Original Paper

Cellular Physiology and Biochemistry

Cell Physiol Biochem 2011;28:315-322

Accepted: July 07, 2011

Chronic β-adrenoceptor Antagonists Upregulate the Rat Alveolar Macrophage Adrenergic System Through the β_1-Subtype

Yue-ping Guo[a,#], Yan Liu[b,#], Jing-bo Li[a], Yun Huang[c], Han-ping Qi[b], Jing Xie[b], Xiao-guang Cui[a], Zi-yong Yue[a] and Wen-zhi Li[a]

[a]Department of Anesthesiology, The 2nd Affiliated Hospital of Harbin Medical University, [b]Department of Pharmacology, [c]Molecular and Cellular Biochemistry, Harbin Medical University, Harbin, [#]Authors made equal contribution to this study

Key Words

β_1-adrenergic receptor • Propranolol • Atenolol • Alveolar macrophages

Abstract

Background: Previous studies demonstrate that macrophages synthesis and release catecholamines, which regulate the immune responses in an autocrine manner. These responses are mediated in part by β-adrenoceptors expressed on macrophages. Some β-adrenoceptor antagonists are commonly used in clinical conditions. Here we investigated whether the chronic administration of β-adrenoceptor antagonists upregulate adrenergic system of alveolar macrophage and the potential mechanims. Methods: Propranolol (30 mg/kg·d) or atenolol (5 mg/kg·d) was administered by gavage to rats for 4 weeks. Then alveolar macrophages were isolated and the expression of β_1 or β_2-adrenoceptor was detected by flow cytometric analysis. Dopamine β-hydroxylase expression was assessed by Western blot assay and the concentrations of noradrenaline, IL-6, and TNF-α in cell supernatants were measured using ELISA after 2 h or 24 h exposure of alveolar macrophages to 100 ng/ml lipopolysaccharide (LPS). Results: Propranolol increased the mean fluorescence intensity (MFI) of β_1, β_2-adrenoceptor and the frequency of β_1-,β_2-adrenoceptor positive macrophages. However, only the MFI of β_1-adrenoceptor and the frequency of β_1-adrenoceptor positive macrophages were increased by atenolol. Furthermore, both propranolol and atenolol promoted LPS-mediated dopamine β-hydroxylase protein expression and increased noradrenaline production in rat alveolar macrophages. This was accompanied by increased LPS-mediated IL-6 and TNF-α production in cell supernatants of alveolar macrophages. Conclusion: These findings demonstrate that propranolol or atenolol upregulates alveolar macrophage adrenergic system, and the response may be β_1-adrenergic receptor subtype dependent.

Introduction

Macrophages that originate from circulating monocytes can migrate to tissues. The environment of their ultimate destination heavily influences the function

KARGER

Fax +41 61 306 12 34
E-Mail karger@karger.ch
www.karger.com

1015-8987/11/0282-0315$38.00/0

Accessible online at:
www.karger.com/cpb

Wen-zhi Li
Department of Anesthesiology, Harbin Medical University
Harbin 150081 (P.R. China)
Tel. 0086 451 86605029, Fax 0086 451 86605029
E-Mail wenzhili9@126.com

of monocytes that differentiate into macrophages. The alveolar macrophages are present in the alveolar space and the conducting airways. Because of their physical location, they are the first immune cells to encounter inhaled pathogens such as microbiological agents, environmental toxins, or allergens prior to polymorphonuclear neutrophils and monocytes [1]. As the resident phagocytes of the lung, alveolar macrophages are an important source of local pro-inflammatory cytokines such as TNF-α, and induce systemic inflammation in human and animal experiments [2, 3].

It is well established that catecholamines regulate immune and inflammatory responses by acting on specific α- and β-adrenoceptors on immune cells [4]. Moreover, macrophages including alveolar macrophages have been identified as a new source of catecholamines [5]. By expressing catecholamine-generating and degrading enzymes which can generate, release and inactivate epinephrine and norepinephrine, macrophages may regulate the inflammatory responses in an autocrine manner [6].

