Modulation of natural killer cell function by alpha-adrenoreceptor-coupled signalling

Jing Xiao [1], Hui-Wei Huang [1], Yu-Ping Peng [1], Jing-Yin Bao [1], Yan Huang [1], Yi-Hua Qiu [1,2]

1 Department of Physiology, School of Medicine, Nantong University, Nantong, China
2 The Key Laboratory of Neuroregeneration of Jiangsu Province, Nantong University, Nantong, China

Jing Xiao and Hui-Wei Huang contributed equally to this study

Correspondence to: Yu-Ping Peng, Department of Physiology, School of Medicine, Nantong University 19 Qixiu Road, Nantong 226001, China.
TEL: +86-513-85051722; FAX: +86-513-85051714; E-MAIL: yppeng@ntu.edu.cn

Yi-Hua Qiu, Department of Physiology, School of Medicine, Nantong University 19 Qixiu Road, Nantong 226001, China.
TEL: +86-513-85051723; FAX: +86-513-85051876; E-MAIL: yhqiu@ntu.edu.cn

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Abstract

OBJECTIVE: Our previous work has shown that α-adrenoreceptor (α-AR)-coupled signaling modulates T lymphocyte function. Here, we investigate the expression of α_1- and α_2-ARs in natural killer (NK) cells and roles of the two subtypes of α-ARs and their coupled signals in modulation of NK cell function.

METHODS: NK cells were purified by Ficoll-Isopaque one-step gradient centrifugation and in discontinuous Percoll density gradients from splenic cells of rats. The mRNA expressions of α_1-ARs and α_2-ARs in NK cells were measured by reverse transcription-polymerase chain reaction (RT-PCR). Flow cytometry was employed to detect the cytotoxicity of NK cells.

RESULTS: NK cells expressed both α_1-AR and α_2-AR mRNAs. Phenylephrine, a selective α_1-AR agonist, increased the cytotoxicity of NK cells. This effect of phenylephrine was reduced by corynanthine, a selective α_1-AR antagonist, and was blocked by PLC inhibitor U-73122, but not by PKA inhibitor H-89. Clonidine, a selective α_2-AR agonist, also enhanced the cytotoxicity of NK cells. This action of clonidine was blocked by α_2-AR antagonist yohimbine or by PKA inhibitor H-89, but not by PLC inhibitor U-73122.

CONCLUSIONS: NK cells express α_1- and α_2-ARs. Activation of the either subtype of α-ARs augments NK cell function. This action of α_1-ARs is transduced by PLC, while α_2-AR effect is mediated by PKA signaling.

INTRODUCTION

Evidence accumulated over the last two decades indicates that the sympathetic nervous system (SNS) innervates all lymphoid organs, while catecholamines (CAs), the end products of SNS, modulate several immune parameters. CAs mediate their effects on target cells via stimulation of relevant receptors. There are two types of receptors that bind NE, namely the α-adrenoreceptors (α-ARs) and the β-ARs. Studies reveal that almost all types of lymphocytes, including T, B and natural killer (NK) cells, express β-ARs (Sanders *et al.* 1997; Kohm & Sanders, 2001; Elenkov *et al.* 2000; Yu *et al.* 2007). Maisel *et al.* (1989, 1990) confirm that lymphocyte subsets differ in their β_2-AR

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density, with NK cells having the greatest number of receptors. In contrast to the well established presence of β-ARs on lymphocytes, only a few studies report the presence of α-ARs on the lymphocytes (Tayebati *et al.* 2000; Sanders, 2002; Bao *et al.* 2007; Pešić *et al.* 2009).

Ligand binding studies reveal that human NK (CD16+) cells express β_2-, α_1-, α_2- but not β_1-ARs (Jetschmann *et al.* 1997). NK cells play an important role in tumor recognition and elimination by acting as effectors of the innate immune surveillance (Trinchieri, 1989). In vivo β-adrenergic stimulation suppresses NK activity and compromises resistance to tumor metastasis in rats (Shakhar & Ben-Eliyahu, 1998). Stresses including surgery and cold suppress the NK cell cytotoxicity which is reversed by the blockade of β-ARs (Jiang *et al.* 2004; Benish *et al.* 2008; Glasner *et al.* 2010). Recent studies including our laboratory (Peng *et al.* 2004; Goldfarb *et al.* 2009) show CAs exerts a suppressive action in modulating NK cell cytotoxicity mainly through β-ARs. It indicates β-ARs play an important role in NK cell function. However, effects of α-AR stimulation on immune cells are not as clear as those of β-AR stimulation, although some studies including our previous ones propose that α-ARs mediate certain changes of immune parameters (García *et al.* 2003; Peng *et al.* 2004). In addition, evidence from our laboratory (Bao *et al.* 2007) showed that T lymphocytes from rat mesenteric lymph nodes expressed α-AR subtypes, and α_2-ARs participated in modulation of T cell function. Still, little is known regarding the expression of α-ARs in murine NK cells and the role of it involved in modulating NK cell cytotoxicity.

