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Biochemical and Biophysical Research Communications 346 (2006) 339–344
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Specific type IV phosphodiesterase inhibitor ameliorates cerulein-induced pancreatitis in rats ☆

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Received 19 May 2006
Available online 2 June 2006

Abstract

Background and aims: Type IV phosphodiesterase is a key enzyme to metabolize intracellular adenosine 3′,5′-cyclic monophosphate (cAMP) expressed in inflammatory cells. The specific type IV phosphodiesterase inhibitor that increases intracellular cAMP is known to be potent suppressor of proinflammatory cytokines. However, the effect of phosphodiesterase inhibitors on the development of pancreatitis has not been well understood. In the present study, we examined the effect of a specific type IV phosphodiesterase inhibitor on experimentally induced pancreatitis.

Methods: Severity of cerulein-induced pancreatitis and pancreatic proinflammatory cytokine levels were studied with or without pretreatment with a specific type IV phosphodiesterase inhibitor (rolipram) in Sprague–Dawley rats.

Results: Administration of rolipram clearly ameliorated severity of pancreatitis evaluated by edema, serum amylase ($P < 0.05$), and lipase levels ($P < 0.05$) in rats. Also, the level of pancreatic proinflammatory cytokine (interleukin-1β (IL-1β)) was significantly reduced when rats were treated with rolipram prior cerulein injection ($P < 0.05$).

Conclusions: Our results demonstrated that intracellular cAMP and pancreatic proinflammatory cytokine level, which are regulated by type IV phosphodiesterase, might play an important role in the pathogenesis of acute pancreatitis.

Keywords: cAMP; Type IV phosphodiesterase inhibitor; Pancreatitis; Cerulein

In the pathogenesis of acute pancreatitis, many factors including activated pancreatic enzymes, cytokines, chemokines, free radicals, blood flow, and neurogenic factors have been reported, although the precise mechanism are still not well understood. On the other hand, adenosine receptor agonists have been shown to modulate various pathophysiologic conditions through receptor-mediated mechanism. Recent studies indicated that some nonspecific adenosine receptor (A1, A2a, A2b, and A3) agonists modulate the severity of pancreatitis [1]. It has been also suggested that each adenosine receptor has different function in view of their biochemical actions to adenosine and biological action. When adenosine or selective agonists bind to A1 receptors, intracellular cAMP level is decreased by inhibition of adenylyl cyclase in polymorphonuclear leukocyte (PMNL). And PMNL adherence to endothelium is enhanced [2]. In addition, oxidative activity and chemotaxis [3,4] are also increased. In opposite, the stimulation of A2a adenosine receptors increases intracellular cAMP mediated by enhancement of adenylyl cyclase activity is known to decrease the adherence of stimulated PMNL to endothelium [5], to reduce the release of reactive oxygen species [3,6], and to restore cytokine-inhibited PMNL

☆ *Abbreviations:* cAMP, adenosine 3′,5′-cyclic monophosphate; IL-1β, interleukin-1β; PMNL, polymorphonuclear leukocyte; ATP, adenosine triphosphate; TNF-α, tumor necrosis factor-α; ELISA, enzyme-linked immunosorbent assay; 5′-AMP, 5′-adenosine monophosphate; PKA, protein kinase A.

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doi:10.1016/j.bbrc.2006.05.133

migration [7]. Thus, role of A1 adenosine receptors is thought to be inflammatory and A2a receptors are anti-inflammatory. Also in the pancreas, it has been reported that adenosine A1 agonist has exacerbated acute pancreatitis in rats [1]. On the other hand, precise function of adenosine A2a receptor stimulation has not been well understood because of lack of the specific agonist although excessive adenosine triphosphate (ATP) catabolism has been reported to occur during pancreatitis [8–11].