Moreover, macrophages highly express β-adrenergic receptor. Accumulated data have shown that the immunomodulatory effects of exogenous or endogenous catecholamine are mediated by β-adrenoceptor [7, 8]. Many β-adrenoceptor antagonists, such as propranolol (β-adrenoceptor nonselective, blocking both β_1 and β_2-adrenoceptor) and atenolol (β_1-adrenoceptor selective, blocking β_1-adrenoceptor), are commonly used in a number of clinical conditions, including arterial hypertension, ischemic heart diseases, congestive heart failure and cardiac arrhythmias. In view of this, we hypothesized that chronic administration of β-adrenoceptor antagonists may modify catecholamines synthesis and β-adrenoceptor expression, thereby modulating the inflammatory response of macrophage. In the present study we employed rat alveolar macrophage as a model to investigate whether β-adrenoceptor antagonists upregulate adrenergic system of alveolar macrophage, by examining the level of dopamine β-hydroxylase, noradrenalin, and β-adrenoceptors, and the production of cytokines. In addition, we chose non-selective β-adrenoreceptor antagonist propranolol and specific β_1-adrenoceptor antagonist atenolol as the target drugs to find out whether this response is dependent on the specific β-adrenoceptors.

Materials and Methods

Animals and Reagents

Wistar rats (250-300 g) used in this study were purchased from the Experimental Animal Center of Harbin Medical University, China. All experimental procedures and protocols regarding the experimental animals had previously been approved by Animal Care Committee of Harbin Medical University, China. The animals were conditioned at an animal facility for one week before the experiments. All animals had free access to food and water.

Sodium pentobarbital was purchased from Shanghai Chemical Regent, China. Lipopolysaccharide (LPS), propranolol hydrochloride, and atenolol hydrochloride were obtained from Sigma, USA. Biotin mouse anti-rat mononuclear phagocyte antibody, PE-conjugated Streptavidin, fluorescein isothiocyanate (FITC)-conjugated anti-β_1 and anti-β_2 adrenergic receptor antibodies were purchased from BD PharMingen (San Diego,CA, USA). BCA Protein Assay Kit was pruchased from Bio-Rad (Mississauga, ON, Canada). Rabbit polyclonal antibodies against dopamine β-hydroxylase and Rabbit anti-glyceraldehyde-3-phosphate dehydrogenase (GAPDH) were obtained from Abcam (Cambridge, MA, USA). The enzyme-linked immunosorbent assay (ELISA) kit was purchased from R&D Systems (Abingdon, UK). Cell lysis buffer containing phosphatase inhibitors was purchased from Cell Signaling Technologies (Beverley, MA, USA).

Drug administration

Thirty Wistar rats of either sex were randomly divided into three groups: control, propranolol, and atenolol. Animals were administered either propranolol at 30 mg/kg·d, atenolol at 5 mg/kg·d, or an equal volume of saline by gavage for 4 weeks. The average daily intake of both water and food did not differ significantly among all groups.

Isolation of alveolar macrophages

Rats were pretreated by gavage with drug or saline for 4 weeks before the experiment. On the day of the experiment, rats were anaesthetized by introperitoneal injection of sodium pentobarbital (40 mg/kg) and killed by exsanguination. The whole lung was lavaged by instilling and withdrawing 10 ml sterile saline three times in situ. The lavaged fluid was centrifuged (400 g for 5 min at 4°C). The pellets were washed twice with cold PBS and resuspended in PBS at 1×10^6 cells/ml. Then the cells were incubated in 6-well plates in 2 ml RPMI-1640 medium without FBS. The purity of the cell suspension was >95% by use of a modified Wright-Giemsa stain. We identified macrophages according to the morphology, as reported previously [9]. Alveolar macrophages were then subjected to flow cytometric analysis (FCA), Enzyme-linked immunosorbent assay (ELISA), or Western blot assay with or without stimulated with LPS as described below.