As mentioned above, α- and β- ARs exist on lymphocytes, but the signal-transduction pathways coupled to ARs are complex. As is well known, the classical pathway of β-ARs-G protein-AC-cAMP-PKA is involved in the lymphocyte modulation. Stimulation of the β_2-AR on NK cells is linked to increased intracellular cAMP (Benish *et al.* 2008). Our previous study showed that α-AR-coupled signal transduction mechanisms mediated the lymphocyte function and apoptosis (Bao *et al.* 2007; Jiang *et al.* 2009). However, it is unclear whether an α-AR-coupled signal transduction pathway is implicated in the modulation of NK cell cytotoxicity.

This study was undertaken to detect the expression of α_1- and α_2-AR in NK cells. In addition, we explored the roles of the α-ARs subtypes and the intracellular signal transduction pathways coupled with α-ARs in mediating the modulation of NK cell cytotoxicity against YAC-1 so as to well comprehend neural-endocrine-immune interactions.

MATERIALS AND METHODS

Cell separation and purification

Spleen were from Sprague-Dawley rats (Center of Experimental Animals, Nantong University, China) weighing 180–250 g with males and females. NK cells from the spleen were isolated as described by Konjevi *et al.* (1997). Briefly, the spleen was harvested by celiotomy, and single cell suspensions were obtained by gently squeezing the spleen. Splenic mononuclear cells were isolated by Ficoll-Isopaque one-step gradient centrifugation. The interface mononuclear cells were harvested and purified in discontinuous Percoll density gradients. The cell suspensions were washed twice with the RPMI 1640 culture medium and resuspended in RPMI 1640 medium containing 10% heat-inactivated calf serum. The cell suspensions were incubated in a culture flask at 37 °C in 5% CO_2 for 2 h. Non-adherent cells were collected and resuspended in the complete culture medium at the final concentration of 4×10^6 cells/ml. Drugs used in this study, i.e., corynanthine, yohimbine, U-73122 and H-89 (all from Sigma), were first added to the cell suspensions of 4×10^6 cells/ml respectively according to the different experimental aims. Phenylephrine and clonidine (Sigma) were then added to the suspensions 30 min later respectively. Control groups were not exposed to drugs.

Reverse transcription polymerase chain reaction

The expression of α_1-AR and α_2-AR mRNAs was examined using reverse transcription polymerase chain reaction (RT-PCR) method. Total RNA in the purified NK cells was extracted with Trizol reagent (Bio Basic Inc.) according to the manufacturer's instructions. The concentration of RNA was quantitated by absorbance at 260 and 280 nm. Single-stranded cDNA was prepared using 5 μg of total RNA, 200 U MMLV reverse transcriptase (Fermentas), 4 μl MMLV buffer (Promega), 2 μl of 10 mM dNTP (Promega), 1 μl oligo (dT)18 (Amresco) and 500 μU Rnasin (Amresco) in 0.5 μl solution, and incubated for 90 min at 42 °C. An amplification of the resulting cDNA sequence was carried out by using PCR. And Oligonucleotide sequences of primers used in PCR for amplification of specific fragments were 5'-GCTCCTTCTACATCCCGCTCG-3' (sense) and 5'-AGGGGAGCCAACATAAGATGA-3' (antisense) for α_1-AR gene, 5'-CTGGCAGCCGTGGTGGGTTTCCTC-3' (sense) and 5'-GTCGGGCCGGCGGTAGAAAGAGAC-3' (antisense) for α_2-AR gene. The predicted sizes of the PCR-amplified products were 300 bp for α_1-ARs, and 426 bp for α_2-ARs. The conditions for the reaction were as follows: the tubes were incubated in a thermal cycler at 95 °C for 5 min, 94 °C for 1 min, 59 °C (α_1-ARs)/62 °C (α_2-ARs) for 1 min, 72 °C for 1 min (36 cycles) and 72 °C for 5 min, then held at 4 °C. The amplified products were resolved by electrophoresis in 1.5% agarose gel, stained with ethidium bromide. The level of each PCR product was semi-quantitatively evaluated by an image analysis system (ShineTech GelAnalyse 2.3).

