Galanin Mediates the Pathogenesis of Cerulein-Induced Acute Pancreatitis in the Mouse

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Objectives: Acute pancreatitis (AP) is characterized by pancreatic microcirculatory and secretory disturbances. As galanin can modulate pancreatic vascular perfusion, we sought to determine if galanin plays a role in AP.

Methods: Acute pancreatitis was induced in wild-type and galanin gene knockout mice by intraperitoneal injections of cerulein. The severity of AP was evaluated (plasma amylase and lipase, myeloperoxidase activity, and acinar cell necrosis) with and without treatment with galanin or the antagonist galantide. Galanin receptor messenger RNA expression in mouse pancreas was measured by reverse transcription–polymerase chain reaction and Western blot analysis.

Results: Galantide ameliorated AP, reducing all indices by 25% to 40%, whereas galanin was without effect. In galanin knockout mice, all indices of AP were reduced 25% to 50% compared with wild-type littermates. Galanin administration to the knockout mice exacerbated AP such that it was comparable with the AP induced in the wild-type mice. Conversely, administration of galantide to the galanin knockout mice did not affect the AP, whereas AP was ameliorated in the wild-type mice. The 3 galanin receptor subtypes are expressed in mouse pancreas, with receptor subtype 3 expression predominating.

Conclusions: These data implicate a role for galanin in AP and suggest a potential clinical application for galanin antagonists in treatment.

Key Words: acute pancreatitis, galanin, galanin receptors, galantide

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Acute pancreatitis (AP) is a common clinical condition with an annual incidence of 5 to 40 per 100,000 population and an overall mortality approaching 1.5 per 100,000 population.[1,2] Approximately one third of the patients develop pancreatic necrosis, which is associated with a mortality rate of approximately 30%.[2,3] Currently, there is no specific treatment of AP.

The pathogenesis of AP is incompletely understood. Although numerous triggering agents have been identified and early cellular events have been defined in animal studies, the complete mechanism(s) remain to be elucidated.[4]

Galanin is a known sympathetic neurotransmitter that acts in the central and peripheral nervous systems.[5] Galanin immunoreactivity is present in many nerve fibers in the pancreas of many species including human and is closely associated with the pancreatic blood vessels. Galanin is a vasoactive compound, and its cardiovascular effects have been described.[6] Some pancreatic islet cells show immunoreactivity for galanin.[7] In addition, galanin modulates insulin secretion and, in some species, pancreatic exocrine secretion.[8,9] Galanin acts via 3 G protein–coupled receptors designated as galanin receptors 1 (GALR1), 2 (GALR2), and 3 (GALR3).[5] The messenger RNA (mRNA) expression and cellular localization of these receptors in the pancreas are poorly characterized.

We recently showed that galanin can regulate pancreatic vascular perfusion.[10] Microvascular changes in AP are well documented, and impaired pancreatic vascular perfusion is thought to play an important role in the development of pancreatic necrosis in AP.[11,12] Therefore, we hypothesized that galanin may play a role in AP. To undertake this study, we chose the commonly used cerulein hyperstimulation model in mice. An advantage of using mice was the availability of galanin knockout mice that could be used in this investigation.[13]

The aims of our studies were to determine if (1) exogenous galanin and the galanin antagonist galantide modulate the severity of AP in a cerulein hyperstimulation model in mice, (2) the severity of cerulein-induced AP in galanin gene knockout mice is less than that displayed by their wild-type littermates, and (3) the 3 galanin receptor subtypes are expressed in the mouse pancreas.

MATERIALS AND METHODS

These following studies were approved by the Animal Welfare Committee of the Flinders University.

Female Balb C mice weighing 15 to 24 g were used. The day before the experiment, the mice were randomly assigned to various experimental groups (see later). A blood sample was collected to determine the baseline plasma amylase and lipase activities as previously described.[14] Mice were fasted overnight with free access to water before AP induction.

Induction of AP

Mice received 7 intraperitoneal injections of cerulein (50 μg/kg; American Peptide Company, Sunnyvale, Calif) in 0.15 mL of saline at hourly intervals for 6 hours.[15] Buprenorphine HCl (0.1 mg/kg; Reckitt Benckiser Health Care UK Ltd, Hull, United Kingdom) was administered subcutaneously to provide analgesia. Our previous studies have shown that this analgesic agent does not influence cerulein-induced AP in mice.[14] Mice were anesthetized 12 hours after the first cerulein injection, a blood sample was collected, and the mice were then euthanized by exsanguination. Pancreata were harvested for subsequent assessment of myeloperoxidase (MPO) activity and histological examination to determine pancreatic damage.