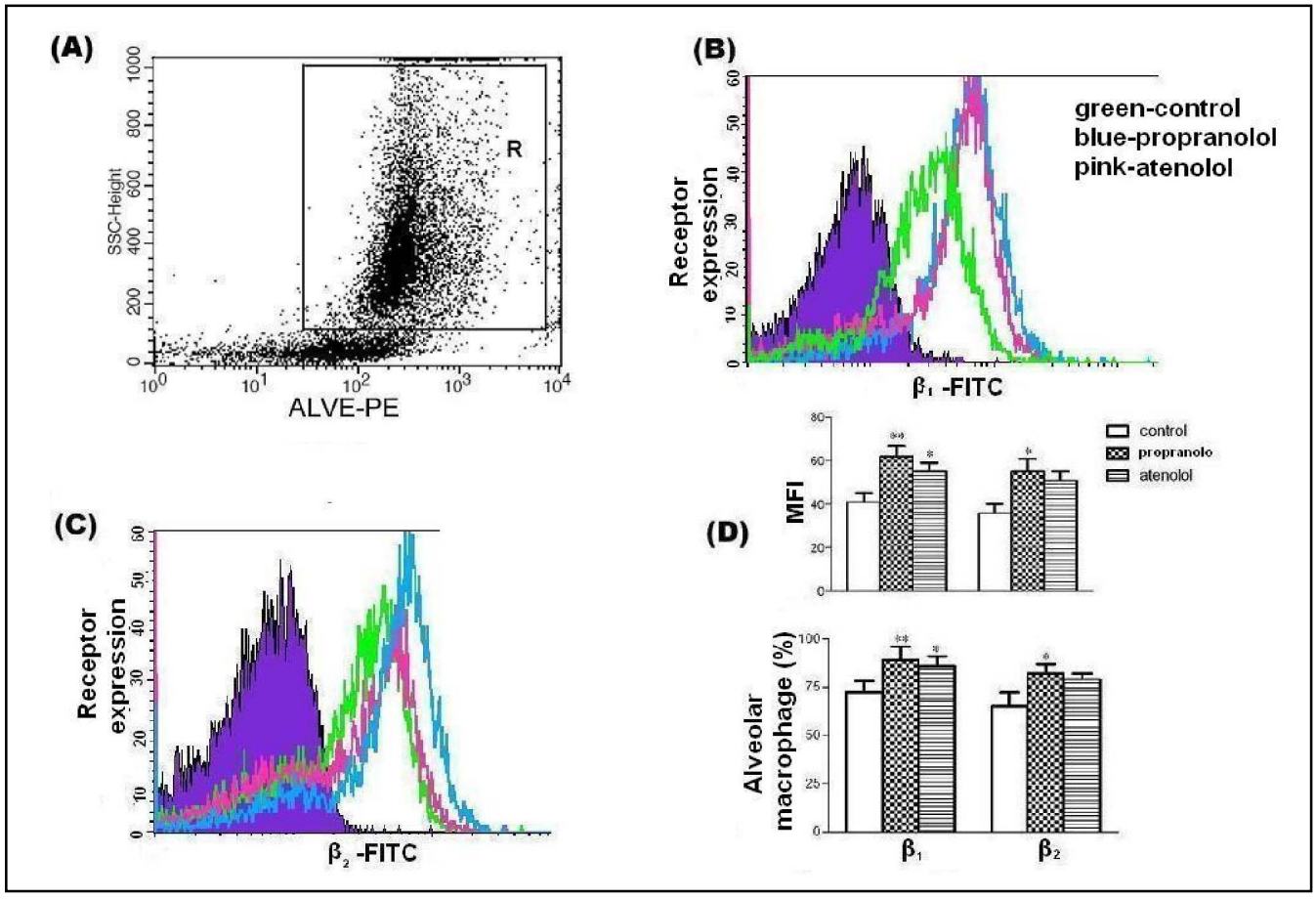

Fig. 1. Propranolol or atenolol treatment upregulates the expression of β_1- and β_2 -adrenoceptors in rat alveolar macrophages. Alveolar macrophages were obtained from rats administered either propranolol (30 mg/kg·d), atenolol (5 mg/kg·d), or an equal volume of saline by gavage for 4 weeks. (A) Alveolar macrophages were gated as indicated in dot plot (gate R). (B), (C) Flow cytometric analysis of β_1- or β_2 - adrenoceptors expression in alveolar macrophages from propranolol administered (blue line), atenolol administered (pink line), and saline administered control (green line) rats. Negative control samples with the omission of primary antibodies (purple shaded area) were used to define background staining. (D) Flow cytometric analysis of the percentage of β_1 and β_2- adrenoceptors positive cells within alveolar macrophages from propranolol, atenolol and saline administered rats and mean fluorescence intensity (MFI) of β_1- and β_2- adrenoceptors immunostaining on these cells. The results were expressed as mean ± SEM (n=6 independent experiments). *P<0.05, **P<0.01 vs. control.

Flow cytometric analysis

The expression of β_1- or β_2- adrenergic receptor was detected by FCA in alveolar macrophages unstimulated with LPS. The alveolar macrophages (5×10^5 cells/sample) were suspended in 100 µl PBS. Then, the cells were incubated with 20 µl of FITC-conjugated anti- β_1 or anti- β_2 adrenergic receptor antibody for 30 min at 4 °C in the dark. The cells were washed with PBS three times, resuspended in 100 µl PBS and incubated with biotin-conjugated mouse anti-rat mononuclear phagocyte antibody and PE- conjugated Streptavidin for 30 min at 4 °C in the dark. Next the cells were washed with PBS two times, resuspended in 500 µl PBS and analyzed with a fluorescence-activated cell sorting Calibur (BD Biosciences, San Jose, CA). The data were processed using the CELL QUEST program (BD Biosciences).

