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Characterization of Polyamine Homeostasis in L-Ornithine–Induced Acute Pancreatitis in Rats

AQ1 *György Biczó, MSc,* Péter Hegyi, MD, PhD,* Riitta Sinervirta,† Sándor Berczi, MD,‡ Sándor Dósa, MD,‡ Andrea Siska, PhD,§ Béla Iványi, MD, PhD, DSc,‡ Viktória Venglovecz, PhD,|| Tamás Takács, MD, PhD, DSc,* Leena Alhonen, PhD,† and Zoltán Rakonczay Jr, MD, PhD**

Objectives: L-Ornithine is a precursor of polyamine synthesis that is essential for cell survival. In contrast, intraperitoneal (IP) administration of a large dose of L-ornithine results in death of pancreatic acinar cells in rats. We investigated changes in pancreatic and extrapancreatic polyamine homeostasis after injection of L-ornithine and tested the effects of the stable polyamine analogue methylspermidine (MeSpd) on L-ornithine–induced pancreatitis.

Methods: Male Wistar rats were injected IP with 3 g/kg L-ornithine and were untreated, pretreated, or treated with 50 mg/kg MeSpd IP. Rats were killed after 0 to 168 hours for determinations of polyamines and activities of ornithine decarboxylase and spermidine/spermine N^1-acetyltransferase (SSAT). Pancreatitis severity was assessed by measuring standard laboratory and histological parameters.

Results: Injection of L-ornithine paradoxically induced pancreatic spermidine catabolism, possibly via activation of SSAT, after (>6 hours) appearance of the first histological signs of acute pancreatitis. Polyamine levels generally increased in the lung and liver with the exception of lung spermidine levels, which decreased. Methylspermidine did not influence polyamine levels and SSAT activity and did not ameliorate the severity of L-ornithine–induced pancreatitis.

Conclusions: L-Ornithine–induced pancreatitis was associated with activation of pancreatic polyamine catabolism. However, administration of a metabolically stable polyamine analogue did not affect disease severity.

Key Words: polyamines, pancreatitis, L-ornithine

Abbreviations: AST - aspartate aminotransferase, HSP72 - heat shock protein 72, IL-1β - interleukin 1β, IP - intraperitoneal(ly), MPO - myeloperoxidase, SSAT - spermidine/spermine N^1-acetyltransferase, ODC - ornithine decarboxylase, MeSpd - 1-methylspermidine, PS - physiological saline

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Polyamines (putrescine, spermidine, and spermine) are essential for normal function and survival of cells.[1] They are positively charged molecules and have been shown to interact with DNA, RNA, proteins, and acidic phospholipids in membranes. Polyamines have been implicated in the regulation of several membrane-bound enzymes, such as adenylate cyclase, and ion channels. Furthermore, they may also have antioxidant properties. Physiologically, polyamines are synthesized from L-arginine (via L-ornithine) and L-methionine (via *S*-adenosylmethionine [SAM]). Polyamine metabolism is highly regulated by 3 key enzymes (Fig. 1).[2] Ornithine decarboxylase (ODC) and SAM decarboxylase mediate polyamine biosynthesis, whereas polyamine catabolism is controlled by the rate-limiting spermidine/spermine N^1-acetyltransferase (SSAT). F1

The pancreas contains the highest concentration of spermidine in the mammalian body, but the exact mechanism how spermidine contributes to the maintenance of pancreatic integrity is unclear.[3–4] In addition, the importance of polyamines in the pancreas is highlighted by the fact that activation of polyamine catabolism through an overexpression of SSAT in a transgenic rat line resulted in severe acute pancreatitis.[5] It has been shown that pancreatic SSAT activation and subsequent polyamine catabolism are characteristic features of experimental acute pancreatitis models and also of human pancreatic tissue samples from patients with acute pancreatitis.[6–8]

We have recently described that intraperitoneal (IP) injection of large doses of L-ornithine results in severe acute necrotizing pancreatitis in rats.[9] As L-ornithine is a precursor of polyamines, the question arises how the biosynthesis and catabolism of polyamines change in L-ornithine–induced acute pancreatitis. Furthermore, as we observed pancreatic spermidine catabolism in rats with L-ornithine–induced pancreatitis, we also tested the effects of the metabolically stable polyamine analogue 1-methylspermidine (MeSpd) administration on the disease as a treatment to compensate for the loss natural spermidine.

MATERIALS AND METHODS

Materials

MeSpd was synthesized from 3-aminobutanol as described earlier.[10] The polyamine analogue was dissolved in physiological saline (PS; 25 mg/mL, pH 7.4). All other chemicals were from Sigma-Aldrich (Munich, Germany) unless stated otherwise.

Experimental Protocols

Animals

Male Wistar rats weighing 180 to 220 g were used. The animals were kept at a constant room temperature of 23°C with a 12-hour light-dark cycle and were allowed free access to water and standard laboratory chow (Biofarm, Zagyvaszántó, Hungary). In each experimental group, 5 to 8 rats were used. The experiments performed in this study were approved by the Animal Care Committee of the University of Szeged.

Characterization of Polyamine Homeostasis in L-Ornithine–Induced Acute Pancreatitis

Pancreatitis was induced by IP injection with 3 g/kg L-ornithine. Polyamine homeostasis was studied at 6, 24, 72, and

From the *First Department of Medicine, University of Szeged, Szeged, Hungary; †Department of Biotechnology and Molecular Medicine, A. I. Virtanen Institute for Molecular Sciences, Biocenter Kuopio, University of Kuopio, Kuopio, Finland; and Departments of ‡Pathology, §Clinical Chemistry, and ||Pharmacology and Pharmacotherapy, University of Szeged, Szeged, Hungary.

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Reprints: Zoltán Rakonczay Jr, MD, PhD, First Department of Medicine, University of Szeged, PO Box 427, H-6701 Szeged, Hungary (e-mail: raz@in1st.szote.u[illegible]d.hu).

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Methylspermidine was synthesized by Dr Nikolay Grigorenko (Engelhardt Institute of Molecular Biology, Russian Academy of Sciences, Moscow, Russia).

