NEUROPEPTIDE RELEASE AUGMENTS SERUM ALBUMIN LOSS AND REDUCES ULTRAFILTRATION IN PERITONEAL DIALYSIS

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◆ **Background:** The triggers of the acute local inflammatory response to peritoneal dialysis (PD) fluid exposure remain unknown. In the present study, we investigated the effects of neurogenic inflammation and mast cell degranulation on water and solute transport in experimental PD.

◆ **Methods:** Single 2-hour dwells in rats with PD catheters were studied. Histamine and the neuropeptides substance P and calcitonin gene-related peptide (CGRP) were measured in PD fluid samples by ELISA. Radiolabeled albumin (¹²⁵I and ¹³¹I respectively) was used as an intraperitoneal (IP) and intravascular tracer. Glucose and urea concentrations were measured in plasma and PD fluid. The effects of varying the volume and osmolarity of a lactate-buffered PD fluid were compared and related to the effects of pharmacologic intervention.

◆ **Results:** Application of 20 mL 3.9% glucose PD fluid induced an IP histamine release during the first 30 minutes, blockable by the mast cell stabilizer doxantrazole and the substance P neurokinin-1 receptor (NK1R)-blocker spantide. Histamine release was also inhibited at a reduced PD volume (14 mL), but was not affected by normalizing the PD fluid osmolarity. Blockade of NK1R also reduced plasma albumin leakage to the peritoneal cavity. Inhibition of CGRP receptors by CGRP8-37 improved osmotic (transcapillary) and net ultrafiltration and reduced the dialysate urea concentration. Neuropeptide release was not clearly related to activation of the TrpV1 receptor, the classic trigger of neurogenic inflammation.

◆ **Conclusions:** Neuropeptide release exaggerated albumin loss and reduced ultrafiltration in this rat PD model. Intervention aimed at the neuropeptide action substantially improved PD efficiency.

Peritoneal dialysis (PD) treatment has several advantages over hemodialysis, but suffers from shorter technique survival partly because of chronic inflammation, fibrosis, and angiogenesis in the peritoneal membrane. Experimental PD in rats likewise triggers acute inflammation, which is characterized by intraperitoneal (IP) complement activation, cytokine release, and neutrophil recruitment. The mechanisms behind PD-induced peritoneal inflammation are not well described and deserve further attention.

In animal models, all fluids—even physiologically neutral fluids (for example, saline or Hanks buffer)—seem to initiate inflammatory reactions when distributed intraperitoneally (1–4). Recently developed PD fluids are usually referred to as “biocompatible” because of composition or sterilization technique, or both. Comparative studies show that such fluids may induce fewer signs of chronic inflammation (5); however, there is no evidence that they do not trigger a local acute inflammatory reaction.

Hypothetically, mechanisms that trigger fluid-induced peritoneal inflammation may include mechanical effects (distension and shear) and osmotic effects (colloid or crystalloid) in addition to dilution of key signaling biomolecules. In PD fluids, components such as glucose and lactate also interact with cellular energy metabolism. Because the triggering mechanisms are unknown, so are the inflammatory processes involved in the initiation of the acute response to PD. Our attention has focused on two phenomena that may induce inflammatory reactions in response to minor mechanical or osmotic stimulation (or both): neurogenic inflammation mediated by the activation of nociceptive afferent nerves, and the non-immunoglobulin E receptor-mediated mast cell response.

The term “neurogenic inflammation” refers to the activation of a subset of primary sensory neurons, C and Aδ fibers. These neurons are normally called “polymodal nociceptors” because they sense thermal, chemical,