Stimulation of alveolar macrophages with LPS

Alveolar macrophages (1×10^6 cells /ml) were incubated in 6-well plates in 2 ml RPMI-1640 medium without FBS, and stimulated with 100 ng/ml LPS at 37 °C for 2 h or 24 h. The supernatants of the alveolar macrophage were collected at indicated time points after stimulation and centrifuged at 500 g to remove cellular debris. The supernatants were then divided into aliquots and frozen at -80 °C before ELISA of cytokines and noradrenaline. Cells were lysed and subjected to Western blot assay for detection of dopamine β-hydroxylase protein.

Western blot analysis

Alveolar macrophages were lysed using cell lysis buffer containing phosphatase inhibitors. The amount of protein in

the cell lysates were quantified using the BCA Protein Assay Reagent. Proteins were separated by electrophoresis on 10% SDS-polyacrylamide gels and transferred to nitrocellulose membranes (Amersham, Piscataway, NJ). Membranes were blocked with 5% non-fat milk at room temperature for 1 h and incubated with the primary antibody at 4 °C for 12 h. The membrane was washed three times with PBS containing 0.1% Tween 20, followed by incubation with the secondary antibody at room temperature for 1 h in the dark. The membrane was then washed three times with PBS and scanned by an Odyssey infrared imaging system (LI-COR, Lincoln, NB) at a wavelength of 800 nm.

Cytokine and noradrenaline Measurements by ELISA

The levels of TNF-α, IL-6, or noradrenaline in the cell-free supernatants were determined by ELISA kits according to the manufacturer's instructions. Samples were assayed in triplicate for each condition.

Statistical analysis

The data were expressed as mean ± SEM. ANOVA analysis was performed using SPSS13.0. A two-tailed $P < 0.05$ was taken to indicate a statistically significant difference.

Results

Chronic propranolol or atenolol treatment regulate the expression of β_1-and/or β_2-adrenoceptors in rat alveolar macrophages

FCA analysis of unstimulated alveolar macrophages (Fig. 1A) showed that β_1-and β_2-adrenoceptors are constitutively expressed on alveolar macrophages (Fig 1. B, C). Non-selective β-adrenoreceptor antagonist propranolol treatment significantly increased the mean fluorescence intensity (MFI) of β_1-and β_2-adrenoceptor and increased the frequency of β_1- and β_2- adrenoceptor-positive macrophages. In contrast, specific β_1-adrenoceptor antagonist atenolol treatment increased MFI of β_1-adrenoceptor and the frequency of β_1- adrenoceptor-positive macrophages, but only slightly (statistically insignificantly) increased the β_2- adrenoceptor-positive macrophages and its MFI (Fig. 1D). These data indicate that chronic treatment with different β-adrenoceptor antagonists led to subtype-specific regulation of β_1- and β_2-adrenoceptors.

Chronic propranolol or atenolol treatment had no significant effect on rat alveolar macrophages without stimulated with LPS

Without stimulated with LPS, propranolol or atenolol had no significant effect on the expression of dopamine β-hydroxylase, the secretion of noradrenaline, IL-6 and

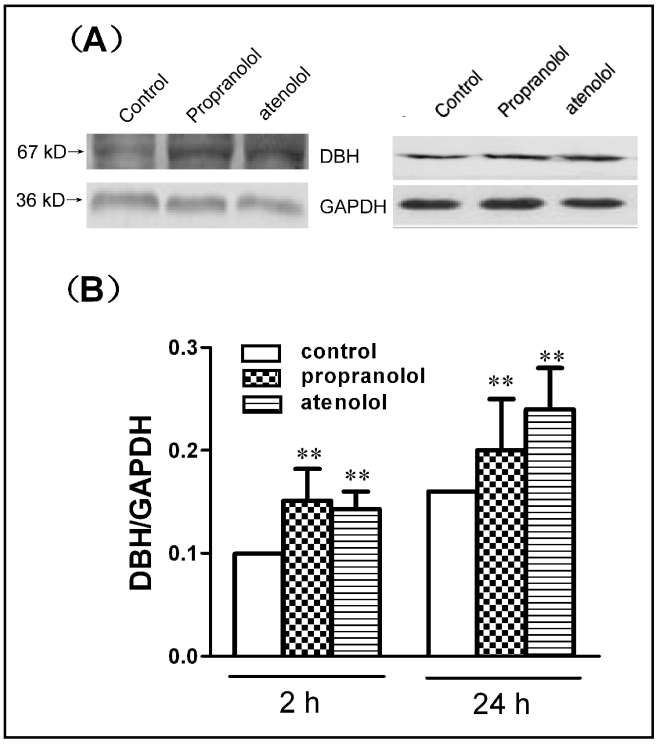

Fig. 2. Propranolol or atenolol treatment upregulates the expression of dopamine β-hydroxylase in rat alveolar macrophages. (A) Western blots showing dopamine β-hydroxylase protein level in alveolar macrophages isolated from different groups of rats 2 h or 24 h after stimulation with LPS (100 ng/ml). (B) Densitometric analysis of the relative protein level of dopamine β-hydroxylase as shown in (A). The data were expressed as mean± SEM and normalized to GAPDH (n=6 independent experiments). *P<0.05, **P<0.01 vs. control. DBH, dopamine β-hydroxylase.