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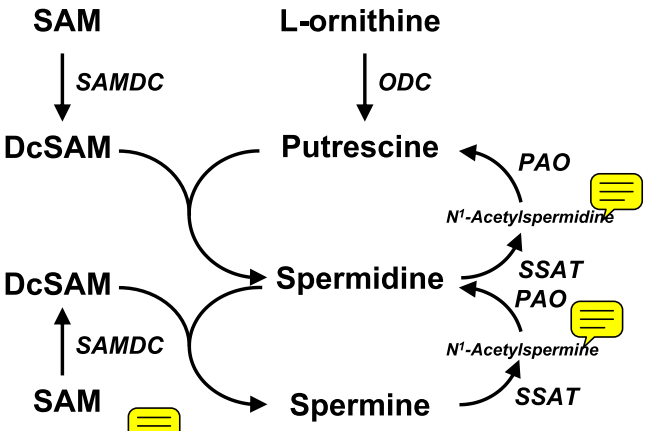

FIGURE 1. Rate-limiting enzymes of polyamine metabolism. Ornithine decarboxylase and SAM decarboxylase mediate polyamine biosynthesis, whereas polyamine catabolism is controlled by SSAT. DcSAM indicates decarboxylated SAM; PAO, polyamine oxidase.

168 hours after the initiation of pancreatitis (n = 5–6). The control animals received PS IP and were killed 24 hours after the injection (n = 5).

Effects of MeSpd on L-Ornithine–Induced Acute Pancreatitis

Rats were divided randomly into 6 groups. In the O24 group (n = 8), rats were injected with 3 g/kg L-ornithine IP and received PS IP 4 hours before (n = 4) and 4 hours after (n = 4) the L-ornithine treatment. In the OM24 group (n = 6), rats were pretreated with 50 mg/kg MeSpd IP 4 hours before injection with 3 g/kg L-ornithine IP. In the OM24 (n = 6) group, rats received 50 mg/kg MeSpd IP 4 hours after the L-ornithine (3 g/kg) injection. In the O48 (n = 5) group, rats were injected with 3 g/kg L-ornithine IP and received PS IP 4 and 24 hours thereafter. In the OM48 group (n = 5), rats were treated with 50 mg/kg MeSpd IP 4 and 24 hours after the L-ornithine (3 g/kg) treatment. In the control group (n = 5), rats received PS IP instead of L-ornithine and MeSpd. The "24" and "48" labels in the group names indicate the time points in hours at which rats were killed after the injection of L-ornithine.

In both protocols, rats were killed by exsanguination through the abdominal aorta after anaesthetization with 44 mg/kg pentobarbital IP. Tissue samples from pancreas (cleaned from fat and lymph nodes), liver, and lung were frozen in liquid nitrogen and stored at −80°C until use. All blood samples were centrifuged at 2500*g* for 20 minutes, and the serum was stored at −25°C.

Assays

Polyamine Levels and Activities of SSAT and ODC

The levels of the natural polyamines (spermidine, spermine, and putrescine) and the polyamine analogue MeSpd were determined by high-performance liquid chromatography according to the method of Hyvönen et al.[11] Pancreatic ODC and SSAT activities were assayed according to Jänne and Williams-Ashman[12] and Bernacki et al,[13] respectively.

Pancreatic Weight–Body Weight Ratio

The pancreatic weight–body weight (PW/BW) ratio was used to evaluate the degree of pancreatic edema.

Activities of Serum and Pancreatic Amylase, Serum Lipase, Aspartate Aminotransferase, and Serum Concentrations of Creatinine

Laboratory parameters were determined as described previously.[9] Serum and pancreatic amylase activities were determined by an enzymatic colorimetric assay standardized by the International Federation of Clinical Chemistry (Diagnosticum Ltd, Budapest, Hungary). Serum lipase activity was determined by an enzymatic colorimetric method and serum concentration of creatinine by the kinetic colorimetric compensated Jaffé method of Roche Diagnostics GmbH (Mannheim, Germany). Aspartate aminotransferase (ASAT) was measured by an IFCC UV kinetic method (Human GmbH, Wiesbaden, Germany).

Pancreatic Myeloperoxidase Activity

Pancreatic myeloperoxidase (MPO) activity, as a marker of tissue leukocyte infiltration, was assessed using the method of Kuebler et al.[14]

Expression of Pancreatic HSP72 and IκB-α

Western blot analysis of pancreatic heat shock protein 72 (HSP72) and IκB-α expression was performed from the cytosolic fraction of the pancreas homogenate. Pancreatic cytosolic fractions were prepared as described previously.[15] The protein concentration of the homogenate was determined by the method of Bradford.[16] Forty micrograms of protein was loaded per lane. Samples were electrophoresed on an 10% sodium dodecyl sulfate–polyacrylamide gel according to the method of Laemmli.[17] The proteins on the gels were either stained with Coomassie brilliant blue (to demonstrate equal loading of proteins for Western blot analysis) or transferred to a nitrocellulose membrane for 1 hour at 100 V. Equal transfer of proteins was verified by ponceau S staining. Membranes were blocked in 5% nonfat dry milk (Bio-Rad, Hercules, Calif) for 1 hour and incubated with rabbit anti-HSP72 (1:10,000 dilution, characterized previously by Kurucz et al[18]) or rabbit anti–IκB-α (1:200 dilution; Santa Cruz Biotechnology, Santa Cruz, Calif) antibody for an additional 1 or 4 hours (respectively) at room temperature. The immunoreactive protein was visualized by enhanced chemiluminescence, using horseradish peroxidase–coupled anti–rabbit immunoglobulin (Dako, Glostrup, Denmark) at 1:10,000 dilution.

Pancreatic Interleukin 1β Concentration

The proinflammatory interleukin 1β (IL-1β) concentrations were measured in the pancreatic cytosolic fractions with an enzyme-linked immunosorbent assay kit (R&D Systems, Minneapolis, Minn) according to the manufacturer's instructions.

Histological Examination

A portion of the pancreas was fixed in 6% neutral formaldehyde solution and subsequently embedded in paraffin. Sections were cut at 4-μm thickness and stained with hematoxylin and eosin. The slides were coded and read by 2 independent observers who were blind to the experimental protocol. Pancreatic tissue injury was evaluated as described previously.[9] Briefly, semiquantitative grading of interstitial edema (0–3), vascular congestion (0–1), leukocyte adhesion (0–3), and infiltration (0–4), apoptosis (0–3), necrosis (0–4), and regeneration (0–2) of acinar cells was determined in each animal.

Statistical Analysis

Results are expressed as means ± SEM. Experiments were evaluated by using the analysis of variance followed by Dunnett

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multiple-comparisons post hoc test. Values of $P < 0.05$ were considered significant.