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osmotic, and mechanical stimuli (6). The TrpV1 receptor, recognized as the trigger of neurogenic inflammation, is a member of a family of polymodal receptors and calcium channels (TrpV1 – 4) that constitute a heat-sensitive, nociceptive subgroup of the Trp receptors (6). The TrpV1 receptor activates axon reflexes subsequently leading to a release of neuropeptides with proinflammatory properties. The most prominent mediators of the neurogenic inflammatory response, the neuropeptides substance P (SP) and calcitonin gene-related peptide (CGRP), affect microvascular tone and permeability. Substance P directly stimulates neurokinin-1 receptor (NK1R) on endothelial cells (7), thereby opening intercellular gaps that allow the passage of proteins into the interstitial space. Calcitonin gene-related peptide produces a receptor-mediated relaxation of vascular smooth muscle, leading to local hyperemia. As inferred from experiments using pharmacologic tools, SP can lead to a significant increase in the secretion of the proinflammatory cytokines interleukin (IL) 1 β , IL-6, tumor necrosis factor α , and nerve growth factor, and CGRP is associated with secretion of nerve growth factor (8). The proinflammatory cytokines represent a link between acute neurogenic inflammation and a chronic inflammatory response and may establish a feedback loop that perpetuates inflammation (6).

Neurogenic inflammation is connected to mast cell activation because mast cells express NK1Rs, and endogenous levels of SP are able to trigger mast cell degranulation (9). Mast cells may also be triggered directly by (for example) hyperosmolarity, releasing a number of inflammatory mediators, histamine being the most well-known. Histamine mediates vasodilatation and increased microvascular permeability. In the long run, mast cell activity may participate in the development of fibrosis and angiogenesis (10,11), key characteristics of the chronic peritoneal reaction to PD (12). There is evidence from the literature that mast cells are involved in the response to

PD (13), but to our knowledge, there are no published studies on neurogenic inflammation in PD.

Changes in microvascular tone and permeability induced by neurogenic inflammation or mast cell activation, or both, may obviously interact with diffusion and osmosis in PD. Consequently, the effects of neurogenic inflammation have a strong potential to affect the transperitoneal transport processes that determine PD efficiency.

The focus of the present study was to investigate the importance of neurogenic inflammation, direct or indirect via mast cells, in terms of ultrafiltration (UF), solute transport, and clearance of albumin from the vascular bed into the IP cavity. Single dwells of PD fluid, administered through an implanted silicone catheter, were studied in an established rat model of experimental PD.

METHODS

EXPERIMENTAL PROTOCOL

Through an implanted catheter, 55 rats underwent single 2-hour dwells of filter-sterilized lactate-buffered 3.9% glucose PD fluid administered by infusion.

Seven different treatments were evaluated and, in various combinations, were compared with a standard PD control treatment. The standard PD dwells (20 mL, 3.9% glucose, 483 mOsm/kg) were compared with low-volume dwells (14 mL, 3.9% glucose, 483 mOsm/kg) and dwells of isotonic (20 mL, 0.5% glucose, 294 mOsm/kg) PD fluid. In addition, 5 different pharmacologic treatments were used to create positive and negative controls for mast cell degranulation and to block TrpV1 receptors and neuropeptide receptors. Drugs were injected either intravenously or subcutaneously before the PD dwells or were added to the PD fluid (Table 1). Human serum albumin labeled with 2.5 kBq ¹²⁵I was added to the PD fluid as an IP volume marker.

TABLE 1
Summary of the Pharmacologic Treatment Protocols

Substance	Dose ^a	Action	Administration	Reference
SB366791	0.5 mg/kg	TrpV1 blocker	Intravenously 30 minutes before PD	14
Spantide	300 μ g/kg	NK1R blocker	Intravenously 20 minutes before PD	15
CGRP8-37	30 μ g/kg	CGRP blocker	Intravenously 20 minutes before PD	16
C48/80	0.6 mg/kg	Mast cell degranulator	Added to the PD fluid	17
Doxantrazole	30 mg/kg	Mast cell stabilizer	Subcutaneously 1 hour before PD	18

TrpV1 = transient receptor potential cation channel subfamily V member 1; PD = peritoneal dialysis; NK1R = neurokinin-1 receptor; CGRP = calcitonin gene-related peptide receptor.

^a Presented per kilogram body weight and chosen for maximum activity according to references from the literature. In addition, protocols based on isotonic PD fluid and low-volume dwells (14 mL PD fluid) were used in the study.