Pancreatic tissue for MPO estimation was weighed and stored at −80°C before extraction and assay. Plasma amylase

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and lipase (IU/L) and MPO (U/mg of wet weight pancreatic tissue extracted or U/mg protein) activities were measured as previously described.[14] Segments of pancreata were fixed overnight in a 10% buffered formalin solution (Orion Laboratories Pty Ltd, Perth, Australia) before standard hematoxylin and eosin processing.

Histological Examination

Pancreatic sections (5 μm) were examined by an independent and experienced pathologist unaware of the experimental details. Fifteen randomly chosen microscopic fields were examined per section. The histological scoring was based on the method described by Niederau et al.[16] The degree of edema, inflammatory infiltrate, and vacuolization and the number of necrotic cells were assessed.

Treatment Groups

Galanin or galantide (American Peptide Company) solutions for injection were prepared in saline containing 0.01% bovine serum albumin (Sigma-Aldrich, St Louis, Mo). In separate groups of mice (n = 5−6), AP was induced with and without coadministration of galanin (10 nmol/kg) or galantide (10, 20, or 40 nmol/kg) with each cerulein injection. Control groups (n = 3−7 per group) received 7 hourly injections (0.15 mL) of saline (containing bovine serum albumin as previously described), galanin (10 nmol/kg), or galantide (10, 20, or 40 nmol/kg). A separate group (n = 5) was coadministered with galantide (40 nmol/kg) commencing 2 hours after AP induction (second to seventh injections).

Galanin Gene Knockout Mouse Studies

Acute pancreatitis was induced in galanin gene knockout mice and their wild-type littermates with cerulein as described previously. Separate groups of mice received galanin (10 nmol/kg) or galantide (20 nmol/kg) with each cerulein injection (n = 6−9 per group). Galanin gene knockout and wild-type littermate control groups (n = 3−4) received 7 hourly saline injections. Plasma amylase and lipase and MPO activities and histological assessment of pancreatic damage were performed as described previously.

Messenger RNA and Protein Studies

To determine the relative expression of the 3 galanin receptor subtypes in the mouse pancreas, 9 mice were fasted overnight and euthanized by cervical dislocation, and their brains and pancreata harvested, snap frozen in liquid nitrogen, and stored at −70°C. As the 3 galanin receptor subtypes are expressed in the mouse brain, this tissue was used as a positive control.[17–19] The RNA was extracted, subjected to deoxyribonuclease I (Ambion, Austin, Tex) treatment, and then underwent reverse transcription (RT) and polymerase chain reaction (PCR) as described previously.[14] Ribosomal 18S was selected as the housekeeping gene. The following primer sequences were used: GALR1: forward primer (FP); GGCAGCTTATTCTCCA CAGC, reverse primer (RP): TGATCTTCAGTAGACCCAC GAG; GALR2-FP: CAGATTGCGAGAGTGGTGACATAG, RP: CGGACAGGGTTAGTCTAGTC; GALR3-FP: ACCACC ACCGCCTTCATC, RP: TTGCTGACAGGATGCAGAAG; and 18S ribosomal RNA-FP: CCGCAGCTAGGAATAATGGA, RP: AGTCGGCATCGTTTATGGTC. Real-time PCR was performed on a Rotor-Gene 3000 (Corbett Life Science, Brisbane, Australia). Polymerase chain reaction products were sequenced at the Southpath and Flinders Sequencing Facility, Flinders Medical Centre, South Australia, Australia. The amplification efficiency of each primer pair was calculated from a real-time PCR dilution curve generated using serial 2-fold dilutions of genomic DNA. Quantitative real-time reverse transcription–PCR analysis was then performed as described previously.[14] A genomic DNA reference was used to account for minor differences in levels of detection between the 3 galanin receptor subtype primer pairs and allow for relative comparison of expression of the 3 receptor subtypes.[17]