Flow cytometric assay for assessment of NK cell cytotoxicity

The flow cytometric cytotoxicity assay of NK cells was performed as described by Papadopoulos *et al.* (1994). Effector cells (NK cells) were separated and purified as mentioned above. Target cells for detection of NK cell cytotoxicity were YAC-1 cell line (Shanghai Institute of Biochemistry and Cell Biology, Chinese Academy of Sciences), a Moloney leukemia virus induced mouse lymphoma, with noted sensitivity to NK cells. The YAC-1 cells were maintained in continuous suspension culture in the complete culture medium at a concentration of about 8×10^5 cells/ml at 37 °C in a humidified 5% CO_2 incubator. All cultures were split 24 h before use to ensure that the YAC-1 cells were in an exponential growth phase during the assays. The experiments employed two fluorescent stains, calcein acetoxymethyl (CAM, from CALBIOCHEM) and ethidium homodimer-1 (EH-1, from Fluka). CAM, which readily enters cells and is converted by intracellular esterase activity to calcein that produces an intense green (530 nm) signal, was firstly added to the YAC-1 cells at a concentration of 100 nM, which was incubated for 15 min at 37 °C in 5% CO_2, protected from light. After labeling, the YAC-1 cells were washed twice, counted and adjusted to 4×10^5/ml. Mixtures of the stained YAC-1 cells (0.5 ml of 4×10^5/ml) and the NK cells (0.5 ml of 4×10^6/ml) were incubated at 37 °C in a humidified 5% CO2 incubator for 3 h. Another mixtures of the stained YAC-1 cells (0.5 ml) and the complete culture medium (0.5 ml) were performed in parallel to serve as control for assessment of spontaneously dead YAC-1 cells. EH-1, which binds to DNA of dead cells and emits red fluorescence (617 nm), was then added to the mixtures at a concentration of 200 nM for 15 min at room temperature in the dark. Four groups of cells were thus identified. Intact NK cells were non-fluorescent, dead NK cells emitted red fluorescence, living YAC-1 cells exhibited green fluorescence and dead YAC-1 cells were characterized by double (green-red) fluorescence. Flow cytometry was performed with a FACSCalibur (BD Biosciences) equipped with an argon laser operating at 488 nm. Two parameter dot plots were obtained with cellQuest software (BD Biosciences). In the each tested sample, the spontaneously dead YAC-1 cells were subtracted from the total dead YAC-1 cells and the NK cell cytotoxicity was expressed as the percentage of the specifically dead YAC-1 cells relative to the total YAC-1 cells.

Statistical analysis

The data were expressed as mean ± standard deviation. Statistical analysis was carried out with Statistics Package for Social Science (10.0, USA). The data were subjected to the one-way analysis of variance, followed by Student-Newman-Keul's test to compare the data of all groups relative to each other. Differences were considered statistically significant at $p<0.05$.

RESULTS

Expression of α_1-AR and α_2-AR mRNAs in NK cells

On the electrophoretic gels of PCR-amplified products for α_1-AR and α_2-AR mRNAs of NK cells, a 300-bp band (α_1-AR mRNA) and a 426-bp band (α_2-AR mRNA) were clearly seen (Figure 1). These molecular weights were consistent with the predicted sizes of the PCR-amplified products for α_1-AR mRNA and α_2-AR mRNA. The data showed that NK cells were able to express the two subtypes of α-ARs, α_1-ARs and α_2-ARs.

α_1-AR agonist phenylephrine increases cytotoxicity of NK cells, and this effect is reduced by α_1-AR antagonist corynanthine

NK cells were pretreated with phenylephrine (10^{-8}, 10^{-7}, 10^{-6} and 10^{-5} M). Compared with the group without phenylephrine treatment, the phenylephrine-treated NK cell cytotoxicity was increased significantly, no matter which concentration of phenylephrine mentioned above was used (Figure 2).