TNF-α compared with the baseline in rat alveolar macrophages (dopamine β-hydroxylase: 0.09±0.01, 0.11±0.02 *vs.* 0.1±0.03; noradrenaline: 13.4±4.8 pg/ml, 10.5±5.2 pg/ml *vs* 11.2±4.2 pg/ml; IL-6: 31.5±.2 pg/ml, 34.6±5.2 pg/ml *vs* 30.1±4.8 pg/ml; TNF-α: 24.2±4.5 pg/ml, 25.2±3.3 pg/ml *vs* 24.8±5.7 pg/ml. Results were presented as mean± SEM of 6 individual rats, P 0.05)

Chronic propranolol or atenolol treatment promote LPS-mediated dopamine β-hydroxylase expression in rat alveolar macrophages

To investigate the effects of chronic β-adrenoreceptor antagonist treatment on the expression of catecholamine-generating enzymes in alveolar macrophages, we used propranolol and atenolol to treat rat alveolar macrophages stimulated by LPS. Western blot analysis demonstrated that chronic propranolol or

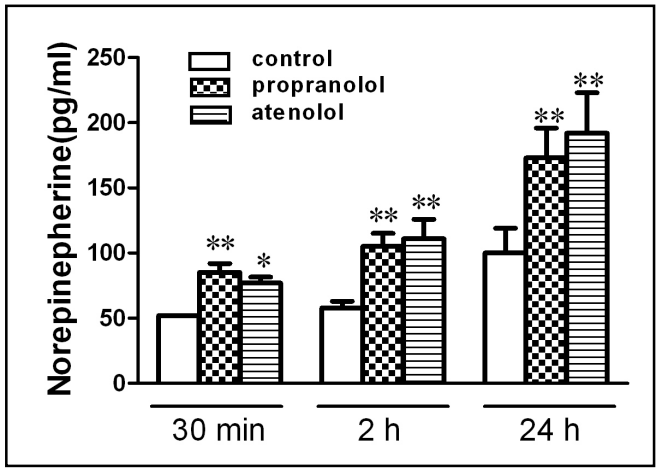

Fig. 3. Propranolol or atenolol treatment promotes the secretion of noradrenaline in rat alveolar macrophages. Noradrenaline level was detected by ELISA in the supernatants of alveolar macrophages isolated from different groups of rats stimulated with LPS (100 ng/ml) for 30 min, 2 h or 24 h. Results were presented as mean ± SEM of 6 individual rats. **P<0.01 vs. control.