Type IV phosphodiesterase inhibitors also have anti-inflammatory effects through increasing level of intracellular cAMP by blocking its catalysis. Recently, Gail reported the marked synergy between specific type IV phosphodiesterase inhibitor, rolipram, and a selective adenosine A2a agonist [12] in PMNL in which both adenosine A2a receptors and type IV phosphodiesterase are abundantly expressed. Therefore, rolipram is thought to be a useful tool to understand the role of intracellular cAMP level in PMNL in the pathogenesis of pancreatitis. Increment of cAMP level in inflammatory cells including PMNL by rolipram has been reported to attenuate some diseases such as colonic mucosal inflammation [13,14], meningitis [2], and arthritis [15,16]. As there has been no report in which the role of type IV phosphodiesterase inhibitor nor adenosine A2a agonist in the pancreas, we examined the effect of rolipram on the development of pancreatitis in vivo.

Materials and methods

Animals

This study was approved by the Akita University Animal Care Committee. Male Sprague–Dawley rats (8-week-old, 250–300 g) were fed on standard laboratory diet and water ad libitum, and kept in cages in temperature (22 ± 2 °C) and humidity (55 ± 5%) controlled room with a 12-h dark–light cycle before and during the experiment. Rats were deprived of food but were allowed access to water 24 h before experiment.

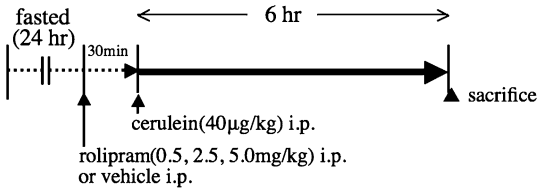
Chemicals

Specific phosphodiesterase IV inhibitor, rolipram, was purchased from Sigma Chemical Co. (St. Louis, MO, USA). Rolipram was dissolved in small volume of dimethyl sulfoxide and then diluted with physiological saline just before injection.

Experimental protocol

Induction of pancreatitis. Pancreatitis was induced as previously described by administration of a single intraperitoneal injection of cerulein (40 μg/kg body weight) [17]. Rats were sacrificed by cervical dislocation under ether anesthesia 6 h after the cerulein injection, and samples of pancreas and blood were rapidly harvested for study. The samples were immediately used for experiments or stored in liquid nitrogen until use.

Effect of specific type IV phosphodiesterase inhibitor on cerulein-induced pancreatitis. Rats were randomized to receive intraperitoneal injection of rolipram (0.5, 2.5, or 5.0 mg/kg, $n = 6$) or vehicle (control group, $n = 6$). Thirty minutes after rolipram injection, rats were injected with cerulein intraperitoneally (40 μg/kg body weight) [17]. Six hours after the cerulein injection, rats were sacrificed to evaluate the severity of pancreatitis comparing with vehicle-treated rats. Six rats were administered intraperitoneally with vehicle instead of cerulein as negative control (Fig. 1).



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Serum amylase was measured by the blue starch method using phadebas amylase test. Serum lipase level was measured according to the method reported by Panteghini [18].

After killing, the pancreas was quickly removed and weighed to evaluate the degree of pancreatic edema [19]. The pancreas-wet weight was expressed as wet pancreas weight per body weight (mg/g).

A part of pancreas in each rat was immediately processed to extraction or frozen in liquid nitrogen until use for cytokine assay.

Histological assessment of pancreatitis. For light microscopy a part of pancreas was fixed in 10% buffered formalin and embedded in paraffin wax. The tissue section was stained with hematoxylin and eosin. The histologic assessment of infiltration of inflammatory cells was carried out with reference to a scale ranging from 1 to 3 as minimal to maximal alterations, and the grading of interstitial edema was based on the percentage involvement of examined area (absence of lesions = 0, involvement of 1–10% = 1, 11–25% = 2, 26–50% = 3, >51% = 4). Two pathologists who are blind in the groups performed the histological assessment.