Five minutes before the PD dwell, 10 kBq ^{131}I -labeled albumin was injected intravenously to serve as an indicator of albumin clearance from the intravascular space to the IP space. Immediately before the PD dwell, a blood sample was obtained from the tip of each animal's tail. During the first 30 minutes of the dwell, 0.5 mL PD fluid samples were collected via the PD catheter every 5 minutes. Later, samples were collected after 60 and 120 minutes' dwell time. Sampling was performed under general anesthesia and was preceded by 1 – 2 minutes of gentle standardized abdominal massage to simulate the mixing action of voluntary movements in a conscious animal. After the 120-minute sample, an extra 10 mL of PD fluid without the volume marker was infused and a last PD fluid sample was collected. The animal was humanely killed after a second blood sample had been obtained by cardiac puncture.

All samples were collected in chilled tubes and were immediately centrifuged (10 000 rpm for 120 s) to remove cells, thus avoiding mast cell degranulation *ex vivo* in the samples. The PD fluid and blood samples were supplemented with ethylenediaminetetraacetic acid at a final concentration of 10 mmol/L and with a protease inhibitor cocktail and phenylmethanesulfonyl fluoride (0.1 mmol/L). Analysis of retrieved fluid included quantitation of IP and intravascular volume markers, histamine, neuropeptides, glucose, and urea. The resulting data were used to calculate UF, solute transport, distribution of the PD fluid between the IP cavity and the peritoneal tissue, clearance of albumin from blood to dialysate, and secretion of histamine and neuropeptides.

ANIMALS, SURGICAL PROCEDURES, AND ANESTHESIA

The study protocol was approved by the Gothenburg ethical committee, and it also adhered to the US National Institutes of Health *Guide for the Care and Use of Laboratory Animals*. Male Sprague–Dawley rats weighing between 300 g and 400 g were used in the experiments. A 7 French silicone catheter (RenaSil SIL-080: Braintree Scientific, Braintree, MA, USA) was autoclaved and implanted under sterile conditions and general anesthesia 1 week before the experiment (19).

General anesthesia was induced and maintained by inhalation of isofluran (Forane: Baxter Medical AB, Kista, Sweden) in room air. During the experiments, the animals were anaesthetized during the intravenous injection of albumin, the PD fluid infusion, and the subsequent 30 minutes of dwell time and sampling. They were allowed to wake and subsequently underwent short durations of anesthesia during the 60-minute sampling and finalization of the experiment at 120 minutes' dwell time. At the

end of the experiment, the animals were killed by cutting the thorax and heart.

FLUIDS AND ADDITIVES

The PD fluid used in the exposures was laboratory-made and filter-sterilized (Nalgene 0.2 μm SFCA 150 mL: Nalgene Nunc International, New York, NY, USA). A solution of 5.4 g/L NaCl, 0.051 g/L $\text{MgCl}_2 \times 6\text{H}_2\text{O}$, and 0.198 g/L $\text{CaCl}_2 \times 2\text{H}_2\text{O}$, buffered with 40 mmol/L lactate (7.5 g/L 60% sodium lactate syrup) was used to create the PD fluids used in the study. An isotonic (294 mOsm) PD fluid was created by adding 5.0 g/L D-glucose (0.5%); all other treatment protocols were performed using a standard PD fluid composition with 3.9% (39 g/L) D-glucose.

The ethylenediaminetetraacetic acid (ED4SS: Sigma–Aldrich Sweden AB, Stockholm, Sweden) mixed with phenylmethanesulfonyl fluoride (78830: Fluka BioChemika, Stockholm, Sweden) and protease inhibitor cocktail for general use (P2714-1BTL: Sigma–Aldrich) was added to samples to prevent coagulation and to minimize the effect of proteases.

Various drugs were added to the PD fluid or were administered intravenously before the experiments as indicated in Table 1.