Two concentrations of phenylephrine (10^{-7} and 10^{-5} M) were used and for each concentration of phenylephrine, three concentrations of corynanthine (10^{-8}, 10^{-7} or 10^{-6} M and 10^{-6}, 10^{-5} or 10^{-4} M, respectively) acted with it on the NK cells. As exhibited above, phenylephrine alone caused an increase in NK cell cytotoxicity (Figure 3). The cotreatment of the NK cell with corynanthine and phenylephrine resulted in a reduction of NK cell cytotoxicity in comparison with that of phenylephrine exposure alone, although they did not completely reach the level of the control lacking any treatment (Figure 3).

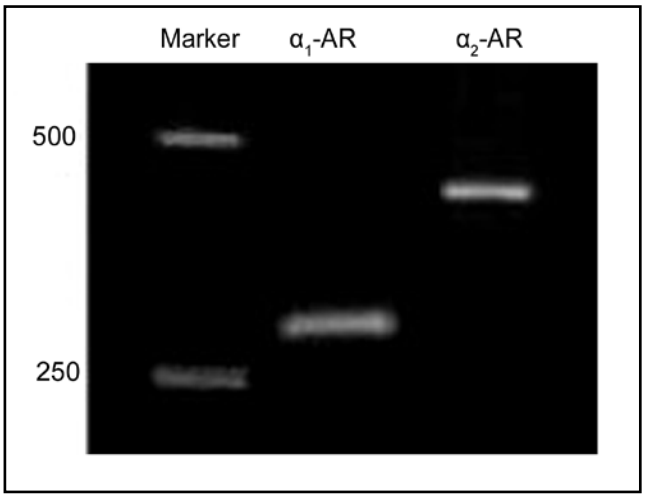

Fig. 1. NK cells express α_1-AR and α_2-AR mRNAs. NK cells were separated from spleen of rats and purified by Ficoll-Isopaque one-step gradient centrifugation and in discontinuous Percoll density gradients. RT-PCR was used to detect α_1-AR and α_2-AR mRNAs in the purified NK cells. The electrophoretic bands of 300-bp for α_1-AR mRNA and 426-bp for α_2-AR mRNA are clearly shown and the molecular weights are consistent with the predicted sizes of the PCR-amplified products.