Cytokine levels in the pancreas. This experiment was performed at 4 °C. A part of pancreas in each rat was immediately frozen in liquid nitrogen for cytokine assay. The frozen tissues were homogenized with 10 volumes of ice-cold 0.1 M phosphate-buffered saline, pH 7.4. The homogenate was centrifuged at 14000*g* for 20 min, and the supernatant was assayed for tumor necrosis factor-α (TNF-α) and IL1-β. The TNF-α and IL1-β are assayed using enzyme-linked immunosorbent assay (ELISA) kit which are commercially available (R&D Systems, Minneapolis, MN, USA).

Data analysis. All data were expressed as means ± SEM. Statistical analysis was performed using the two-tailed Student's *t* test for unpaired values as appropriate; *P* values <0.05 were considered to be statistically significant.

Results

Effect of specific type IV phosphodiesterase inhibitor (rolipram) on cerulein-induced pancreatitis

Severe pancreatic swelling, evaluated by pancreas wet weight/body weight, was observed after cerulein injection in vehicle-treated rats (11.21 ± 1.61 mg/g) compared with control which receive vehicle injection instead of cerulein as negative control (1.99 ± 0.30 mg/g) (Fig. 2). Pretreatment of rats with rolipram significantly suppressed the severity of pancreatic swelling in dose range of 0.5–5.0 mg/kg ($P < 0.05$ in all doses of rolipram). Significant increment in serum amylase (36076 ± 2828 IU/L) and lipase level (2881 ± 514 IU/L) was observed 6 h after cerulein injection in vehicle-treated control rats compared with cerulein (−) control rats (amylase: 3824 ± 139 IU/L, lipase:

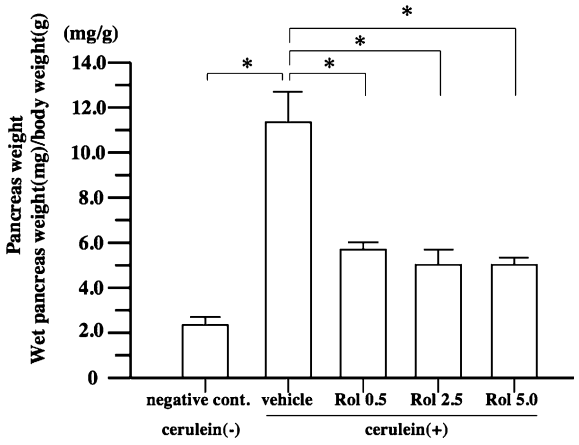

Fig. 2. Effect of rolipram on cerulein-induced pancreatic swelling. Six hours after cerulein injection, rats were sacrificed and the pancreatic swelling was evaluated with or without (vehicle) pretreatment with rolipram (0.5, 2.5, or 5.0 mg/kg, $n = 6$ in each group). $^*P < 0.05$, significant difference compared with vehicle-treated group or negative control (without cerulein injection).