IODINE-LABELED ALBUMIN PREPARATIONS

The IP tracer ^{125}I -labeled human serum albumin was commercially available (DRN-IT20S-M2: Tyco Healthcare, Mansfield, MA, USA).

The intravascular tracer ^{131}I -albumin was prepared by iodination of bovine serum albumin [BSA (A9647-100G: Sigma–Aldrich)]: 750 μL phosphate-buffered saline (PBS), 50 μL 20 mg/mL BSA, and 200 μL ^{131}I sodium iodide [10 MBq/mL (83067: GE Healthcare, Piscataway, NJ, USA)] was mixed in Iodo-Gen pre-coated iodination tubes (28601: Pierce, Rockford, IL, USA). The mixture was incubated in room temperature for 15 minutes and occasionally swirled. During incubation, PD-10 columns, Sephadex G-25 M (17-0851-01: GE Healthcare Bio-Sciences AB, Uppsala, Sweden), were equilibrated with 20 mL PBS. The ^{131}I -conjugated albumin was then removed from the reaction mixture by a single passage through the PD-10 column. Radioactivity was measured in a Beckman gamma counter (Beckman Coulter, Miami, FL, USA).

ANALYSES OF HISTAMINE, SP, CGRP, GLUCOSE, AND UREA

Concentrations of histamine, SP, and CGRP were determined using commercially available ELISA kits (Histamine ELISA RE59221: IBL International, Hamburg, Germany;

Assay Designs Substance P EIA kit 900-018: Enzo Life Sciences, Farmingdale, NY, USA; CGRP EIA kit 589001: Cayman Chemical, Ann Arbor, MI, USA).

Glucose concentrations in dialysate and plasma were determined by applying the Glucose Hexokinase II Reagent kit (Advia Chemistry 04903429: Bayer Healthcare, Uxbridge, UK) to samples diluted 1:40 with sterile water. Measurements of absorbance at 340 nm were performed in 96-well plates. Glucose concentrations were recalculated as end-to-initial dialysate concentration ratios (D/D_0) at 30, 60, and 120 minutes' dwell time.

Urea was measured using Quantichrom Urea Assay kits (DIUR-500; BioAssay Systems, Hayward, CA, USA) according to the manufacturer's directions. The calculated dialysate-to-average plasma concentration ratios (D/P) at 30, 60, and 120 minutes were used to analyze transperitoneal urea transport.

DETERMINATION OF UF VOLUME AND ALBUMIN CLEARANCE

Radiolabeled albumin was used as an IP volume marker to allow for measurements of UF and PD fluid reabsorption. Thus, 10 kBq ^{125}I -labeled human serum albumin was added to the PD fluid in combination with 1 mg of unlabeled BSA that blocked surface adsorption. At the beginning of the experiment, 50 kBq ^{131}I -conjugated human serum albumin in 0.1 mL PBS was injected intravenously. The concentrations of these volume markers were determined by gamma counting of plasma and PD fluid samples.

At the end of the experiment, 10 mL of PD fluid without additives was injected through the catheter after the 2-hour sample had been collected. The dilution of tracer induced by this injection was used to calculate the final IP fluid volume (19). Osmotic UF and net UF were calculated by combining data from the tracer dilution during the dwell with the measured final IP fluid volume and the known total activity of tracer infused. Net UF was defined as the IP volume at 120 minutes' dwell time, and osmotic UF was calculated from the dilution of the volume tracer (^{125}I) at each sampling time. The difference between osmotic UF and net UF at the end of the dwell is accounted for mainly by redistribution of PD fluid to the peritoneal tissues [also known as "fluid loss" (20) or "reabsorption" (21)]. Albumin clearance from plasma to dialysate was determined from the measured concentrations of ^{131}I in plasma at the beginning and end of the dwell and from the PD fluid sampled during the dwell.

STATISTICS

The Student t-test was used to evaluate differences between treatments. The chosen level of significance was

$p \leq 5\%$. A sequentially rejective Bonferroni procedure was used to compensate for multiple comparisons. Measured variables are presented in the text as mean $\pm$ standard error of the mean.