RESULTS

Polyamine Homeostasis in L-Ornithine–Induced Acute Pancreatitis

Pancreas

Pancreatic spermidine content significantly decreased 24 to 168 hours after the injection of 3 g/kg L-ornithine, whereas spermine content significantly increased (by 1.6-fold) only at 72 hours (Figs. 2A, B). Considering the different pool sizes of spermidine and spermine, the net effect was a significant depletion of the total polyamine pool beginning from 24 hours after injection of L-ornithine. Pancreatic ODC activity significantly increased at 24 hours (Fig. 2C); SSAT activity significantly increased from 24 hours and peaked at 72 hours with an almost 10-fold maximum elevation and remained significantly higher than the activity in the control group until 168 hours (Fig. 2D). Surprisingly, putrescine was not detectable in the pancreas, although putrescine accumulation should be an evident consequence of simultaneously increased activities of SSAT and ODC (if synthesis would not override catabolism).

Liver

Hepatic putrescine level showed a 26-fold elevation at 6 hours after L-ornithine injection, but thereafter fell back to control values (Fig. 3A). Hepatic spermidine level significantly increased from 6 to 72 hours (Fig. 3B), and spermine content also showed significant elevation from 24 to 72 hours (Fig. 3C).

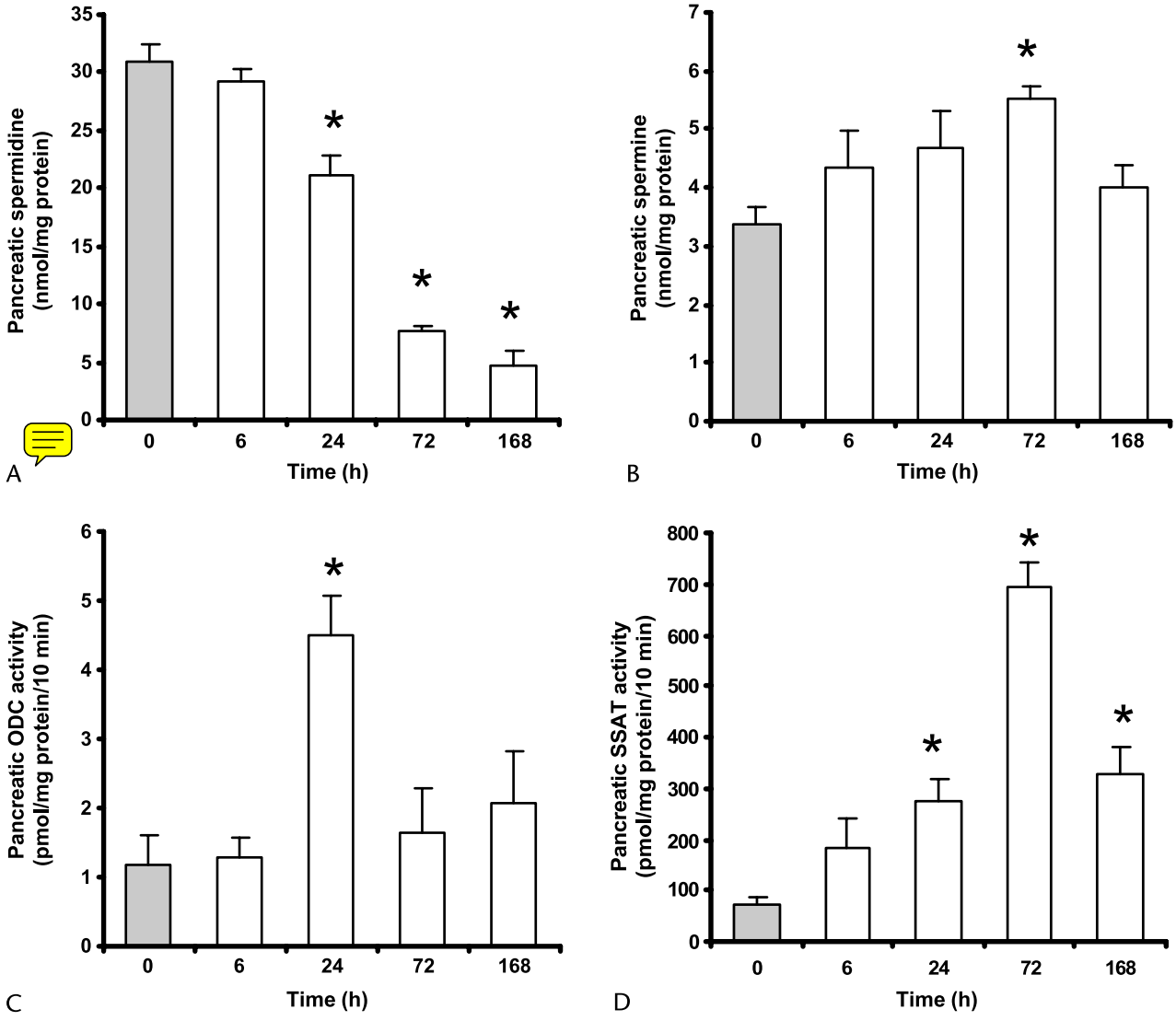

FIGURE 2. Time-course changes in pancreatic polyamine homeostasis after IP administration of 3 g/kg L-ornithine. Pancreatic (A) spermidine and (B) spermine levels and activities of (C) ODC and (D) SSAT are shown. Data are presented as means ± SEM, n = 5–6. *Significant difference ($P < 0.05$) versus the control group (0 hour, gray column).