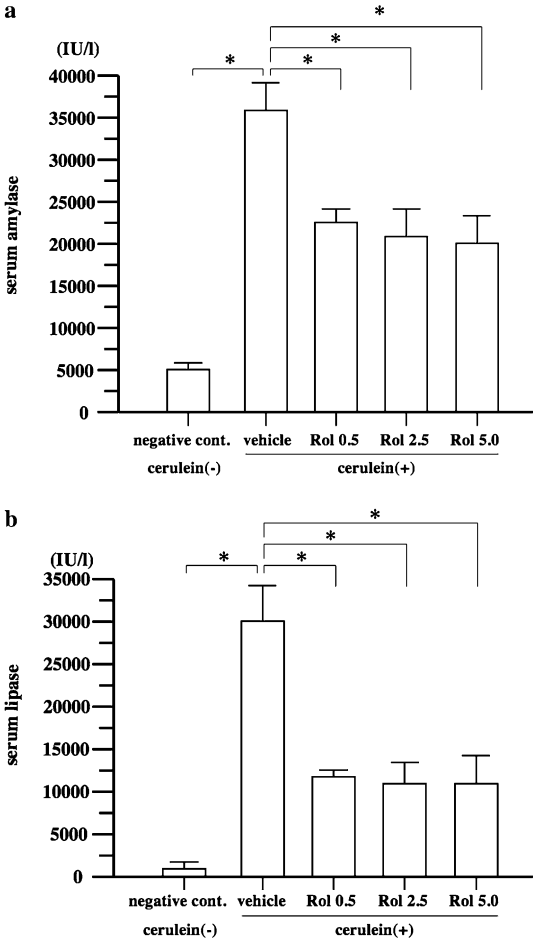

Fig. 3. Serum amylase and lipase levels after cerulein injection. Six hours after cerulein injection, rats were sacrificed, and serum amylase (a) and lipase (b) activities were assayed with or without (vehicle) pretreatment with rolipram (Rol 0.5: 0.5 mg/kg, Rol 2.5: 2.5 mg/kg or Rol 5.0: 5.0 mg/kg, $n = 6$ in each group). Data are means ± SEM. $^*P < 0.05$ compared with vehicle-treated group or negative control (without cerulein injection).

5 ± 1 IU/L). In rolipram-treated rats, elevation of these two enzymes after cerulein injection was significantly suppressed to about 40–60% in dose range of 0.5–5.0 mg/kg compared with vehicle-treated group ($P < 0.05$ in all doses of rolipram) (Fig. 3).

Macroscopic findings (Fig. 4a) showed apparent amelioration of pancreatic swelling in rolipram-treated rats compared with vehicle-treated rats. Also, by histological assessment, degree of edema and inflammatory cell infiltration were clearly ameliorated by rolipram treatment compared with vehicle-treated control rats (Fig. 4b and Table 1). It could be noted that inhibition of inflammatory cell infiltration was especially significant in rolipram-treated rats.

Cytokine levels in the pancreas after development of pancreatitis

As shown in Fig. 5, elevated level of IL-1β level in the pancreas 6 h after cerulein injection (2426.9 ± 123.3 pg/g) was significantly suppressed to 1709.4 ± 131.1 pg/g (0.5 mg/kg, $P < 0.05$), 1132.5 ± 217.4 pg/g (2.5 mg/kg, $P < 0.05$), and 911.3 ± 89.5 pg/g (5.0 mg/kg, $P < 0.05$) by rolipram treatment in a dose-dependent manner. Slightly elevated level of intrapancreatic TNF-α was observed ($P < 0.05$ compared with negative control (without cerulein)). Rolipram showed tendency to suppress the elevation of TNF-α although it was not statistically significant.

Discussion

Rolipram is a specific and strong inhibitor of type IV phosphodiesterase, an enzyme that catalyzes cAMP to 5′-adenosine monophosphate (5′-AMP). Therefore, addition of rolipram to cell causes an increment in intracellular cAMP which presumably enhances the activity of cAMP-dependent protein kinase A (PKA), and reduces production of proinflammatory cytokines such as IL-1β and TNF-α [20]. The potential usefulness of selective type IV phosphodiesterase inhibitors as novel anti-asthmatic and anti-inflammatory agents has been reported [21,22]. In addition, rolipram has been reported to inhibit experimentally induced colitis [13,14] and arthritis [15,16] in animal models. These actions are thought to be mediated by inhibition of diverse leukocyte functions including secretion of proinflammatory cytokines and adherence of leukocytes to endothelium through increment of intracellular cAMP [23,24].

Adenosine has been reported to inhibit the production of reactive oxygen species from stimulated PMNL [25]. Further studies have indicated that binding of adenosine to A2a adenosine receptors are essential to inhibit these leukocyte functions and to exhibit anti-inflammatory actions [5,7,26]. The main biological action resulted from adenosine A2a receptor stimulation is known to increase intracellular cAMP level and to reduce diverse leukocyte