RESULTS

HISTAMINE RELEASE EARLY IN THE PD DWELL

A typical PD dwell caused a rapid release of histamine into the IP fluid space. At 20 mL PD volume, the histamine concentration peaked transiently during the first 30 minutes (Figure 1). Compared with the release induced by the mast cell degranulating compound C48/80, the histamine release induced by PD fluid was small, approximately 8% (Figure 1). A comparison of the concentration

Figure 1 — Histamine release pattern in 2-hour dwells using 14 mL and 20 mL peritoneal dialysis (PD) fluid. Upper panel: In response to 20 mL of PD fluid, the intraperitoneal histamine concentration peaks within the first 30 minutes and then approaches a plateau, suggesting a steady state between the plasma space and the intraperitoneal fluid volume. Lower panel: The peak histamine concentration in response to 20 mL PD fluid reaches approximately 8% of the value induced by the mast cell degranulator C48/80, used as a positive control.

of released histamine at the 2 PD fluid volumes (14 mL, 20 mL) showed a significant difference at 15 minutes' dwell time.

RELEASE OF NEUROPEPTIDES SP AND CGRP

Attempts to measure SP and CGRP in the PD fluid samples failed. Measured concentrations were at the detection limits of the respective assays and could indicate no differences between treatments.

INTERVENTIONS WITH HISTAMINE RELEASE

Assessment of histamine release was based on IP histamine per kilogram body weight, averaged over the first 30 minutes' dwell time (Figure 2). It showed that the histamine release at 14 mL PD volume was significantly smaller than that at 20 mL PD volume. At 20 mL volume, the mast cell stabilizer doxantrazole significantly reduced the histamine release. So did the competitive NK1R blocker spantide, suggesting that histamine release was induced by SP, the known ligand of NK1R. However, the TrpV1 receptor inhibitor SB366791 did not significantly affect histamine release. A PD fluid with reduced

Figure 2 — Histamine release, calculated as the average quantity of histamine per kilogram body weight (b.w.) released during the first 30 minutes of dwell time. Low-volume peritoneal dialysis (PD) dwells (14 mL instead of 20 mL) significantly reduced histamine release, as did treatment with the mast cell stabilizer doxantrazole and the substance P receptor antagonist spantide at normal PD volumes (20 mL). Addition of the TrpV1 receptor blocker SB366791 did not significantly affect histamine release compared with that during control dwells with 20 mL PD fluid without additional treatment. * Significant at the 5% level after correction for multiple comparisons.

glucose content (0.5%)—and thus physiologic osmolarity (294 mOsm/kg)—did not differ from the hyperosmolar 3.9% glucose standard PD fluid with respect to histamine release.

PLASMA ALBUMIN LOSS

The albumin loss from plasma was measured as the clearance of ^{131}I BSA into the PD fluid over the entire dwell, given that an initial evaluation suggested that albumin clearance occurred at a constant rate over the 120 minutes' dwell time. Various pharmacologic interventions were compared at 20 mL PD volume. The NK1R antagonism by spantide significantly reduced albumin clearance (Figure 3), but neither the TrpV1 receptor blocker SB366791 nor the mast cell stabilizer doxantrazole had any significant effect. However, degranulation of the mast cells by administration of C48/80 increased the albumin clearance significantly (Figure 3).

UF OF PD FLUID

Blockage of CGRP receptors by CGRP8-37 significantly increased osmotic UF and net UF at 120 minutes (Table 2, Figure 4).

The TrpV1 receptor blocker SB366791 significantly reduced osmotic UF at 30, 60, and 120 minutes, but

Figure 3 — Plasma albumin leakage, calculated as the average clearance of labeled bovine serum albumin from plasma to the intraperitoneal space during 2-hour dwells with 20 mL standard peritoneal dialysis (PD) fluid. Mast cell degranulation by C48/80 significantly increased albumin leakage, and addition of the substance P receptor antagonist spantide significantly reduced albumin leakage compared with control dwells of fluid without additional treatment. The mast cell stabilizer doxantrazole and the TrpV1 receptor blocker SB366791 had no significant effects. b.w. = body weight. * Significant at the 5% level after correction for multiple comparisons.