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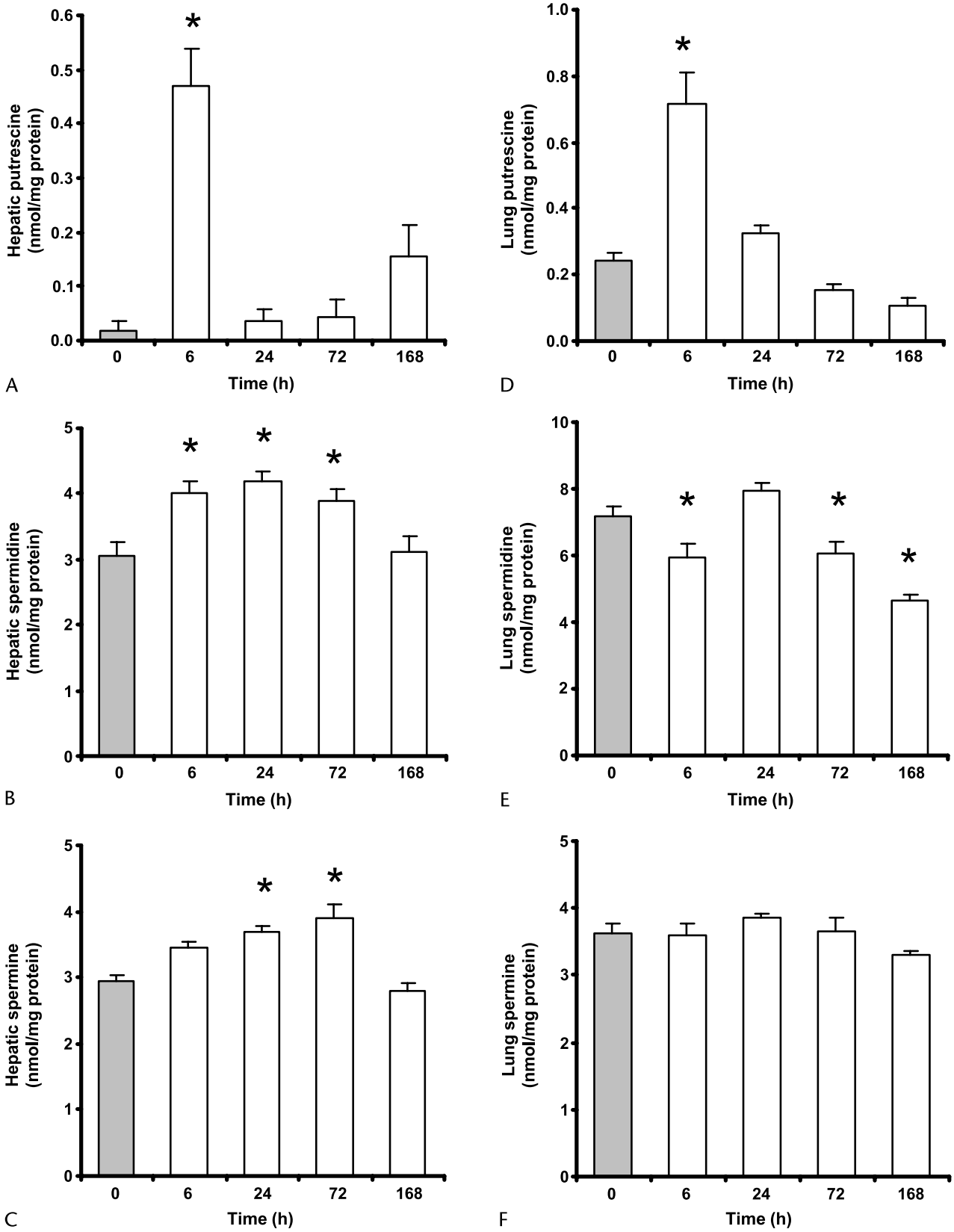

FIGURE 3. Time-course changes in hepatic and lung polyamine pools after IP administration of 3 g/kg L-ornithine. The diagrams demonstrate hepatic (A) putrescine, (B) spermidine, and (C) spermine levels; and lung (D) putrescine, (E) spermidine, and (F) spermine levels. Data are shown as means ± SEM, n = 5–6. *Significant difference ($P < 0.05$) versus the control group (0 hour, gray column).

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Lung

Similarly to that observed in the liver, lung putrescine levels showed a significant peak at 6 hours after L-ornithine injection (Fig. 3D). In contrast to the changes observed in the hepatic polyamine pools, lung spermidine content showed significantly decreased levels at 6, 72, and 168 hours (Fig. 3E). Lung spermine content was not significantly altered at the investigated time points (Fig. 3F).

Effects of the Synthetic Polyamine Analogue 1-MeSpd on L-Ornithine–Induced Acute Pancreatitis

Pancreatic SSAT Activity, Putrescine, Spermidine, MeSpd, and Spermine Content

Methylspermidine accumulated in the pancreas as a result of both pretreatment (MO24: 4.53 ± 1.79 nmol/mg protein) and