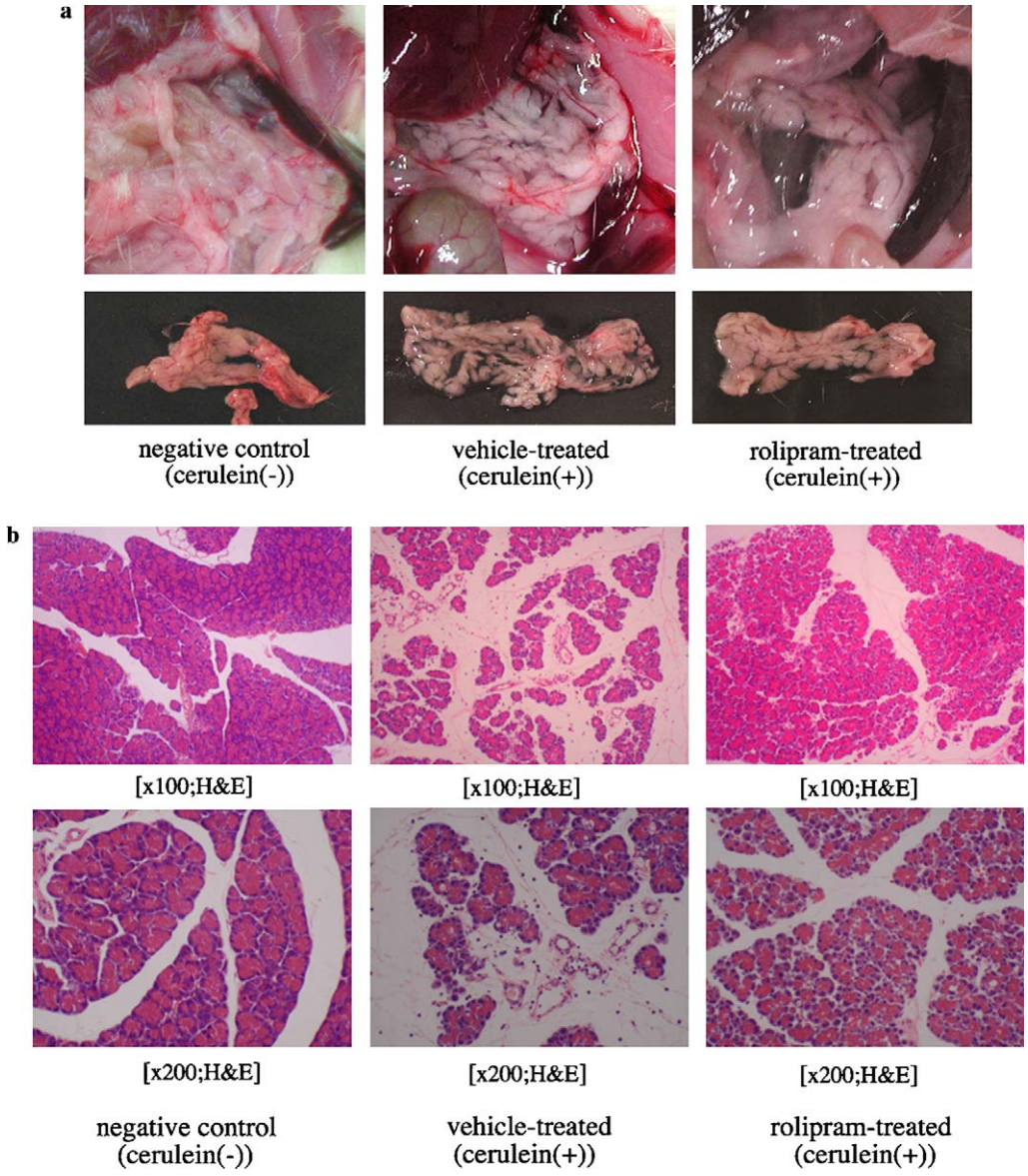

Fig. 4. Macroscopic findings (panel a) and microscopic view (panel b) of the pancreas 6 h after cerulein injection. Remarkable swelling, edema and inflammatory cell infiltration were observed in vehicle-treated group. Severity of pancreatitis was apparently ameliorated in rats treated with rolipram.