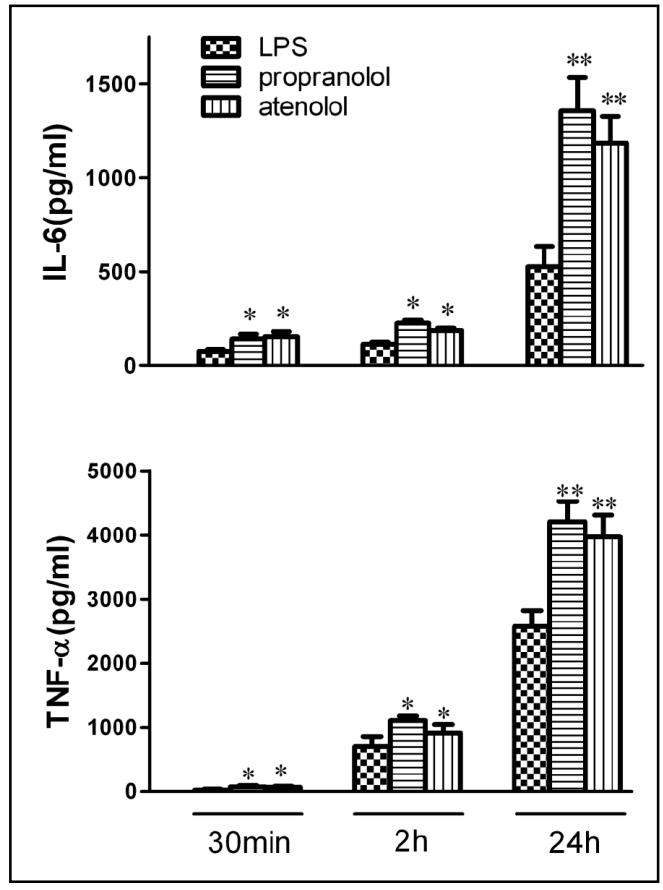

Fig. 4. Propranolol or atenolol treatment promotes the secretion of IL-6 and TNF-α in rat alveolar macrophages. IL-6 and TNF-α levels were detected by ELISA in the supernatants of alveolar macrophages isolated from different groups of rats stimulated with LPS (100 ng/ml) for 30 min, 2 h or 24 h. Results were presented as mean± SEM of 6 individual rats. *P<0.05, **P<0.01 vs. control.

atenolol treatment clearly upregulated the dopamine β-hydroxylase protein level 2 h and 24 h after LPS treatment. (Fig. 2 A, B). These data indicate that both non-selective β-adrenoreceptor antagonist and specific β_1-adrenoreceptor antagonist led to increased expression of catecholamine-generating enzymes in alveolar macrophages in response to LPS.

Chronic propranolol or atenolol treatment promote LPS-mediated noradrenaline production in rat alveolar macrophages

To investigate whether the stimulatory effect of chronic β-adrenoceptor antagonist on LPS-induced expression of dopamine β-hydroxylase in alveolar macrophages would result in the activation of noradrenaline production, we examined noradrenaline level in the cell supernatants at 30 min, 2 h and 24 h after exposure of macrophages to LPS. As shown in Fig. 3, chronic propranolol or atenolol treatment increased noradrenaline level significantly in cell supernatants at all time points, compared to the control, suggesting that β-adrenoceptor antagonist stimulates the production of catecholamine in alveolar macrophage.

Chronic propranolol or atenolol treatment promote LPS-mediated cytokine production in rat alveolar macrophages

To investigate whether the upregulation of noradrenaline secretion in alveolar macrophage by chronic β-adrenoceptor antagonist was accompanied by increased cytokine production, we examined the levels of IL-6 and TNF-α in cell supernatants at 30 min, 2 h and 24 h after incubated with LPS. Chronic propranolol or atenolol treatment increased IL-6 level at 30 min, 2 h and 24 h after exposure of macrophages to LPS, compared to macrophages from time-matched control animals (Fig. 4). In the case of TNF-α, chronic propranolol or atenolol treatment resulted in enhanced production of TNF-α at all time points after exposure of macrophages to LPS (Fig. 4).

Discussion

In the present study, we showed that chronic administration of propranolol or atenolol led to increased expression of the catecholamine generating enzyme dopamine β-hydroxylase at protein level, increased noradrenaline release, increased expression of β-adrenoceptor, and enhanced release of pro-inflammatory cytokines TNF-α, IL-6 in rat alveolar macrophage after exposure to LPS *in vitro*. These results indicate that propranolol and atenolol can cause alterations in immune response, which may be β_1-adrenoceptors subtype dependent.

β-adrenoceptor plays important roles in cardiac function, and represents one of therapeutic targets for cardiovascular diseases such as hypertension, heart failure, arrhythmias and angina [10, 11]. Many β-adrenoceptor antagonists have been clinically used for the therapy of cardiovascular diseases, which act by blocking the binding of catecholamine to β-adrenergic receptor sites [12]. However, there are some differences between the individual agents that are of clinical importance. Therefore, in this study, we chose non-selective β-adrenoreceptor antagonist propranolol and specific β_1-adrenoceptor antagonist atenolol as the target drugs to investigate whether the chronic administration of β-adrenoceptor antagonists modulates the immune response of macrophage.

Receptor up-regulation is a phenomenon of receptor-mediated adaptation well observed upon antagonist therapy [13]. Up-regulation of β-adrenoceptors has been recognized clinically as well as in experimental studies, but the reports are conflicting. For example, chronic treatment with propranolol upregulated β-adrenoceptors in rat heart [14], but such up-regulation was not observed in other studies [15-17]. Thus, the changes of β-adrenoceptor in β-adrenoceptor antagonist therapy remain controversial, in spite of its clinical significance.

In the present study, we observed that prolonged β-adrenoceptor blockade upregulated receptor expression in unstimulated alveolar macrophages. This up-regulation revealed the occurrence of subtype-specific regulation for β_1- and β_2-adrenoceptors. The expression of both β_1- and β_2-adrenoceptors was significantly increased by non-selective β-adrenoreceptor antagonist propranolol. On the other hand, a drastic increase in β_1-adrenoceptor but no change in β_2-adrenoceptor was observed in specific β_1-adrenoceptor antagonist atenolol treated rats. Such a subtype-selective modulation of β_1- and β_2-adrenoceptors should be taken into consideration because these different changes in alveolar macrophages after chronic treatment with different β-adrenoceptor antagonists may be relevant to the different responses and efficacies observed in the antagonist therapy [18].