Western Blot Analysis

Whole frozen pancreas or brain was homogenized in 2 to 5 mL of 20-mmol/L Tris-HCl (pH 7.4), 150-mmol/L NaCl, 1-mmol/L ethylene diamine tetraacetic acid, 1-mmol/L ethylene glycol tetraacetic acid, 1% Triton X-100, 1% sodium dodecyl sulfate (SDS), and 10% protease inhibitor cocktail (Sigma-Aldrich). After incubation on ice for 30 minutes, the homogenate was centrifuged at 2000*g* for 10 minutes at 4°C. One hundred microliters of supernatant was precipitated with 500 μL of ice cold 5% trichloroacetic acid, incubated on ice for 10 minutes, and centrifuged at 10,000*g* for 2 minutes. The protein pellet was dissolved in 100 μL of phosphate-buffered saline (PBS; pH 7.4) and the protein concentration measured using the Bio-Rad Protein Assay (Bio-Rad, Hercules, Calif). A volume of supernatant equivalent to 300 μg of total protein was transferred to a new tube and then centrifuged at 100,000*g* for 1 hour at 4°C to form a pellet of membrane-bound protein. This pellet was redissolved in the homogenization buffer described previously. An equal volume of 2× sample reducing buffer (60-mmol/L Tris-HCl [pH 6.8], 10% glycerol, 2% SDS, 5% 2-mercaptoethanol, and 0.05% bromophenol blue) was added, and the sample was incubated at 99°C for 10 minutes. The entire sample was separated by SDS–polyacrylamide gel electrophoresis and transferred to a nitrocellulose membrane (Amersham Biosciences UK Limited, Buckinghamshire, United

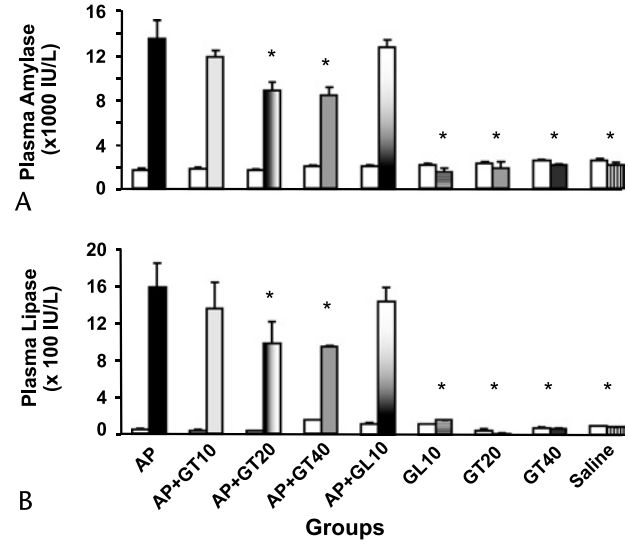

FIGURE 1. Acute pancreatitis (AP)–induced hyperenzymemia in a cerulein mouse model with and without treatment with galantide or galanin. Activities of plasma amylase (A) and lipase (B; IU/L). The pre-AP induction activity of both plasma enzymes is represented by the open bars (A, B). Groups of mice with AP were treated with galantide at 10 (AP + GT10), 20 (AP + GT20), or 40 nmol/kg (AP + GT40) or with galanin (10 nmol/kg; AP + GL10). The control groups received galanin (10 nmol/kg; GL10), galantide (20 and 40 nmol/kg; GT20 and GT40, respectively), or saline. Data are presented as mean ± SEM. *$P < 0.05$ compared with AP-alone group.