Table 1
Histological score of pancreas after cerulein injection

Group	Score	
	Inflammatory cells infiltration	Edema
Negative control	0	0
Cerulein		
Vehicle	3	3–4
Rol 0.5	1–2	2–3
Rol 2.5	0–1	2
Rol 5.0	0	1–2

The histologic assessment of neutrophil infiltration was carried out with reference to a scale ranging from 1 to 3 as minimal to maximal alterations, and the grading of necrosis and interstitial edema was based on the percentage involvement of examined area (absence of lesions = 0, involvement of 1–10% = 1, 11–25% = 2, 26–50 = 3, >51% = 4).

functions. In view of the action to increase intracellular cAMP levels in inflammatory cells, both adenosine A2a agonist and rolipram have similar action. Also, both adenosine A2a receptor and type IV phosphodiesterase are mainly expressed in inflammatory cells including macrophages and PMNL [1]. In opposite, stimulation of A1 adenosine receptors decreases intracellular cAMP level and induces adherence of PMNL to endothelium, and increases oxidative activity and chemotaxis of inflammatory cells [3,4].

In the studies for the pancreas, adenosine has been known to play an important role in the pathogenesis of pancreatitis modulating inflammatory pathways [27]. Recently, it has been reported that adenosine A1 receptor agonist exacerbated cerulein-induced acute pancreatitis in rats [1]. However, precise function of adenosine A2a receptor stimulation has not been well understood because of

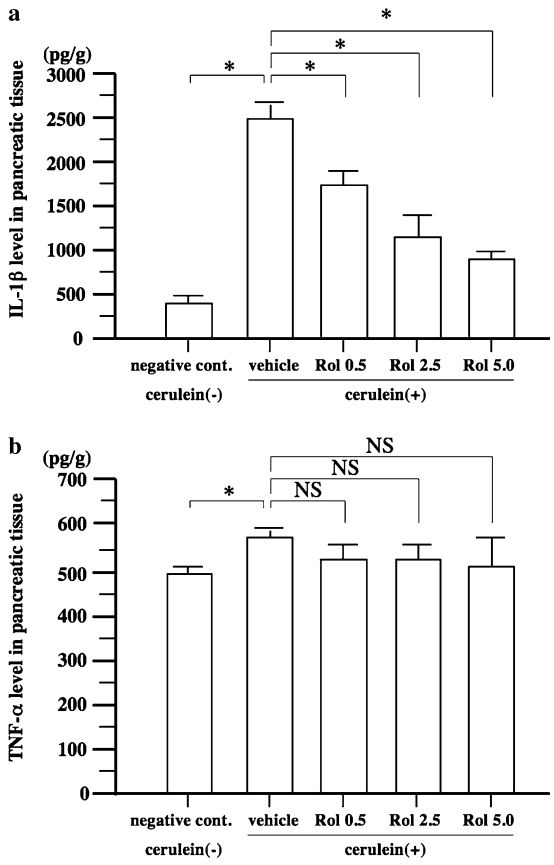

Fig. 5. Cytokine levels in the pancreas after development of pancreatitis. Six hours after cerulein injection, rats were sacrificed, and pancreatic IL-1β (a) and TNF-α (b) levels were assayed with or without (vehicle) pretreatment with rolipram (Rol 0.5: 0.5 mg/kg, Rol 2.5: 2.5 mg/kg or Rol 5.0: 5.0 mg/kg, $n = 6$ in each group). Data are means ± SEM. $^*P < 0.05$ compared with vehicle-treated group or negative control (without cerulein injection); NS, statistically no significant difference.

absence of the specific agonist although excessive ATP catabolism has been reported to occur during pancreatitis [8–11].