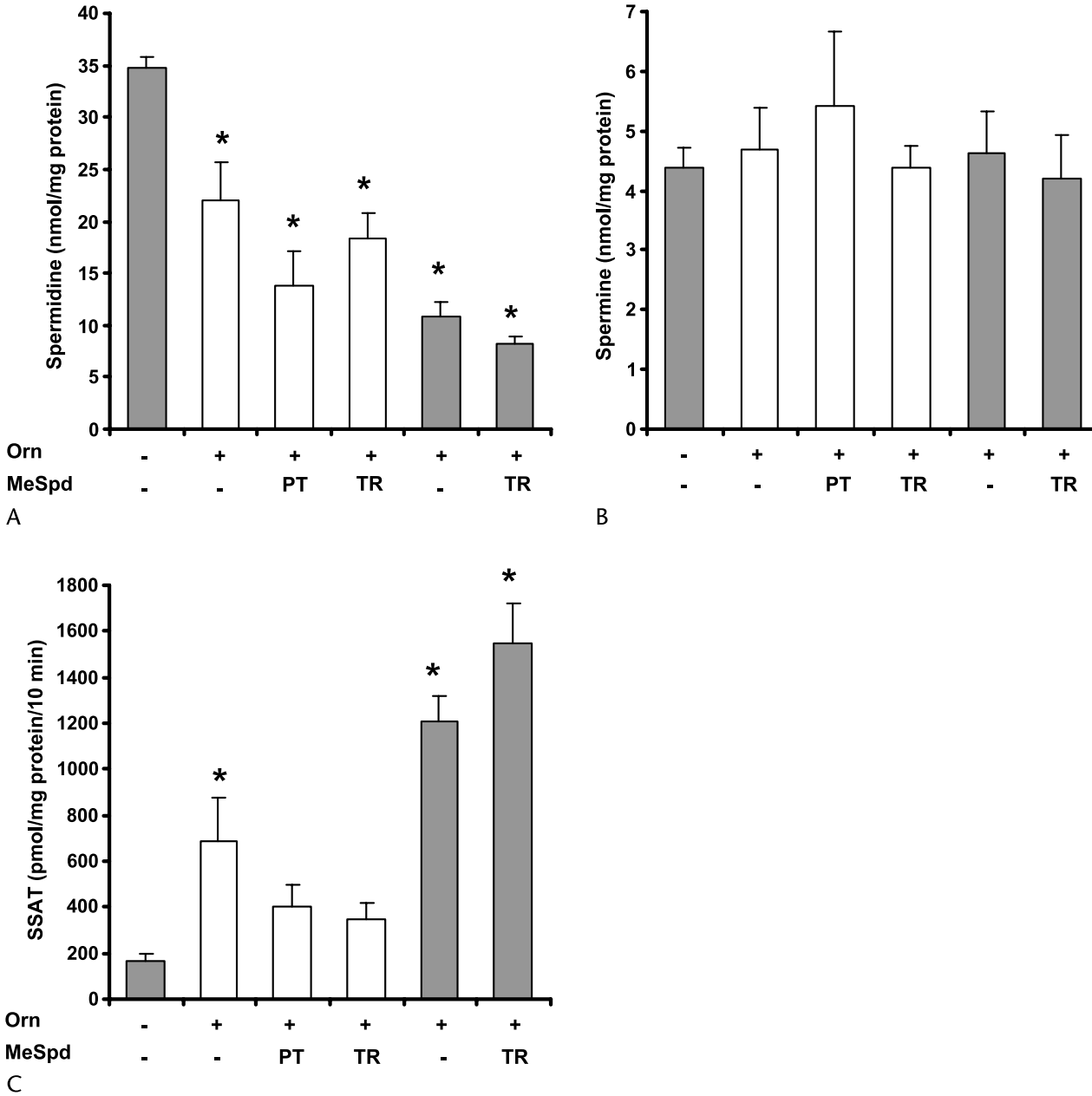

FIGURE 4. Effects of ~~1-MeSpd~~ (MeSpd) administration on changes in pancreatic polyamine homeostasis in L-ornithine–induced acute pancreatitis. The diagrams demonstrate pancreatic (A) spermidine and (B) spermine levels and (C) SSAT activity. Groups of rats were injected IP with 3 g/kg L-ornithine (Orn +) or PS (Orn −) and were untreated (MeSpd −), pretreated (MeSpd PT), or treated (MeSpd TR) with 50 mg/kg MeSpd IP. The white and gray bars indicate groups of animals killed at 24 or 48 hours (respectively) after the L-ornithine or PS injection. For a more detailed experimental protocol, see Materials and Methods. Data are shown as means ± SEM, n = 5–8. *Significant difference ($P < 0.05$) versus the control group (Orn −, MeSpd −).

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Fig 5 4/C

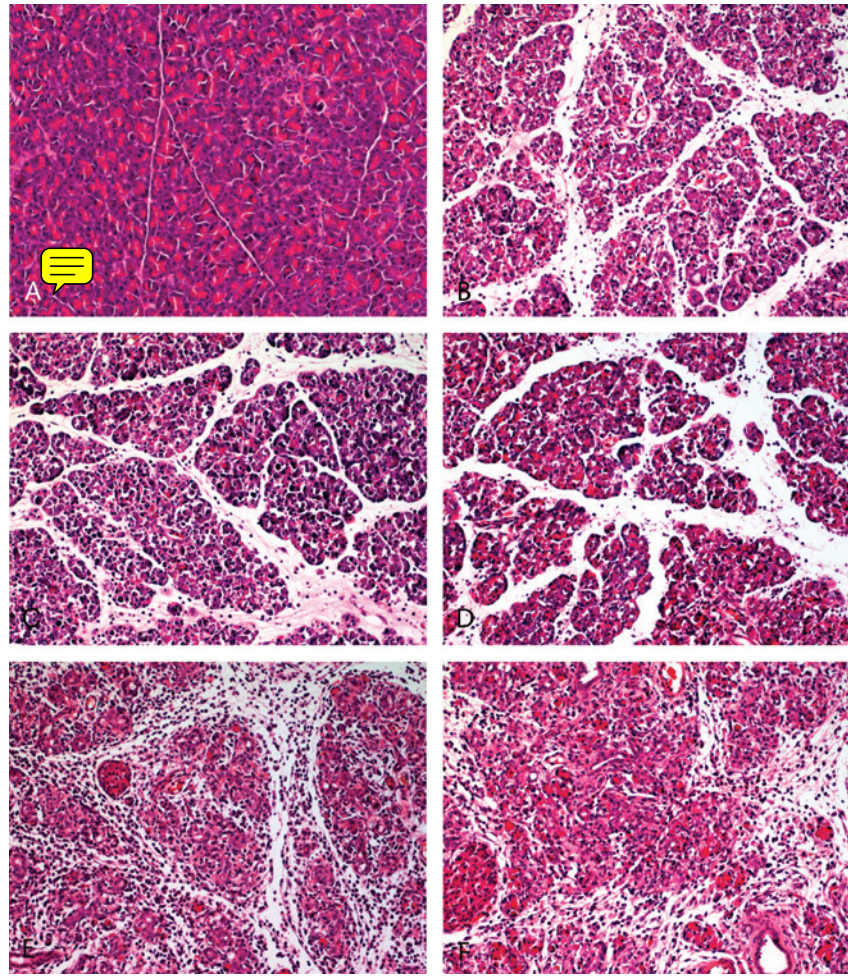

FIGURE 5. Effects of MeSpd on pancreatic morphological damage in L-ornithine–induced acute pancreatitis. The images show representative hematoxylin and eosin images of pancreata of rats injected IP with (A) PS or (B–F) 3 g/kg L-ornithine, which were (A, B, E) untreated, (C) pretreated, or (D, F) treated (twice in case of F) with 50 mg/kg MeSpd IP. Animals were killed at (A–D) 24 hours or (E–F) 48 hours after the injection of L-ornithine or PS. Original magnification ×200.

TABLE 1. Effects of 1-MeSpd on Histological Parameters in L-Ornithine–Induced Acute Pancreatitis

MeSpd	—	PT	TR	—	TR
Time of Sacrifice, h	24			48	
Vascular congestion	0.6 ± 0.2*	1.0 ± 0.0*	0.8 ± 0.2*	0.8 ± 0.2*	1.0 ± 0.0*
Leukocyte adherence	3.0 ± 0.0*	3.0 ± 0.0*	2.7 ± 0.2*	2.8 ± 0.2*	2.8 ± 0.2*
Interstitial edema	2.8 ± 0.2*	3.0 ± 0.0*	2.8 ± 0.2*	3.0 ± 0.0*	2.8 ± 0.2*
Leukocyte infiltration	2.9 ± 0.5*	2.8 ± 0.3*	3.5 ± 0.3*	4.0 ± 0.0*	3.4 ± 0.2*
Vacuolization	0.3 ± 0.2	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.2 ± 0.2
Necrosis	4.0 ± 0.0*	4.0 ± 0.0*	3.8 ± 0.2*	0.0 ± 0.0	0.0 ± 0.0
Apoptosis	1.8 ± 0.5*	1.7 ± 0.4*	1.3 ± 0.5*	0.0 ± 0.0	0.2 ± 0.2
Regeneration	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	1.8 ± 0.2*	2.0 ± 0.0*
Total	15.3 ± 0.5*	15.5 ± 0.6*	15.0 ± 0.8*	12.4 ± 0.2*	12.4 ± 0.5*

Rats were injected IP with 3 g/kg L-ornithine and were untreated (MeSpd —), pretreated (MeSpd PT), or treated (MeSpd TR, twice in case of the animals killed at 48 hours) with 50 mg/kg MeSpd IP. Animals were killed at 24 or 48 hours after the injection of L-ornithine. Values for control animals (injected IP with PS instead of L-ornithine and MeSpd) were 0.0 ± 0.0.