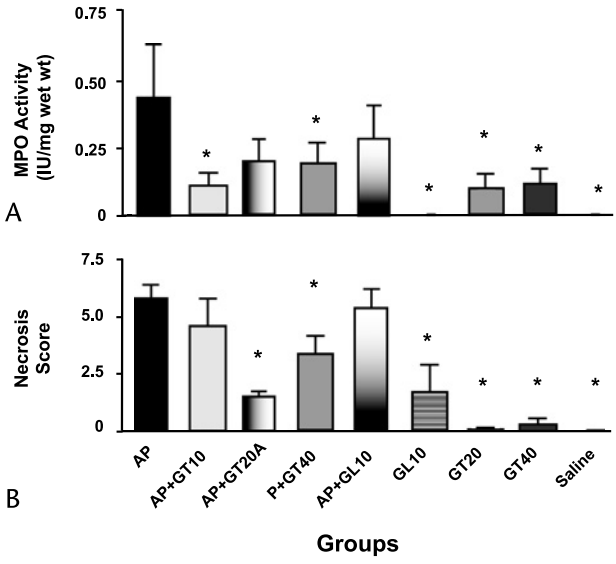

FIGURE 2. Acute pancreatitis (AP)–induced myeloperoxidase (MPO) activity and acinar cell necrosis in a cerulein mouse model with and without treatment with galantide or galanin. Pancreatic MPO (A; IU/mg wet weight of tissue) and acinar cell necrosis score (B), respectively. Groups of mice with AP were treated with galantide at 10 (AP + GT10), 20 (AP + GT20), or 40 nmol/kg (AP + GT40) or with galanin (10 nmol/kg; AP + GL10). The control groups received galanin (10 nmol/kg; GL10), galantide (20 and 40 nmol/kg; GT20 and GT40, respectively), or saline. Data are presented as mean ± SEM. *$P < 0.05$ compared with AP-alone group.

Kingdom) in 20-mmol/L Tris-HCl (pH 7.6), 150-mmol/L glycine, and 16% vol/vol methanol. The nitrocellulose membranes were blocked with 3% wt/vol nonfat dry milk in PBS. Primary antisera (rabbit polyclonal) for GALR1, GALR2, or GALR3 (Alpha Diagnostics, San Antonio, Tex) were added (1:1000 dilution) and incubated overnight at 4°C. The nitrocellulose membranes were washed in PBS (pH7.4) containing 0.05% Tween 20 (Tris-buffered saline) incubated with horseradish peroxidase–conjugated rabbit secondary antisera (1:2000 dilution, Sigma-Aldrich) for 2 hours at room temperature, followed by a repeated Tris-buffered saline wash. Antibody binding signal was detected using enhanced chemiluminescence (GE Healthcare Bio-Sciences Pty Ltd, Sydney, Australia).

Statistical Analysis

The statistical analysis used SPSS (version 11.5; SPSS Inc, Chicago, Ill). All data are expressed as mean ± SEM of number (n) of animals. The data were analyzed using Mann-Whitney *U* test (for 2 groups) or Kruskal-Wallis test (3 or more groups). Statistical significance was accepted at the $P < 0.05$ level. Absence of error bars in any of the figures indicates that the SEM was too small to illustrate.

RESULTS

Cerulein-Induced AP in Mice

The AP-induced hyperenzymemia was significantly reduced to 60% to 65% of the AP-alone group by galantide (20 or 40 nmol/kg) administration at AP induction ($P < 0.05$; F1 Figs. 1A, B). Galanin (10 nmol/kg) and galantide (10 nmol/kg) administration, however, did not significantly affect AP-induced hyperenzymemia. Delaying galantide treatment to 2 hours after AP induction significantly reduced the plasma hyperamylasemia (AP alone, 13,405 ± 1656 IU/L compared with galantide treated, 8492 ± 1529 IU/L [n = 5]; $P < 0.05$). The plasma enzyme activities were not different from the pretreatment levels in the control groups (Figs. 1A, B). AQ4

Pancreatic MPO activity for the various groups is illustrated in Figure 2A. The MPO activity in the AP group was significantly greater than the control groups. Treatment with galantide (10 or 40 nmol/kg) reduced the MPO activity by approximately 70% and 60% of that in the AP group ($P < 0.05$). The MPO activities of the galanin (10 nmol/kg)- and galantide (20 nmol/kg)-treated AP groups were not statistically different from the AP-alone group. When galantide (40 nmol/kg) administration was delayed by 2 hours, there was no statistical difference in the MPO activity (AP alone, 0.46 ± 0.17 U/mg wet weight tissue compared with galantide treated, 0.44 ± 0.13 U/mg wet weight tissue [n = 5]). The MPO activities in the control groups were not statistically different from the saline group. F2 AQ5 AQ6

The mean acinar cell necrosis score in the AP group was 5.8 (Fig. 2B). Galantide treatments (20 and 40 nmol/kg) significantly reduced the necrosis score by 75% ($P < 0.001$) and 45% ($P < 0.05$), respectively. In contrast, after treatment with galanin and galantide (10 nmol/kg), no significant difference in the acinar cell necrosis score was observed compared with the AP-alone group. The necrosis score for the control groups were not statistically different from the saline group (Fig. 2B). Delaying galantide treatment (40 nmol/kg) by 2 hours after AP onset

