2PO4, 1.2 mM MgSO4, 25 mM NaHCO3, 8.3 mM glucose, 2.5 mM CaCl2), continuously gassed with 95% O2-5% CO2. Transepithelial electrical resistance was monitored throughout the experiment to assess tissue viability. After 20 min, fluorescein isothiocyanate-labeled 4-kDa dextran was added at the mucosal side (2.2 mg/ml as final concentration). At the same time, vasopressin (10⁻⁹ to 10⁻⁷ M), SSR149415 (10⁻⁶ M), or vehicle was added in the

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serosal compartment. Fluorescence was measured at the serosal side after 60 min. The role of mast cells was investigated in groups of mice pretreated with either doxantrazole or sodium cromoglycate. Doxantrazole (10 mg/kg in NaHCO₃ 5%, ip route) was injected 2 h before euthanasia (12). Sodium cromoglycate (100 mg/kg) was dissolved in saline and injected by intraperitoneal route 12 h and 30 min before euthanasia (17).

**Statistical Analysis**

Results are expressed as means ± SE. Statistical analysis was performed by analysis of variance (one-way or two-way, as appropriate, with Bonferroni correction for multiple comparisons). A P value <0.05 was considered significant. Calculations were performed using GraphPad Prism (version 4.0, GraphPad Software, San Diego, CA).

**RESULTS**

V₁b Receptor Is Expressed by Human and Rat Tissues

V₁b receptor was detected by immunohistochemistry in normal human colon, ulcerative colitis, and Crohn’s disease cases. Positive labeling was found in enterocytes, both at the membrane and into the cytoplasm (Fig. 1). Moreover, staining was seen in selected cell bodies in a subset of ganglia of the enteric nervous system (Fig. 1). However, no difference in staining intensity and localization was observed between normal and inflammatory conditions (Fig. 1).

Interestingly, rat tissues showed the same staining localization as shown by in situ hybridization (Fig. 2A) and confirmed by immunohistochemistry (not shown). Seven days after the induction of colitis, an upregulation of the V₁b receptor expression was observed in rat enterocytes, and in the areas of mucosal regeneration. Regarding the enteric nervous system, no staining difference was observed in inflammatory conditions (Fig. 2B).

**Effect of SSR149415 on Experimental Colitis in Rats**

Body weight gain of noninflamed controls was 25.5 ± 1.9% at the end of the 8-day period of observation. After intrarectal DNBS, the body weight gain was significantly reduced with respect to noninflamed controls (11.2 ± 2.9%, *P* < 0.001). Treatment with SSR149415 at the doses of 3, 10, and 30 mg/kg did not reduce this impairment in body weight gain (data not shown).

Macroscopic and microscopic damage scores were significantly (*P* < 0.001) increased by intrarectal DNBS (Figs. 3 and 4). Oral SSR149415 at 3, 10, and 30 mg/kg significantly reduced macroscopic damage produced by DNBS (Fig. 3A); the three doses induced a global similar effect. Likewise, microscopic damage scores and TNF-α tissue levels were also lowered by SSR149415 pretreatment (Fig. 3, B and C). Finally, animals receiving SSR149415 exhibited lower MPO levels at the dose of 10 mg/kg (5.5 ± 1.23 vs. 18.3 ± 2.9 U/mg with DNBS, *P* < 0.01, *n* = 8), whereas the doses of 3 and 30 mg/kg decreased

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**Fig. 1.** V₁b receptor labeling by immunohistochemistry in human colonic biopsies from healthy subjects (A), ulcerative colitis patients (B), and Crohn’s disease patients (C). The V₁b receptor is present in enterocytes and some ganglia of the enteric nervous system. No difference of expression is observed under normal and inflammatory conditions.
activity without reaching significance ($P = 0.0963$ and $P = 0.0536$, respectively, $n = 8$).