*Significant difference ($P < 0.05$) versus the control group.

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treatment (OM24: 10.42 ± 1.35 nmol/mg protein, OM48: 8.74 ± 1.18 nmol/mg protein). Pancreatic spermidine content significantly decreased in the L-ornithine–treated groups (Fig. 4A), whereas spermine contents did not show any alteration (Fig. 4B). Pancreatic SSAT activity significantly increased in response to L-ornithine injection by more than 4-fold at 24 hours and more than 7-fold at 48 hours (Fig. 4C). Putrescine was not present in detectable amounts in any of the groups (results not shown). Methylspermidine administration did not affect any of these parameters.

Histological Examination

Interstitial edema, vascular congestion, leukocyte adherence, and infiltration and necrosis of acinar cells greatly increased at 24 and 48 hours in response to L-ornithine injection (Fig. 5). Apoptosis of acinar cells was detected only at 24 hours. Methylspermidine administration did not ameliorate any of the investigated histological parameters (Fig. 5; Table 1).

Serum and Pancreatic Amylase Activities, Serum Lipase Activity, and PW/BW Ratio

Serum amylase activities did not increase significantly in response to L-ornithine injection. However, MeSpd treatment in the OM24 group significantly increased serum amylase activity, and it was also significant versus the O24 group (Fig. 6A). Pancreatic contents of amylase significantly decreased in the L-ornithine–treated groups versus the control group except in the

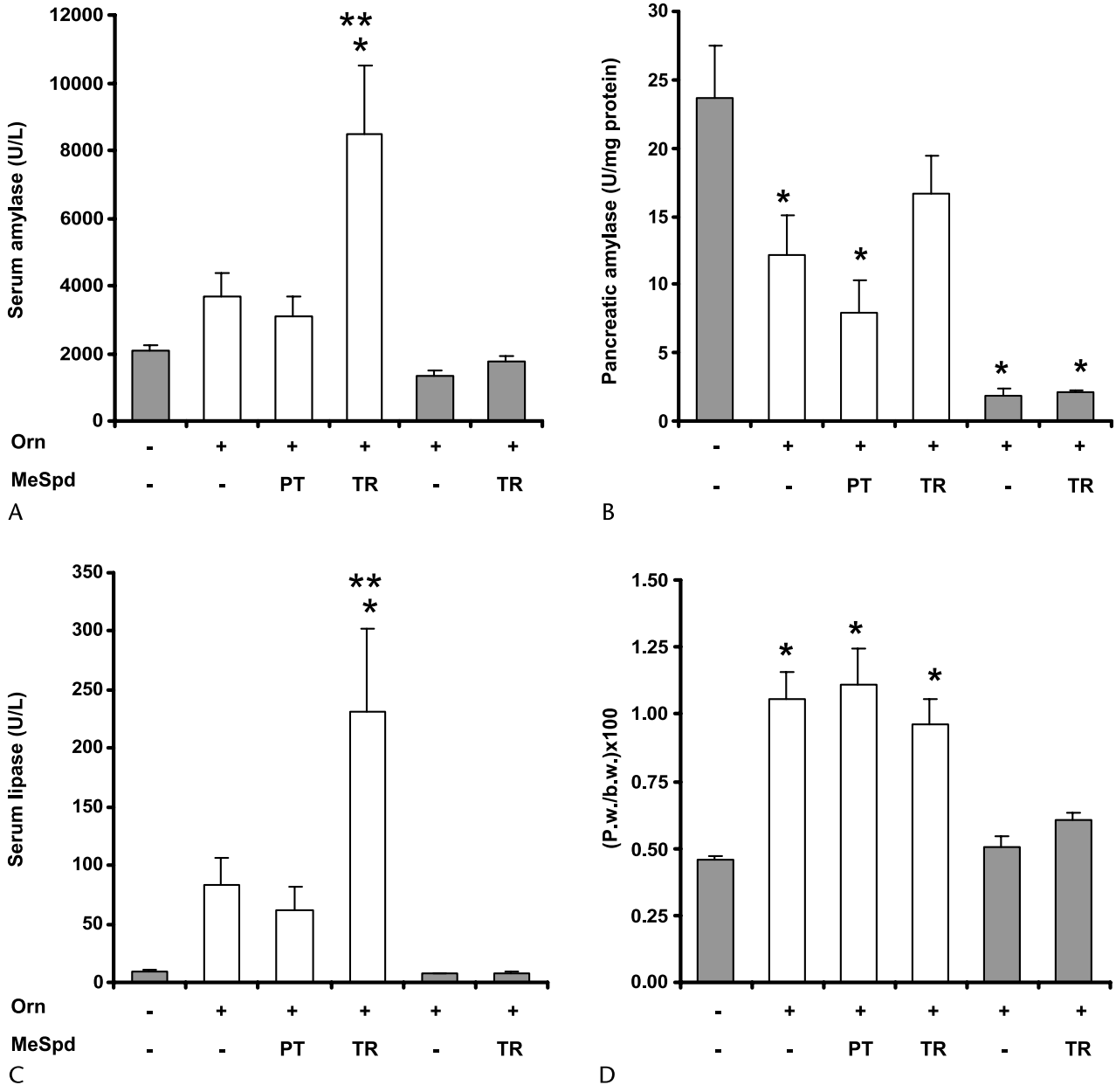

FIGURE 6. Effects of MeSpd on the activities of serum and pancreatic digestive enzymes and PW/BW ratio in L-ornithine–induced acute pancreatitis. The bar charts demonstrate activities of (A) serum and (B) pancreatic amylase, (C) serum lipase, and (D) PW/BW ratio. See legend of Figure 4 for the labeling of the bars. Data are shown as means ± SEM, n = 5–8. Significant difference ($P < 0.05$) versus the *control (Orn −, MeSpd −) or **Orn 24 hours (Orn +, MeSpd −, white bar) group.

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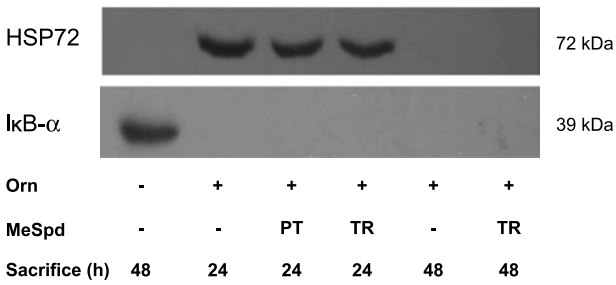

FIGURE 7. Effects of MeSpd on pancreatic HSP72 and IκB-α levels in L-ornithine–induced acute pancreatitis. The figure shows representative Western immunoblot analysis of protein lysates (40 μg/lane) from the pancreata of rats administered PS, L-ornithine, and/or MeSpd. See legend of Figure 4 for the labeling of groups. Rats were killed, as indicated, at 24 or 48 hours after injection with PS or L-ornithine.