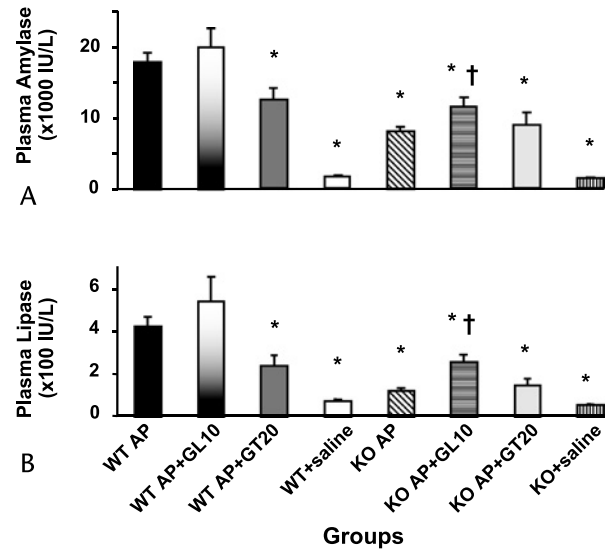

FIGURE 3. Hyperenzymemia induced with cerulein in galanin gene knockout mice (KO) or wild-type littermates (WT) with and without galanin or galantide treatment. The cerulein-induced hyperamylasemia (A) and hyperlipasemia (B) in the KO mouse (KO AP) were significantly less than that induced in the WT littermates (WT AP). Hyperenzymemia was exacerbated by treatment of the KO mouse with galanin (10 nmol/kg; KO AP + GL10) but not in the WT littermates similarly treated (WT AP + GL10). Treatment of the KO mouse with galantide (20 nmol/kg; KO AP + GT20) did not alter the hyperenzymemia; however, galantide treatment reduced the hyperenzymemia in the WT littermates (WT AP + GT20). The plasma enzyme activities in saline control groups for the KO (KO + saline) and WT littermates (WT + saline) were comparable. Data are presented as mean ± SEM. *$P < 0.05$ compared with WT AP-alone group. †$P < 0.5$ compared with KO AP group.

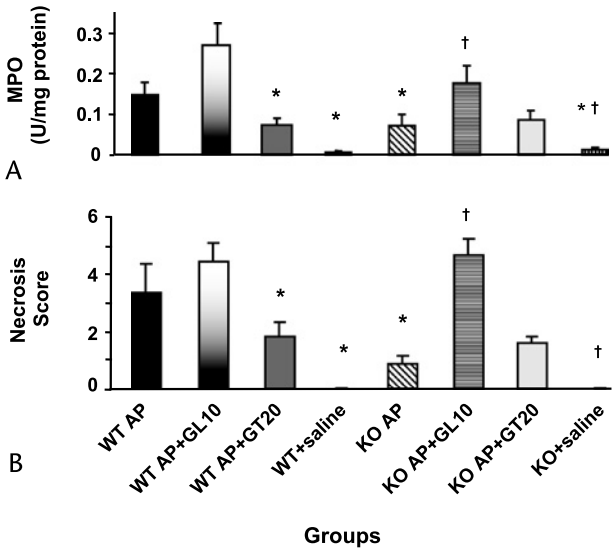

FIGURE 4. Acute pancreatitis–induced myeloperoxidase (MPO) activity and acinar cell necrosis after cerulein administration in galanin gene knockout mice (KO) or wild-type littermates (WT) with and without 10-nmol/kg galanin (GL) or 20-nmol/kg galantide (GT) treatment. A, MPO activity. The AP-induced MPO activity was significantly less in the KO mouse (KO AP) compared with the WT littermates (WT AP). Treatment of the KO mouse with GL and cerulein (KO AP + GL10) increased MPO activity to a level comparable with that induced by cerulein in the WT littermates (WT AP). Treatment with GL did not alter the cerulein-induced MPO activity in the WT littermates (WT AP + GL10). Conversely, treatment of the KO mouse with GT and cerulein (KO AP + GT20) did not affect the MPO activity, whereas it reduced the cerulein-induced MPO activity in WT littermates (WT AP + GT20). The MPO activity in the control groups, KO plus saline (KO + saline) and WT plus saline (WT + saline), was comparable. B, Acinar cell necrosis score. Treatment of the KO mouse with GL and cerulein (KO AP + GL10) increased the acinar cell necrosis score to a level comparable with that induced by cerulein in the WT littermates (WT AP) but GL did not alter the caerulein-induced acinar cell necrosis score in the WT littermates (WT AP + GL10). Conversely, treatment of the KO mouse with GT and cerulein (KO AP + GT20) did not affect the acinar cell necrosis score but reduced the acinar cell necrosis score resulting from cerulein administration to WT littermates (WT AP + GT20). The acinar cell necrosis score in all control groups (WP + saline and KO + saline) was not significantly different. Data are presented as mean ± SEM. *$P < 0.05$ compared with WT AP-alone group. †$P < 0.5$ compared with KO AP group.