**Experimental Colitis in Wild-Type and V1b$^{−/−}$ Mice**

Two days after intrarectal administration of TNBS, severe macroscopic damages (Fig. 5A) associated with high MPO activity (Fig. 5B) and weight loss (Fig. 5C) were observed in WT animals. By contrast, macroscopic damage scores and MPO levels were significantly lower in V1b$^{−/−}$ mice ($366 \pm 82$ U/mg of protein vs. $689 \pm 49$, Fig. 5, $P < 0.05$). Weight loss was also less severe in V1b$^{−/−}$ mice ($P < 0.05$). This strongly suggests that vasopressin via V1b receptor activation is involved in the early phase of mucosal damage triggered by TNBS, since the absence of V1b receptors led to a markedly weaker inflammation. Preventive or curative treatment with SSR149415 (30 mg/kg po) significantly decreased macroscopic damage and MPO activity in WT animals, to levels similar to those of V1b$^{−/−}$ animals ($393 \pm 49$ U/mg of protein, Fig. 5). No additive effect of SSR149415 and V1b deficiency in mice was observed, which illustrates the antagonist specificity (Fig. 5, A and B). Regarding DSS-induced colitis, SSR149415 was devoid of any preventive or curative effect (Supplemental Fig. S1; the online version of this article contains supplemental data).

**Experimental Colitis on Water-Deprived Animals**

Water deprivation was performed to increase circulating vasopressin levels, according to Cristia et al. (9). This resulted in a dramatic mortality in TNBS-treated animals (9 of 10 mice in the group). Administration of either doxantrazole, a mast cell stabilizer, or the V1b antagonist SSR149415 prevented this elevated mortality and significantly reduced the colitis severity assessed by macroscopic damage scores and MPO activity (Fig. 6). Interestingly, the effect of SSR149415 was more...
pronounced in water-deprived animals compared with mice with free access to water (Fig. 6).

**Effect of Vasopressin on Mouse Colonic Paracellular Permeability**

Vasopressin, added at the serosal side of the WT mouse colonic wall, increased colonic permeability to FITC-dextran, used as a marker of the paracellular route. The dose response revealed a bell-shaped curve, the maximal effect being reached at $10^{-8}$ M ($2.30 \pm 0.33$, $P < 0.05$ vs. control, Fig. 7A). Basal permeability was unchanged in V1b$^{-/-}$ animals compared with controls ($0.91 \pm 0.09 \text{ nmol·h}^{-1} \cdot \text{cm}^{-2}$ vs. $0.82 \pm 0.09$, Fig. 7B). The effect of vasopressin was absent in V1b$^{-/-}$ animals (not significant vs. saline, Fig. 7B), and was abolished when WT colonic preparations were pretreated with SSR149415 $10^{-6}$ M ($0.79 \pm 0.13 \text{ nmol·h}^{-1} \cdot \text{cm}^{-2}$, $P < 0.05$ vs. vasopressin, Fig. 7B).

Pretreating WT mice with doxantrazole or sodium cromoglycate assessed the involvement of mast cells. No change in permeability was observed in pretreated animals (Fig. 7C), whereas vasopressin $10^{-8}$ M did not trigger any increase in colonic paracellular permeability in preparations from animals pretreated with either doxantrazole or cromoglycate ($0.76 \pm 0.10$ and $0.91 \pm 0.18 \text{ nmol·h}^{-1} \cdot \text{cm}^{-2}$, respectively; Fig. 7C).

**DISCUSSION**

Our results identify vasopressin as a putative participant of gut inflammation and show that the V1b receptor is a key target in the inflammation process, as shown in TNBS-induced experimental colitis. So far, the role of V1b receptor was poorly documented in the colon, and only RT-PCR experiments reported mRNA expression in the rat small intestine (35). In the present work, we have shown that V1b receptor expression in normal and inflamed human and rat colon tissues shares a similar localization, i.e., enterocytes and in cells of the enteric nervous system, whereas no staining was observed above background in other areas such as lamina propria. Moreover, in V1b-deficient mice, we observed a lower inflammatory response to TNBS and, in two models of hapten-induced experimental colitis, a reduction of gut inflammation under blockade of V1b receptors by SSR149415. In water-deprived animals, mast cell or V1b blockade ameliorated the dramatic mortality.
observed in TNBS-treated mice. Conversely, no improvement of inflammation was observed in DSS-induced colitis. Finally, Ussing chambers experiments revealed that vasopressin was able to increase colonic paracellular permeability of mouse tissue, this effect being dependent on V1b receptor activation and mast cells. Thus these findings report for the first time an original role for vasopressin in participating to inflammation, which can be, at least in part, linked to its ability to alter epithelial barrier.