OM24 group (Fig. 6B). Serum lipase activity significantly increased in the L-ornithine–treated groups at 24 hours versus the control group (Fig. 6C). Serum lipase activity significantly increased in the OM24 group versus the O24 group (Fig. 6C). Pancreatic weight–body weight ratio significantly increased at 24 hours in response to 3 g/kg L-ornithine (Fig. 6D). Methylspermidine administration did not influence PW/BW, either at 24 hours or at 48 hours.

Pancreatic HSP72 and IκB-α Expression

Heat shock proteins are essential cytoprotective molecules that are known to be induced in acute pancreatitis.[18] IκB-α is an inhibitory protein of the proinflammatory transcription factor, nuclear factor κB (NF-κB).[19] Injection of 3 g/kg L-ornithine induced pancreatic HSP72 synthesis at 24 hours (Fig. 7). Pancreatic IκB-α levels decreased significantly 24 and 48 hours after the L-ornithine injection (Fig. 7). Methylspermidine administration did not affect HSP72 and IκB-α levels.

Pancreatic MPO Activity

Pancreatic MPO activity significantly increased in the L-ornithine–treated groups versus the control group (Fig. 8A). Methylspermidine administration did not influence MPO activity in any of the groups.

Pancreatic IL-1β levels

Corresponding to IκB degradation, and consequently to activation of NF-κB, pancreatic IL-1β synthesis showed significantly elevated levels at 24 and 48 hours after the L-ornithine injection (Fig. 8B). Methylspermidine treatments had no significant effects on proinflammatory cytokine levels, although the analogue treatment seemed to partially prevent the elevation of IL-1β level.

Serum Concentrations of Creatinine and ASAT Activity

Significant elevations of serum creatinine concentrations were detected in the O24 and OM24 groups versus the control group (Fig. 8C). Serum ASAT activities significantly increased 24 and 48 hours after L-ornithine injection (Fig. 8D). Methylspermidine administration did not affect serum ASAT activity in any of the groups.

DISCUSSION

The involvement of polyamines in the pathogenesis of acute pancreatitis was first found in transgenic rats overexpressing SSAT.[5] Interestingly, we have also found increased pancreatic spermidine catabolism (possibly mediated via activation of SSAT) in L-ornithine–induced acute pancreatitis. In contrast, polyamine levels generally increased in the lung and liver with the exception of lung spermidine levels, which decreased. Pretreatment or treatment with the stable polyamine analogue MeSpd did not influence the levels of natural polyamines and SSAT activity and did not ameliorate the severity of L-ornithine–induced acute pancreatitis. This may be explained by the finding that the analogue did not accumulate in the pancreas to the level compensating for the most of spermidine depletion with the exception of the treatment group at 24 hours.

Assuming that the direct transformation of L-ornithine into putrescine may secure specific targeting of the polyamine metabolism but consists of a partial utilization of L-ornithine, "detoxification" of the excess amino acid load may also include conversion of L-ornithine to citrulline or glutamic semialdehyde. The influence of these metabolic routes cannot be excluded.

Pancreatic polyamine catabolism seems to be a general feature of acute pancreatitis.[6–8] In the current study on L-ornithine–induced acute pancreatitis, we have shown that polyamine levels are altered only after the first signs of histological damage. Whereas the earliest events (vascular congestion, vacuolization, and apoptosis of acinar cells) in L-ornithine–induced acute pancreatitis pancreas can already be detected at 4 hours,[9] polyamine catabolism started only after 6 hours. The belated elevations in the activity of SSAT and spermidine catabolism suggest that these mechanisms do not take part in the initiation of L-ornithine–induced acute pancreatitis. Nevertheless, polyamine catabolism was apparent at 24 hours after the injection of L-ornithine when the highest degree of pancreatic injury was detected. The earliest time point at which pancreatic polyamine pools and SSAT activity were investigated in the similar L-arginine–induced model was 24 hours.[6] However, in taurocholate-induced pancreatitis, pancreatic SSAT activity already increased from 3 to 6 hours.[8] An interesting difference between the taurocholate-induced, arginine-induced, and the L-ornithine–induced models is that neither spermine catabolism nor putrescine accumulation could be detected in the pancreas despite the elevations in SSAT and ODC activities in the L-ornithine–induced model. As polyamine homeostasis is controlled not only by their metabolism, but also by their secretion and uptake, these findings might be a consequence of altered polyamine transport.[1–2] They may also be an indication of accelerated polyamine metabolic cycle, as described for SSAT transgenic mice,[20] which could in part explain the lack of spermine depletion in L-ornithine–induced pancreatitis where the synthesis dominates over the catabolism.

As acute pancreatitis will affect extrapancreatic organs such as the liver and lung, we also investigated changes of polyamine pools in these tissues. Depletion of spermidine and spermine levels has been reported in red blood cells of rats with taurocholate-induced pancreatitis, and these changes correlated with the extent of pancreatic necrosis.[21] We have demonstrated that changes in polyamine pools in response to L-ornithine injection occur not only in the pancreas and blood, but also in the liver and lung. Interestingly, although a marked accumulation of putrescine was observed in these tissues at 6 hours, subsequent activation of polyamine catabolism was not detected in these organs. Only a moderate decrease was found in lung spermidine levels.

Synthetic α-methylated polyamine analogues, such as MeSpd, are metabolically more stable than natural polyamines as they are not substrates for SSAT and are poor substrates for spermine synthase.[22] Nevertheless, they are supposed to fulfill