significantly reduced the necrosis score (AP alone, 5.8 ± 0.6 compared with galantide treated, 3.8 ± 0.3 [n = 5]; $P < 0.05$).

Acute Pancreatitis in Galanin Gene Knockout Mice

All indices of cerulein-induced AP in the galanin gene knockout mice were significantly reduced compared with AP alone in the wild-type littermates (Figs. 3, 4). Hyperenzymemia in the galanin gene knockout was approximately 30% to 45% of that observed in the wild-type littermates ($P < 0.05$; Fig. 3). Similarly, in the galanin gene knockout, MPO activity was approximately 50% and acinar cell necrosis score was approximately 25% of that in the wild-type littermate AP-alone group ($P < 0.05$; Figs. 4A, B, respectively). Galanin administration to the knockout mice increased the AP indices to levels comparable

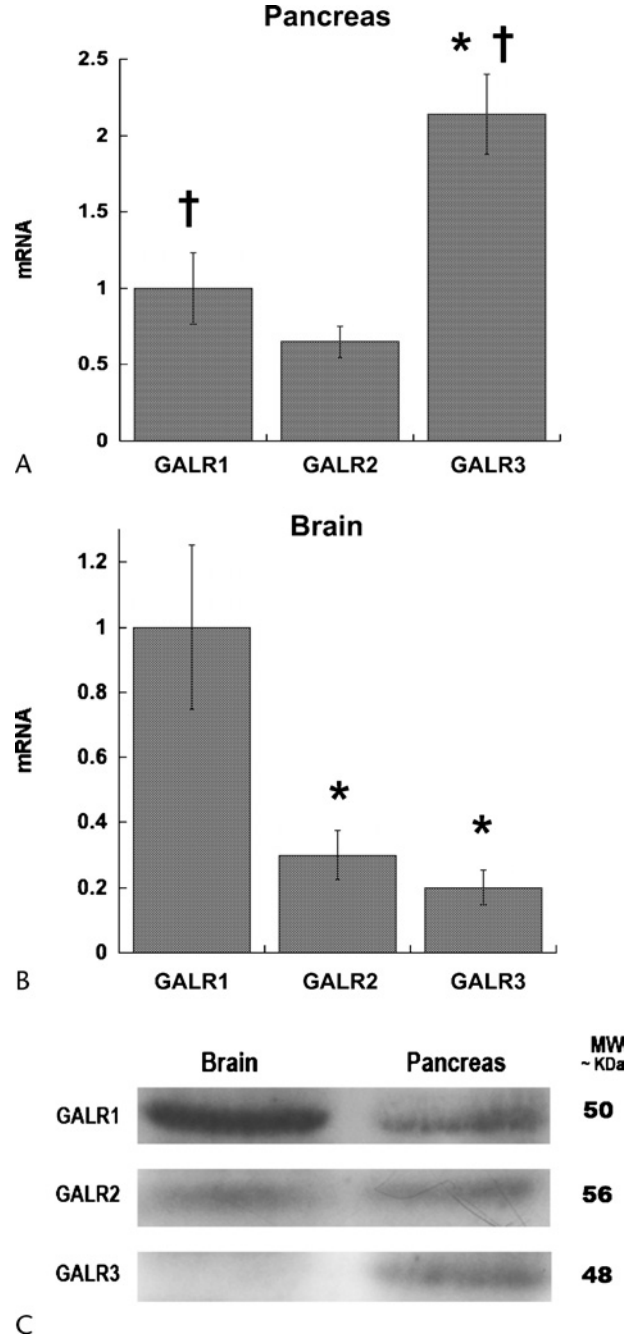

FIGURE 5. The expression of galanin receptor subtype mRNA in the mouse pancreas and brain. Galanin receptor subtype 1 (GALR1) expression (n = 9) was set to 1, and the GALR2 and GALR3 expressions are presented relative to this. A, The expression of mouse pancreatic GALR3 was greater than that of GALR1 and GALR2, normalized to the expression of 18S. B, Mouse brain GALR1 was the most abundantly expressed, and GALR2 and GALR3 were expressed at a similar level and normalized to the expression of 18S. C, Western blot analysis confirmed the presence of proteins corresponding in molecular weight (MW) to the 3 galanin receptor subtypes in mouse pancreas and brain. Data are presented as mean ± SEM. *$P < 0.05$ compared with GALR1. †$P < 0.05$ compared with GALR2.

with those in the wild-type littermate AP-alone group. In contrast, galanin administration did not statistically alter the AP indices in the wild-type littermate mice. Conversely, administration of galantide to the galanin gene knockout mice did not significantly affect the AP indices, whereas all AP indices were reduced in the wild-type littermate mice (Figs. 3, 4).

Galanin Receptor Subtype Expression

The 3 galanin receptor subtypes are expressed in the mouse pancreas (Fig. 5A). GALR3 expression was 2- to 3-fold higher than GALR1 and GALR2 expressions ($P < 0.02$), and GALR1 was more expressed than GALR2 ($P < 0.05$). In contrast, in control brain tissue, GALR1 expression was 4- to 6-fold higher than GALR2 or GALR3 expression ($P < 0.05$; Fig. 5B). Immunodetection on Western blots revealed proteins of the appropriate size for the 3 receptor subtypes in the mouse pancreas and brain (Fig. 5C).

DISCUSSION

We have provided evidence that galanin plays a role in the pathogenesis of cerulein-induced AP in mice. We have demonstrated that prophylactic treatment with galantide ameliorated AP-induced plasma hyperenzymemia, pancreatic MPO activity, and pancreatic acinar cell necrosis. Moreover, delayed galantide treatment ameliorated most indices of AP. Cerulein-induced AP in galanin gene knockout mice was less severe compared with that induced in their wild-type littermates. We showed that the mouse pancreas expresses the 3 known galanin receptor subtypes (mRNA and protein). Taken together, our data implicate galanin in the induction and progression of cerulein-induced AP.