TABLE 2
Transperitoneal Transport

Transport type	Time (min)	Measurement	Control	CGRP8-37	Spantide	SB366791
Fluid ^a	30	Osmotic UF	12.56±2.39	17.20±2.57	10.65±3.60	1.07±3.71 ^b
	60	Osmotic UF	25.39±3.13	29.88±2.08	22.86±11.06	10.51±3.90 ^b
	120	Osmotic UF	30.57±3.08	40.84±2.45 ^b	38.53±6.09	15.54±4.61 ^b
		Redistribution	26.32±1.94	16.54±6.60	30.70±3.07	10.92±5.17 ^b
		Net UF	4.25±3.88	24.30±6.16 ^b	7.83±6.18	4.62±6.92
Solutes ^c	30	D/P urea conc	0.69±0.08	0.51±0.09	0.49±0.09	0.63±0.05
		D/D ₀ glucose conc	0.68±0.04	0.73±0.02	0.89±0.05 ^b	0.85±0.03 ^b
	60	D/P urea conc	0.78±0.11	0.68±0.10	0.86±0.17	0.91±0.06
		D/D ₀ glucose conc	0.62±0.05	0.56±0.05	0.63±0.05	0.56±0.05
	120	D/P urea conc	1.00±0.06	0.63±0.11 ^b	0.89±0.10	0.98±0.07
		D/D ₀ glucose conc	0.23±0.03	0.28±0.02	0.32±0.04	0.28±0.06

^a Presented as osmotic (transcapillary) ultrafiltration (UF), redistribution, and net UF (osmotic UF = net UF + redistribution) in milliliters per kilogram body weight. All values are mean ± standard error of the mean.

^b Significant at the 5% level after correction for multiple comparisons.

^c Presented as end-to-initial dialysate glucose concentration (D/D₀) and dialysate-to-plasma (D/P) urea concentration at 30, 60, and 120 minutes of dwell time. All values are mean ± standard error of the mean.

Figure 4 — Graphic representation of transperitoneal fluid transport over 120 minutes' dwell time (presented as mean ± standard error of the mean in Table 2). Total bar height, corresponding to osmotic (transcapillary) ultrafiltration (UF), is resolved in the Net UF (net volume gain) and Redistribution (clearance of volume marker from the intraperitoneal cavity) table rows. Compared with a standard peritoneal dialysis dwell, + represents a significant increase and – represents a significant decrease of the three transport components. The CGRP receptor antagonist CGRP8-37 significantly increased osmotic UF and net UF; the TrpV1 receptor blocker SB366791 significantly reduced redistribution and osmotic UF. b.w. = body weight.

also reduced the redistribution of PD fluid to the peritoneal tissues. In terms of net UF, the reduced redistribution canceled out the reduced osmotic UF at 120 minutes, and the resulting net UF did not differ significantly from that in the control group (Table 2, Figure 4).

GLUCOSE AND UREA TRANSPORT

At 30 minutes, the D/D₀ glucose was significantly increased by blockade of NK1R (with spantide) or the TrpV1 receptor (with SB366791). The D/P urea at 120 minutes was significantly lower in animals treated with CGRP8-37 than in control animals (Table 2).

DISCUSSION

The present study shows that exposure to PD fluid induces a transient histamine release during the first 30 minutes of a dwell. The histamine source was mast cells, given that the release was significantly inhibited by the mast cell blocker doxantrazole, and the mast cells were triggered by SP binding to NK1R, given that spantide substantially lowered the quantity of released histamine.

In contrast to doxantrazole, spantide significantly reduced plasma albumin clearance (by approximately 50%) into the peritoneal cavity (Figure 3). Reduced clearance without a parallel reduction of osmotic UF [an increased average was in fact seen (Figure 4)] suggests a decrease in permeability for albumin, which is a known effect of SP (7).