Increased plasma levels of vasopressin have been described in various chronic inflammatory states in humans, such as cirrhosis (21), leukemia (23), and early phase of sepsis (25). In the Lewis rat, which displays elevated vasopressin plasma levels, an increased susceptibility to develop a colitis has been observed, which can be counteracted by immunoneutralization of vasopressin (33). By contrast, vasopressin-deficient Brattleboro rats are more resistant to gastrointestinal damage induced by endotoxins (42).

Vasopressin may influence inflammation through several sites of action. Indeed, V1 receptors have been described in peripheral blood mononuclear cells (2) and can trigger IFN-γ secretion by T cells (40). In the experimental DNBS model of colitis in rats, oral SSR149415 (3, 10, and 30 mg/kg) prevented colon macroscopic and microscopic damages produced by DNBS for all the doses tested. In addition, we observed that in this model SSR149415 is able to decrease the mucosal concentration of TNF-α under inflammatory conditions. This is of peculiar relevance at the intestinal level since, together with IFN-γ, TNF-α is a well known disruptor of enterocyte tight

Fig. 6. Effect of SSR149415 and doxantrazole pretreatment on colitis induced by TNBS in mice submitted to water deprivation. Colitis was assessed by macroscopic damage scores (A) and MPO activity (B). Animals submitted to water deprivation and TNBS showed a mortality of 9 of 10 animals; thus the data were not analyzed. Doxantrazole or V1b antagonist preventive treatment prevented the mortality and ameliorated the signs of inflammation. Data are means ± SE, n = 10 – 12 mice per group. *P < 0.05, **P < 0.01, ***P < 0.001 vs. WT/TNBS-treated group.

Fig. 5. Macroscopic damages (A) and myeloperoxidase activity (B) determined in wild-type (WT) and V1b-deficient mice, treated or not with SSR149415 30 mg/kg. C: limited weight loss in V1b-/- animals, compared with WT, submitted to 2,4,6-trinitrobenzene sulfonic acid (TNBS)-induced colitis. Data are expressed as means ± SE, n = 10 – 12 mice per group. *P < 0.05, **P < 0.01, ***P < 0.001 vs. WT/TNBS-treated group.
receptors and an immunomodulatory role triggered by V2 receptor activation. In the context of TNBS/DNBS-induced inflammatory colitis, we corroborate these findings, since 1) a V1b receptor antagonist, SSR149415, reduces the inflammatory parameters both in a curative and a preventive manner, and 2) the colitis severity is weaker in V1b-deficient mice. Water deprivation is known to increase circulating vasopressin levels, and in this context we observed a strong mortality of animals in this group (90%), whereas treatment with the V1b antagonist abolished this lethality and ameliorated the colitis severity.

Conversely, we observed no improvement of inflammatory parameters on DSS-induced colitis. This may be due to the difference of colitis induction between DNBS/TNBS and DSS. Indeed, DNBS and TNBS act as a hapten that will stimulate the local immune cells (31). This is verified in our present experiments by TNF-α mucosal levels, which are lowered under SSR149415 treatment. However, it is supposed that sulfate moieties from DSS are responsible for its toxicity, through H2S and HS− production by enteric microbiota (34). In addition, colitis initiation does not require immune activation, since SCID mice develop a similar inflammation compared with controls (13).