This is the first report of the involvement of galanin in the pathogenesis of AP in mice. The mechanism(s) underlying these effects of galanin and galantide in the context of AP are under active investigation, and several mechanisms may be operating. Other investigators have shown that galanin typically inhibits pancreatic exocrine secretion, and evidence for both neural and nonneural mechanisms have been described.[5,9,20] Some studies have shown that galanin can modulate basal and stimulated acinar cell secretions, in acinar cell preparations, but this is not a consistent observation.[21,22] Galanin may, however, facilitate secretion. Galanin evokes transient rises in intracellular calcium concentration in rat GH3/B6 pituitary cells, consistent with increased secretion.[23] We have shown in preliminary studies with mouse lobules (unpublished data) that in the presence of supraphysiological concentrations of cerulein (as used to induce AP), galanin enhances amylase secretion. This stimulatory effect of galanin is blocked by preincubation with galantide. It is well recognized that pancreatic exocrine secretion is under neurohormonal control.[24] In our mouse lobule preparation, the neurohormonal elements are retained, so the site of action of galanin may not be directly on the acinar cell. Overall, these observations are consistent with the role(s) of galanin in modulating exocrine secretion in AP.

Galanin may also contribute to AP by modulating pancreatic microcirculation. We have shown that exogenous galanin decreases and galantide increases pancreatic vascular perfusion in the Australian possum.[10] There are significant changes in perfusion during induction of AP in possums, consisting of an initial fall in pancreatic vascular perfusion (likely due to the fall in blood pressure during this period, as vascular conductance was not altered), followed by a rebound in pancreatic vascular perfusion and increased vascular conductance 2 hours after AP induction.[25] This rebound was prevented by inducible nitric oxide synthase inhibitors suggesting mediation by nitric oxide.[26] Overall, these findings suggest that galanin may contribute to the early phase of AP by affecting pancreatic microcirculation and that galantide's beneficial effects may, at least in part, be via improving the pancreatic microcirculation. In support of this, our preliminary studies in the Australian possum suggest that exogenous galanin and galantide treatment modulate the microcirculatory disturbance and severity of AP in a possum model.[27] The findings reported here suggest the involvement of galanin in the pathogenesis of AP in the mouse and the possum. Further studies with other species and other forms of AP are required to determine the generality of this observation.