A study from Flierl MA and his colleagues indicates that catecholamines are potent inflammatory activators of macrophages. In their study, a compensatory increase in catecholamine generating enzymes and catecholamines in macrophages has been found in adrenalectomized animals, resulting in enhanced acute inflammatory response [5]. In addition, synovial macrophages wee identified as the source of the secreted noradrenaline [19]. In the present study, we detected dopamine β-hydroxylase expression, noradrenaline levels, and cytokines production in rat alveolar macrophages without LPS stimulated. We found no significant effect of propranolol or atenolol on the expression of dopamine β-hydroxylase, the secretion of noradrenaline, IL-6 and TNF-α. Then macrophages were exposure to LPS. As expected, chronic propranolol or atenolol treatment obviously upregulated the dopamine β-hydroxylase protein level and increased noradrenaline level 2 h and 24 h after LPS treatment. These effects may be associated with reduced sympathetic tone by β-adrenoceptor antagonists, because it has been reported that β-adrenoceptor antagonists attenuate sympathetic tone [20]. These data indicate that β-adrenoreceptor antagonist stimulates alveolar macrophages to express catecholamine-generating enzymes and increase noradrenaline level.

During acute infection, lung alveolar macrophage is the primary producer of cytokines, such as TNF-α and IL-6, which contribute to the antimicrobe activity of the innate immune system [21, 22]. Furthermore, IL-6 is a pro-inflammatory cytokine that affects the secretion of several neuroendocrine hormones [23, 24]. Here, we show that non-selective β-adrenoreceptor antagonist propranolol treatment induced the production of IL-6 and TNF-α in rat alveolar macrophages exposure to LPS. The selective β_1-adrenoreceptor antagonist, atenolol, also induced a similar pattern of cytokine production.

Taken together, the increased adrenoceptor expression on alveolar macrophages, the increased levels of dopamine β-hydroxylase and noradrenaline in these cells, and increased secretion of IL-6 and TNF-α suggest that chronic administration of propranolol or atenolol promotes the local inflammatory response

stimulated by LPS. Furthermore, the subtype-specific regulation of β_1- and β_2-adrenoceptors of non-selective β-adrenoreceptor and specific β_1-adrenoceptor antagonist on alveolar macrophages indicate that β_1-adrenoreceptor mediates the upregulation of adrenergic system in alveolar macrophage. This is consistent with a recent study which reported a unique synergistic pro-inflammatory response mediated through a β_1-adrenoreceptor cAMP-dependent mechanism in LPS-challenged monocytic cells [25]. Although we could not rule out any contributions of β_2-adrenoreceptor stimulation in our study, our data suggest that this response appears largely, if not exclusively, to be mediated via a β_1-adrenoreceptor subtype mechanism. Further *in vivo* studies are needed to confirm that chronic administration of β-adrenoreceptor antagonists promote the local inflammatory response.

Acknowledgements

This work was supported by the National Natural Science Foundation of China (No. 30973531).

References

1 Marriott HM, Dockrell DH: The role of the macrophage in lung disease mediated by bacteria. Exp Lung Res 2007;33:493-505.

2 Chao J, Wood JG, Gonzalez NC: Alveolar hypoxia, alveolar macrophages, and systemic inflammation. Respir Res 2009;10:54.

3 Gonzalez NC, Allen J, Blanco VG, Schmidt EJ, van Rooijen N, Wood JG: Alveolar macrophages are necessary for the systemic inflammation of acute alveolar hypoxia. J Appl Physiol 2007;103:1386-1394.

4 Deng J, Muthu K, Gamelli R, Shankar R, Jones SB: Adrenergic modulation of splenic macrophage cytokine release in polymicrobial sepsis. Am J Physiol Cell Physiol 2004;287:C730-736.

5 Flierl MA, Rittirsch D, Nadeau BA, Chen AJ, Sarma JV, Zetoune FS, McGuire SR, List RP, Day DE, Hoesel LM, Gao H, Van Rooijen N, Huber-Lang MS, Neubig RR, Ward PA: Phagocyte-derived catecholamines enhance acute inflammatory injury. Nature 2007;449:721-725.