Indirectly, those findings suggest that PD fluid exposure leads to liberation of SP that, in turn, increases plasma albumin loss by mechanisms that do not depend on histamine. In parallel, histamine is excreted from SP-stimulated mast cells, also a known SP effect, but

the histamine level is too low to affect plasma albumin leakage significantly (a higher histamine concentration after challenge with C48/80 increased plasma albumin leakage). This role of histamine in PD was observed earlier in the rabbit (22).

Unfortunately, neither neuropeptide (SP or CGRP) could be measured directly because their IP concentrations were below the detection limit of the assays used in the study. That finding is reasonable given that the neuropeptides act locally on cells close to the nerve terminals and are rapidly degraded by peptidases. A very small fraction of the released SP and CGRP would be expected to reach the IP space, where those substances would be further diluted in the large fluid volume. There was convincing indirect evidence of SP release: The early histamine peak was a distinct reaction to PD fluid exposure, and that reaction was almost extinguished with NK1R blockade.

No corresponding indicator of CGRP release could be detected, although CGRP8-37 treatment significantly affected osmotic UF. Thus, the effects of CGRP8-37 reflected either blockade of normal CGRP baseline activity or inhibition of the effects of CGRP liberation in response to PD fluid exposure. The established role of CGRP outside the nervous system is that of a potent vasodilator, which is consistent with our findings of an increased osmotic UF when CGRP receptors were blocked. According to theory, counteracting CGRP-induced vasodilatation should reduce glucose out-diffusion and thus increase osmotic UF over time. The measurements of glucose and urea concentrations indicate that glucose transport may have been reduced at 30 minutes by TrpV1 and NK1R blockade (Table 2). At 120 minutes, the D/P urea was significantly lower in the CGRP8-37 group (Table 2).

Translating concentration ratios into diffusion rates is complicated because IP solute concentrations are also affected by dilution attributable to UF. Thus, a large increase in the osmotic UF, as seen in the CGRP8-37 group, could hide the glucose concentration increase and amplify the urea concentration decrease caused by a general reduction of the solute diffusion rate. Preliminary theoretical simulations indicate that the increase in osmotic UF (35%), the decrease in D/P urea (30%), and the trend toward an increase in glucose concentration (20%) seen in the CGRP8-37 group are consistent with the effects of a 30% reduction in the solute diffusion rate. We therefore hypothesize that the UF increase in the CGRP8-37 group was a result of a lowered glucose diffusion rate sustaining the osmotic gradient.

Under normal baseline conditions, the regulatory role of CGRP seems to be unimportant (23,24), and administration of CGRP8-37 has little or no effect on regional

vascular beds (16,24,25) and no effect on systemic blood pressure (26). In the present study, however, CGRP8-37 significantly affected osmotic UF, probably by reducing peritoneal microvascular blood flow. It is therefore likely that CGRP was in fact released locally in parallel with SP in response to PD fluid exposure.

The effects of CGRP receptor inhibition could have involved central hemodynamics. In a preliminary evaluation, noninvasive blood pressure measurements (tail cuff) were compared between control and CGRP8-37-treated animals. Our measurements did not indicate any significant differences in blood pressure.

It is well established that the TrpV1 receptor mediates the induction of a subsequent SP and CGRP release, known as the neurogenic inflammatory response. Blocking the TrpV1 receptors yielded some unexpected results. Contrary to the effect of direct blockade of NK1R, TrpV1 receptor blockade did not significantly affect albumin clearance (Figure 3). Concerning fluid transport, blockade of TrpV1 receptors significantly reduced osmotic UF, but CGRP receptor blockade increased osmotic and net UF (Table 2, Figure 4). Blockade of TrpV1 caused a significant reduction in fluid redistribution seen neither after NK1R blockade nor after CGRP receptor blockade. Published evidence suggests that neurogenic inflammation changes the contraction state of the extracellular connective tissue matrix, thereby facilitating edema formation (27). That effect may explain why PD fluid redistribution to the peritoneal tissue was inhibited by TrpV1 blockade in the present study.