Galanin could also contribute to AP via neurogenic inflammation. Neurogenic inflammation as a cause of AP is well recognized.[28] Recent evidence suggests that galanin gene knockout mice may have a deficit in the development of the sensory nerves that participate in neurogenic inflammation in the skin.[29] The galanin gene knockout mice may also have disrupted neutrophil accumulation in response to an inflammatory insult.[29] Our data are not consistent with the role of galanin in neurogenic inflammation where a sensory nerve deficit or compromised neutrophil accumulation is involved because administration of galanin to the galanin gene knockout mice exacerbated the indices of AP to levels comparable to those induced by cerulein in the wild-type littermates.

The galanin receptor subtype(s) that mediate the effects of galanin during AP remain to be defined. The molecular expression of these subtypes in the pancreas has received little attention, and receptor localization studies have not been reported. GALR3 mRNA is expressed in the human pancreas; however, the expression of the other 2 subtypes has not be described.[18] We have demonstrated here that the 3 galanin receptor subtype mRNAs are expressed in the mouse pancreas, with GALR3 as the most highly expressed. This contrasts with the murine brain (also shown here) and skin where GALR1 and GALR2 are the most highly expressed.[30] Overall, these data indicate that there is a potential for signaling through the 3 galanin receptor subtypes in the pancreas and that a greater proportion of GALR3-mediated galanin activity may occur in the pancreas. Interestingly, galanin modulates inflammatory edema formation via GALR3 in mouse skin microvasculature.[31] We have preliminary data suggesting that the cerulein-induced AP in mice is mediated, in part, by GALR3.[32] The galanin receptor subtype localization within the pancreas also needs to be established. We have attempted an immunohistochemical study with commercially available antibodies, but this was unsuccessful.

We have measured the expression of the 3 galanin receptor subtypes in the pancreas of the galanin gene knockout mice and found that the 3 receptor subtypes are expressed but at levels approximately 50% of that in the wild-type littermate pancreas (unpublished data). We found that galanin administration with cerulein failed to alter the AP-induced plasma hyperenzymemia in wild-type mice. In contrast, AP-induced hyperenzymemia in the galanin gene knockout mouse was enhanced with exogenous galanin treatment. Together, these observations are consistent with the full occupancy or desensitization of pancreatic galanin receptors by endogenous galanin released during AP in wild-type mice. In the galanin gene knockout mice, the galanin receptors are vacant owing to a lack of endogenous ligand. When exogenous ligand is provided, galanin's contribution to AP is revealed. The hyperenzymemia in the galanin gene knockout mice was not influenced by administration of galantide probably because no endogenous ligand was present to be antagonized by galantide.

Delayed galantide administration was effective in reducing the severity of AP in wild-type mice, suggesting that galanin

receptors may also be involved in later events in this AP model. As pancreatic MPO activity was not reduced by delayed galantide treatment, this suggests that inflammatory cell infiltration and/or inflammatory cell activation was not influenced. It is worth noting, however, that galanin is probably 1 of several players in AP, as prophylactic administration of galantide was not able to completely abrogate AP. Numerous factors have been implicated in AP, such as substance P (acting via NK-1 receptors).[33,34]

The source of endogenous galanin that might participate in AP is unclear. Galanin is abundantly expressed in intrinsic and extrinsic pancreatic neurons.[35] Neuronal stimulation produces levels of galanin immunoreactivity in pancreatic venous effluent in the picomole range, but it is unknown if galanin is released during AP.[36] Galanin immunoreactive pancreatic endocrine cells have been reported.[7] Further studies are required to clarify the source and the kinetics of release of endogenous galanin during AP.

CONCLUSIONS

In conclusion, our data implicate galanin in the induction and progression of experimental AP in mice. The beneficial effect of galantide in AP may be due to modulation of pancreatic vascular perfusion, acinar cell secretion, and/or the inflammatory cascade typifying AP. These findings also suggest a potential clinical application for galanin antagonists in the treatment of AP.

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