In the present study, we demonstrated that specific type IV phosphodiesterase inhibitor, rolipram, clearly ameliorates cerulein-induced pancreatitis. When adenosine A2a receptors on PMNL are stimulated, A2a receptors are coupled through G protein (Gs) to adenylyl cyclase, which converts ATP to cAMP. The cAMP is metabolized to 5′-AMP by type IV phosphodiesterase. And increased level of cAMP is known to reduce PMNL functions such as adherence to endothelial cells, chemotaxis, production of reactive oxygen species, and production of proinflammatory cytokines (Fig. 6) [2]. In our experiments, elevated level of intrapancreatic proinflammatory cytokine (IL-1β) was significantly suppressed, and inflammatory cell infiltration in the pancreas was also clearly suppressed when rats were pretreated with rolipram prior to cerulein administration. In our experimental model, slight elevation of intrapancreatic TNF-α level was observed. Rolipram showed tendency to suppress the elevation of TNF-α although it was not statistically significant. It has been reported that pancreatic ATP decreased by cerulein injection, and the catabolism of ATP closely related to intracellular adenosine accumulation and its release from the cells [8,9,28–30]. However, considering our results and Sato's finding observed during the development of pancreatitis [1], pancreatitis would be modified depending on receptor subtype of adenosine which could regulate intracellular cAMP in PMNL. Although many factors are known to contribute to pancreatic acinar cell damage and its protection, adenosine could be acting as an important factor through the receptors.

In conclusion, our results demonstrated here might suggest the contribution of type IV phosphodiesterase activity to the pathogenesis of acute pancreatitis using specific inhibitor of this enzyme for the first time. Also, intracellular cAMP levels in inflammatory cells might play an essential role in the pathogenesis of acute pancreatitis, and modulation of cAMP levels by drug or gene engineering could be clinically useful for the therapy of pancreatitis.

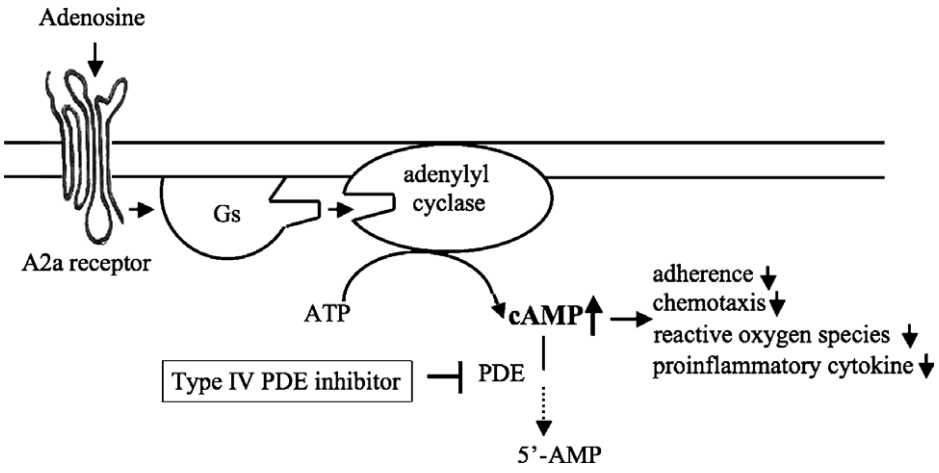

Fig. 6. Inhibition of neutrophil (PMNL) activation. Adenosine binds to adenosine A2a receptors on PMNL. A2a receptors are coupled through G protein (Gs) to adenynyl cyclase, which converts ATP to cAMP. The cAMP is metabolized to 5′-AMP by type IV phosphodiesterase [2]. Rolipram increases the level of intracellular cAMP by blocking its catalysis. High level of intracellular cAMP reduces adherence to endothelial cells, chemotaxis, production of reactive oxygen species, and production of proinflammatory cytokines.

Furthermore, adenosine A2a receptor agonist which also increases intracellular cAMP in inflammatory cells could be also a candidate for new attractive therapy for acute pancreatitis.

Acknowledgments

This work was supported in part by Grant-in-Aid for Scientific Research from the Ministry of Education, Science, Spots and Culture, Japan (to M.O. #14570442).

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