Taken together, the data suggest that TrpV1 receptor activation induced effects other than SP and CGRP release or that a combined release of CGRP and SP after TrpV1 receptor activation led to unexpected combination effects. Consequently, the neuropeptide release seen in response to PD could have been mediated by, for example, TrpV4, considered to be involved in neurogenic inflammatory responses to osmotic challenge (28). According to scarce information, NK1 agonists can also be released from sources outside the nervous system (29), but little is known about the mechanisms involved. Most probably, SP and CGRP were released from nerve endings.

An obvious issue is whether the mechanical or the osmotic effects of the PD fluid dwell, or both, triggered the neuropeptide release. Hyperosmolarity is a known trigger of the neurogenic inflammatory response (30), and some TrpV receptors, especially TrpV2 and TrpV4, are sensitive to shear stress and membrane stretch (31). Comparison of the standard PD fluid (3.9% glucose) to an isotonic PD fluid (0.5% glucose) detected no differences in histamine release, but a reduced volume load significantly reduced the quantity of released histamine (Figure 1).

For technical reasons, IP pressures were not monitored in the present study. However, available data from rats indicate that the distension of peritoneal tissues increases moderately up to volumes of 25 mL/100 g body weight when the IP pressure is 2 mmHg (32). In the present study, histamine release increased significantly when the dwell volume was increased to 20 mL from 14 mL, corresponding to average volume loads of 4.5 mL/100 g and 6 mL/100 g body weight respectively. Values for net UF were generally low in the present study because of the large sampling volume (4 mL per dwell in total) required to allow for high-resolution measurements of histamine and neuropeptides. This methodology reduced the volume increase caused by UF so that maximum IP volumes did not exceed 10 mL/100 g body weight during the first 30 minutes of the dwell. Activation of mechanoreceptors because of tissue stretching seems unlikely at the small distending forces generated in this IP volume interval. Hypothetically, dilution effects leading to colloid osmotic pressure changes (21) and an expansion of the connective tissue interstitium could have activated mechanosensors of the TrpV family.

It is known that an implanted PD catheter, as used in the present experimental model, induces an inflammatory foreign body reaction (33) that probably modulates the peritoneal response to PD fluid exposure. However a pre-implanted catheter is part of PD treatment in humans, and there is also evidence of a foreign-body reaction in that setting. A greater discrepancy in relation to clinical PD is the single-dwell design of our study. In clinical PD, repeated exposure to PD fluid transforms the peritoneal membrane; in our model, the peritoneum was naïve to PD and therefore might have reacted in a different way.

The effects of inhibiting neuropeptides were advantageous for PD efficiency in this animal model. A 47% reduction of average albumin loss in response to SP inhibition and a substantially improved net UF (corresponding to 35% of the PD dwell volume) are great improvements if translated to clinical PD. Positive effects may also include the chronic reaction to PD fluid, given that neurogenic inflammation interacts with the cytokine network responsible for fibrosis and angiogenesis. Although histamine release was unimportant for the clearance of albumin during a single PD dwell, it is likely that histamine has a role in the chronic inflammatory response to PD, characterized by fibrosis and angiogenesis (10–12).

CONCLUSIONS

Experimental PD in a rat model induced a rapid histamine release dependent on the activity of the neuropeptide SP. That response was dependent on PD fluid volume

and was possibly triggered mechanically by colloid osmotic forces exerted by the PD fluid. Blocking the SP receptor NK1R reduced plasma albumin leakage into the peritoneal space by half. Inhibition of the receptors for CGRP, another neuropeptide, substantially improved UF. The TrpV1 receptor, the classic trigger of neurogenic inflammation, could not, however, be connected to the indicated neuropeptide release. If our results translate to humans, interference with neuropeptide effects has the potential to substantially improve PD efficiency.

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DISCLOSURES

All authors declare that no financial conflicts of interest exist.

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