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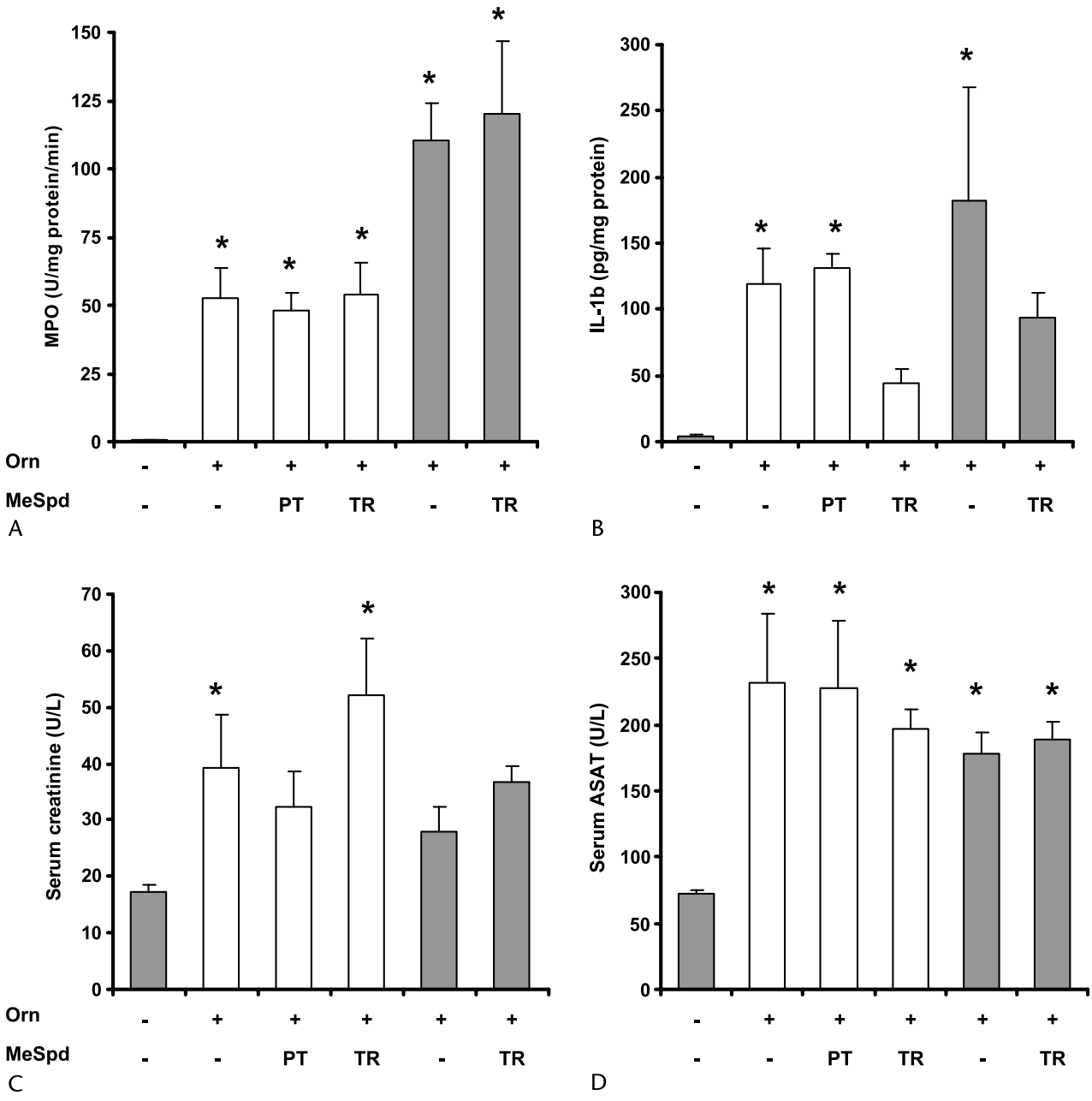

FIGURE 8. Effects of MeSpd on pancreatic parameters reflecting inflammation and extrapancreatic organ involvement in L-ornithine–induced acute pancreatitis. The bar charts demonstrate pancreatic (A) MPO activities, (B) IL-1β levels and ~~(C)~~ serum creatinine concentrations, and (D) ASAT activities. See legend of Figure 4 for the labeling of the bars. Data are shown as means ± SEM, n = 5–8. *Significant difference ($P < 0.05$) versus the control group (Orn −, MeSpd −).

most of the putative cellular functions of natural spermidine and spermine. Methylated derivatives of polyamines are substrates of proteins involved in polyamine transport and accumulate in the pancreas after IP administration. Moreover, they act as substitutes of natural polyamines.[23] Methylspermidine pretreatment totally prevented zinc-induced pancreatitis in transgenic rats overexpressing SSAT as judged by plasma α-amylase activity and histopathology.[24] In this study, we administered MeSpd (to compensate for depleted pancreatic spermidine levels) as a single-injection pretreatment before the induction of L-ornithine–induced pancreatitis or twice as treatment after the induction of pancreatitis. In both cases, MeSpd was ineffective at reducing the severity of the disease. This was demonstrated by measuring several laboratory and histological parameters of acute pancreatitis. Both the pretreatment and treatment with MeSpd led to decreased spermidine levels in the L-ornithine–induced pancreatitic groups, which suggest that catabolism of the natural polyamines further increased in the presence of synthetic polyamine analogue or the analogue replaces the natural spermidine in its binding site in the tissue. The current results are partly in contrast with the earlier studies on L-arginine– and taurocholate-induced

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pancreatitis. Methylspermidine pretreatment was shown to reduce the severity of L-arginine–induced (2.5 g/kg) pancreatitis without preventing SSAT activation and subsequent polyamine catabolism.[6] In contrast (and in accordance with our results), the severity of cerulein-induced (7 × 50 μg/kg) pancreatitis was not affected by MeSpd pretreatment.[6] The common feature is that neither in cerulein-induced pancreatitis nor in L-ornithine–induced pancreatitis did the analogue accumulate to the levels compensating for the depleted polyamine pools. Bismethylspermine treatment (but not pretreatment) ameliorated pancreatic injury at 24 hours after the induction of taurocholate-induced pancreatitis, but it did not ameliorate the late progression of the pancreatic necrosis at 72 hours.[21] Moreover, bismethylspermine treatment resulted in lethal toxicity at 72 hours after induction of pancreatitis.[21] We must note that the infusion of sodium taurodeoxycholate (2%) into the pancreatic duct or IP injection of 2.5 g/kg L-arginine will result in milder pancreatitis versus 3 g/kg L-ornithine. The contrasting results may be attributed to the fact that stable polyamine analogues may be effective only at earlier time points and in less severe forms of necrotizing acute pancreatitis.

In the present study, we have shown that increased SSAT activity and consequent spermidine catabolism in the pancreas are apparent phenomena in L-ornithine–induced acute pancreatitis in rats. However, the fact that these changes occur belatedly and that MeSpd administration did not ameliorate any of the parameters, either in the pretreatment or in treatment group, suggests that activated polyamine catabolism does not play a role in the initiation of pancreatic injury in L-ornithine–induced experimental pancreatitis. The organ-specific responses to L-ornithine and the ~~method~~ of pancreatic damage induction by the basic amino acid need to be investigated. Further studies are needed to reveal the role of polyamine homeostatic processes in the maintenance of pancreatic integrity.

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