6 Engler KL, Rudd ML, Ryan JJ, Stewart JK, Fischer-Stenger K: Autocrine actions of macrophage-derived catecholamines on interleukin-1 beta. J Neuroimmunol 2005;160:87-91.

7 Elenkov IJ, Wilder RL, Chrousos GP, Vizi ES: The sympathetic nerve-an integrative interface between two supersystems: the brain and the immune system. Pharmacol Rev 2000;52:595-638.

8 McCourtie AS, Merry HE, Farivar AS, Goss CH, Mulligan MS: Alveolar macrophage secretory products augment the response of rat pulmonary artery endothelial cells to hypoxia and reoxygenation. Ann Thorac Surg 2008;85:1056-1060.

9 Donnelly LE, Tudhope SJ, Fenwick PS, Barnes PJ: Effects of formoterol and salmeterol on cytokine release from monocyte-derived macrophages. Eur Respir J 2010;36:178-186.

10 Brodde OE: Beta-adrenoceptor blocker treatment and the cardiac beta-adrenoceptor-G-protein(s)-adenylyl cyclase system in chronic heart failure. Naunyn Schmiedebergs Arch Pharmacol 2007;374:361-372.

11 Brodde OE, Bruck H, Leineweber K: Cardiac adrenoceptors: physiological and pathophysiological relevance. J Pharmacol Sci 2006;100:323-337.

12 Bangalore S, Messerli FH, Kostis JB, Pepine CJ: Cardiovascular protection using beta-blockers: a critical review of the evidence. J Am Coll Cardiol 2007;50:563-572.

13 Haeusler G.: Pharmacology of beta-blockers:classical aspects and recent developments. J Cardiovasc Pharmacol 1990;16:S1-S9.

14 Glaubiger G, Lefkowitz RJ: Elevated beta-adrenergic receptor number after chronic propranolol treatment. Biochem Biophys Res Commun 1977;78:720-725.

15 Baker SP, Potter LT. Effect of propranolol on beta-adrenoceptors in rat hearts. Br J Pharmacol 1980;68:8-10.

16 Baker SP, Katovich MJ: Chronic propranolol treatment decreases cardiac beta-adrenoceptors in spontaneously hypertensive rats. Eur J Pharmacol 1982;78:479-482.

17 Tan YY, Summers RJ: beta-Adrenoceptor regulation in rat heart, lung and skin after chronic treatment with (-)-tertatolol or (-)-propranolol. J Auton Pharmacol 1995;15:421-436.

18 Bouzamondo A, Hulot JS, Sanchez P, Cucherat M, Lechat P: Beta-blocker treatment in heart failure. Fundam Clin Pharmacol 2001;15:95-109.

19 Miller LE, Jüsten HP, Schölmerich J, Straub RH: The loss of sympathetic nerve fibers in the synovial tissue of patients with rheumatoid arthritis is accompanied by increased norepinephrine release from synovial macrophages. FASEB J 2000;14:2097-2107.

20 Chiladakis JA, Georgiopoulou E, Alexopoulos D: Autonomic effects of nebivolol versus atenolol in healthy subjects. Cardiovasc Drugs Ther 2004;18:469-473.

21 Jarai R, Kaun C, Weiss TW, Speidl WS, Rychli K, Maurer G, Huber K, Wojta J: Human cardiac fibroblasts express B-type natriuretic peptide: fluvastatin ameliorates its up-regulation by interleukin-1alpha, tumour necrosis factor-alpha and transforming growth factor-beta. J Cell Mol Med 2009;13:4415-4421.

22 Loppnow H, Zhang L, Buerke M, Lautenschläger M, Chen L, Frister A, Schlitt A, Luther T, Song N, Hofmann B, Rose-John S, Silber RE, Müller-Werdan U, Werdan K: Statins potently reduce the cytokine-mediated IL-6 release in SMC/MNC cocultures. J Cell Mol Med 2011;15:994-1004

23 Kageyama K, Kagaya S, Takayasu S, Hanada K, Iwasaki Y, Suda T: Cytokines induce NF-κB, Nurr1 and corticotropin-releasing factor gene transcription in hypothalamic 4B cells. Neuroimmunomodulation 2010;17:305-313.

24 Haedo MR, Gerez J, Fuertes M, Giacomini D, Páez-Pereda M, Labeur M, Renner U, Stalla GK, Arzt E: Regulation of pituitary function by cytokines. Horm Res 2009;72:266-274.

25 Grisanti LA, Evanson J, Marchus E, Jorissen H, Woster AP, DeKrey W, Sauter ER, Combs CK, Porter JE: Pro-inflammatory responses in human monocytes are beta1-adrenergic receptor subtype dependent. Mol Immunol 2010;47:1244-1254.