According to V1b receptor localization, i.e., enterocytes and enteric ganglia, we can hypothesize that in our model vasopressin may regulate inflammation by acting on epithelial cells and/or on the enteric nervous system. We have shown herein that vasopressin increases paracellular permeability of mouse colonic samples in Ussing chambers, this effect being inhibited by SSR149415 and absent in V1b-deficient animals. Moreover, we observed that preventive administration of SSR149415 also reduced colitis severity in mice submitted to water deprivation to increase their vasopressin blood levels. We can thus speculate that SSR149415 limits the crossing of the hapten (DNBS or TNBS) to the internal milieu, which hampers its ability to stimulate the local immune system. This is also relevant with the absence of effect of the compound on DSS-induced colitis. It is well admitted that epithelial barrier breakdown is a key primary event in gut inflammation and IBD (47). Barrier integrity lies on several parameters and is continuously submitted to exogenous and endogenous stimuli. Of interest, one study has shown in a model of endotoxin-induced gastrointestinal damage that a peptide V1 antagonist reduced the severity of mucosal injury (42). Despite the presence of V1b receptor on enterocytes, our data suggest a new mechanism, that vasopressin exerts an indirect effect on colonic barrier function. Indeed, in colonic samples recovered from animals treated with the mast cell stabilizers doxantrazole and sodium cromoglycate, vasopressin was devoid of any effect, suggesting that the mast cell is also a putative target of vasopressin. Mast cells are immune cells playing a pivotal role in the regulation of barrier function. Indeed, in colonic samples recovered from animals treated with the mast cell stabilizers doxantrazole and sodium cromoglycate, vasopressin was devoid of any effect, suggesting that the mast cell is also a putative target of vasopressin. Mast cells are immune cells playing a pivotal role in the regulation of barrier function.
locally release mediators as CRH or nerve growth factor close to mast cells, leading to their degranulation. In addition, we have recently shown, in an animal model of stress, a closer association between mast cells and enteric nerves, associated with an increased colonic permeability (1). One can hypothesize that activation of enteric nerves by vasopressin, through V1b receptors, may be responsible for epithelial barrier impairment in our experimental conditions. Of interest, we show that mast cell blockade by doxantrazole abolished the high mortality observed in water-deprived, TNBS-treated animals and decreased the colitis severity in TNBS-treated mice, either normally hydrated or submitted to water deprivation. Alternatively, a recent paper reported that the known Gq/11-coupled V1b receptors were also able to signal in transfected cells through a G, protein/cAMP pathway depending on the nature of the ligand as well as on its localization within specialized compartments of the plasma membrane. Thus, through a cAMP involved in AQP2 recruitment and permeability, direct V1b receptor activation could modify intestinal barrier function (32). We noticed that vasopressin displayed a bell-shaped dose response regarding permeability. This observation was not unexpected; indeed, some authors have reported similar dose responses, either with vasopressin-(4-9) acting on V1a receptors (29) or [deamino-Cys1,D-Arg8]-vasopressin acting on V2 receptors (48).

A strong V1b receptor expression was denoted in the areas of mucosal regeneration, 7 days after the induction of inflammation. Recently, Cristia et al. (9) have shown that a prolonged vasopressin administration in rats stimulates the proliferation of the colonic myofibroblast layer, this effect involving both V1 and V2 receptors. Trophic effects of vasopressin have been documented through V1a receptors in various cell types (38, 48). Our present observation could denote a proproliferative effect of vasopressin, which might play a role in the resolution of inflammation, which in the resolution of inflammatory in the late phase, by contrast with its early proinflammatory role.

To conclude, our data provide an original proinflammatory role for vasopressin in early stages of colitis, involving V1b receptors and mast cells. Despite the need for further studies to be addressed, our work clearly identifies V1b receptor antagonists as putative new therapeutic agents in gut inflammatory conditions.

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DISCLOSURES

Claudine Serradeil-Le Gal, Anke Schulte, Silke Schroedel, and Marc Pascal are employees of Sanofi-Aventis France, SA.

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