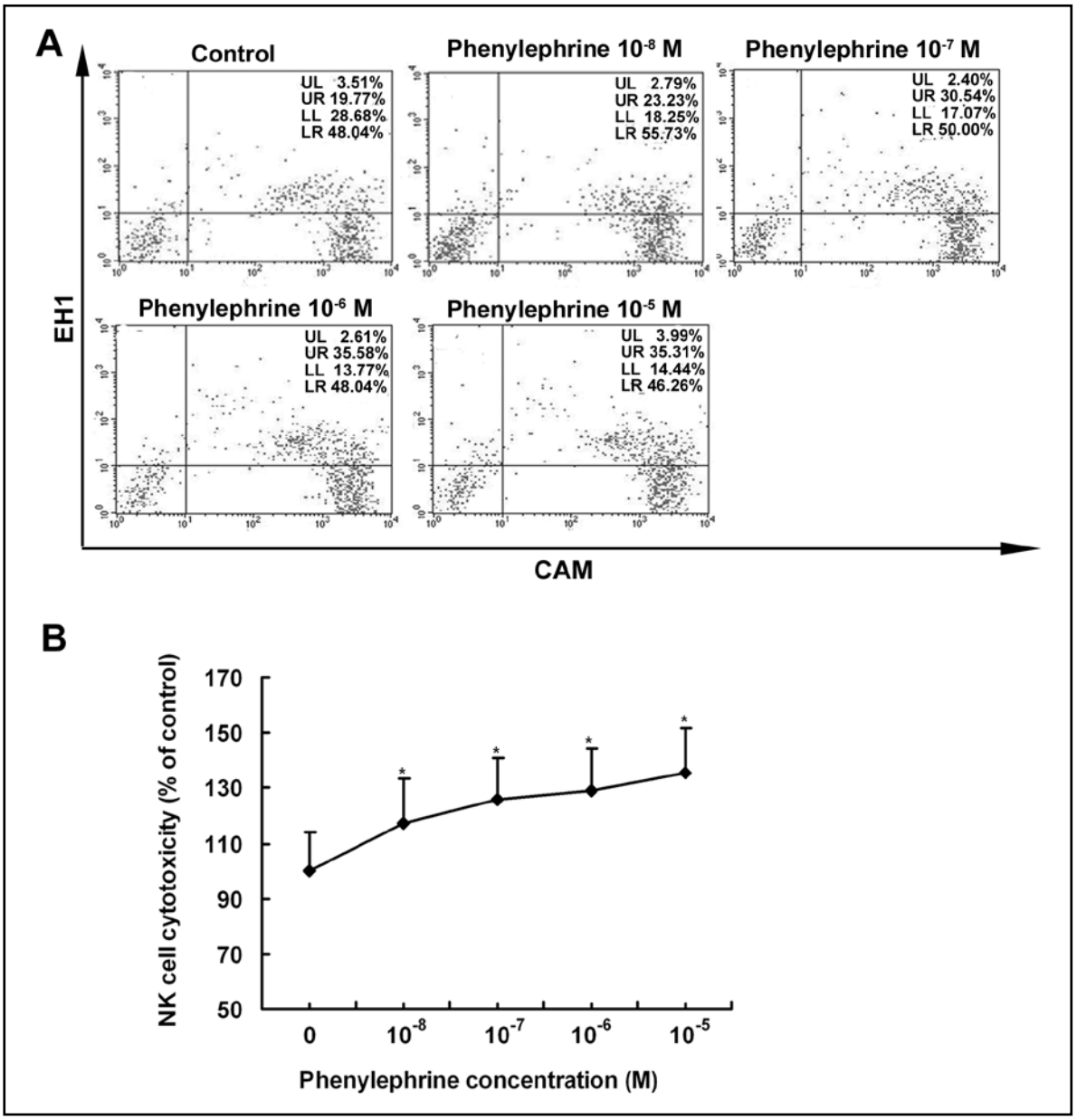

Fig. 2. Activation of α_1-AR increases the cytotoxicity of NK cells. Purified NK cells were exposed to α_1-AR agonist phenylephrine. Flow cytometric assay was used to measure the cytotoxicity of NK cells against YAC-1. (A) is a representative diagram showing fluorescent dot images reflecting cytotoxicity of NK cells. In these plots the abscissa is the log scale of green fluorescence and the ordinate is the log scale of red fluorescence. "UL" represents the upper left plot and reflects the number of dead NK cells labeled by EH-1; "UR" is the upper right plot and reflects the number of dead YAC-1 cells stained by both CAM and EH-1; "LL" denotes the low left plot and reflects the number of intact NK cells labeled by neither CAM nor EH-1; "LR" indicates the low right plot and reflects the number of living YAC-1 cells stained by CAM. (B) is a statistical graph of eight independent experiments. In each tested sample, the spontaneously dead YAC-1 cells were subtracted from the total dead YAC-1 cells and the NK cell cytotoxicity was expressed as the percentage of the specifically dead YAC-1 cells relative to the total YAC-1 cells. NK cell cytotoxicity=$[(UR/(UR+LR))_{killed}-(UR/(UR+LR))_{spontaneous}]\times100\%$. The values are expressed as percentages of control (set at 100%). *$p<0.05$, compared with the zero point (without phenylephrine treatment).

PLC inhibitor U-73122 blocks the enhanced cytotoxicity of NK cells by α_1-AR agonist phenylephrine

The cytotoxicity of NK cells treated with U-73122 (10^{-8}, 10^{-7} or 10^{-6} M) and phenylephrine (10^{-7} M) was decreased compared with that of phenylephrine treatment alone and returned to the level of control without treatment (Figure 4). The results showed that U-73122 blocked the enhanced cytotoxicity of NK cells induced by phenylephrine.

PKA inhibitor H-89 does not alter the increased NK cell cytotoxicity by α_1-AR agonist phenylephrine

NK cells were co-treated with PKA inhibitor H-89 (10^{-8}, 10^{-7} or 10^{-6} M) and phenylephrine (10^{-7} M). Compared with control lacking any treatment, the NK cell cytotoxicity with the phenylephrine exposure alone increased by 45.32%, with a significant difference. After the co-exposure to H-89 and phenylephrine, the NK cell cytotoxicity was still enhanced relative to the con-

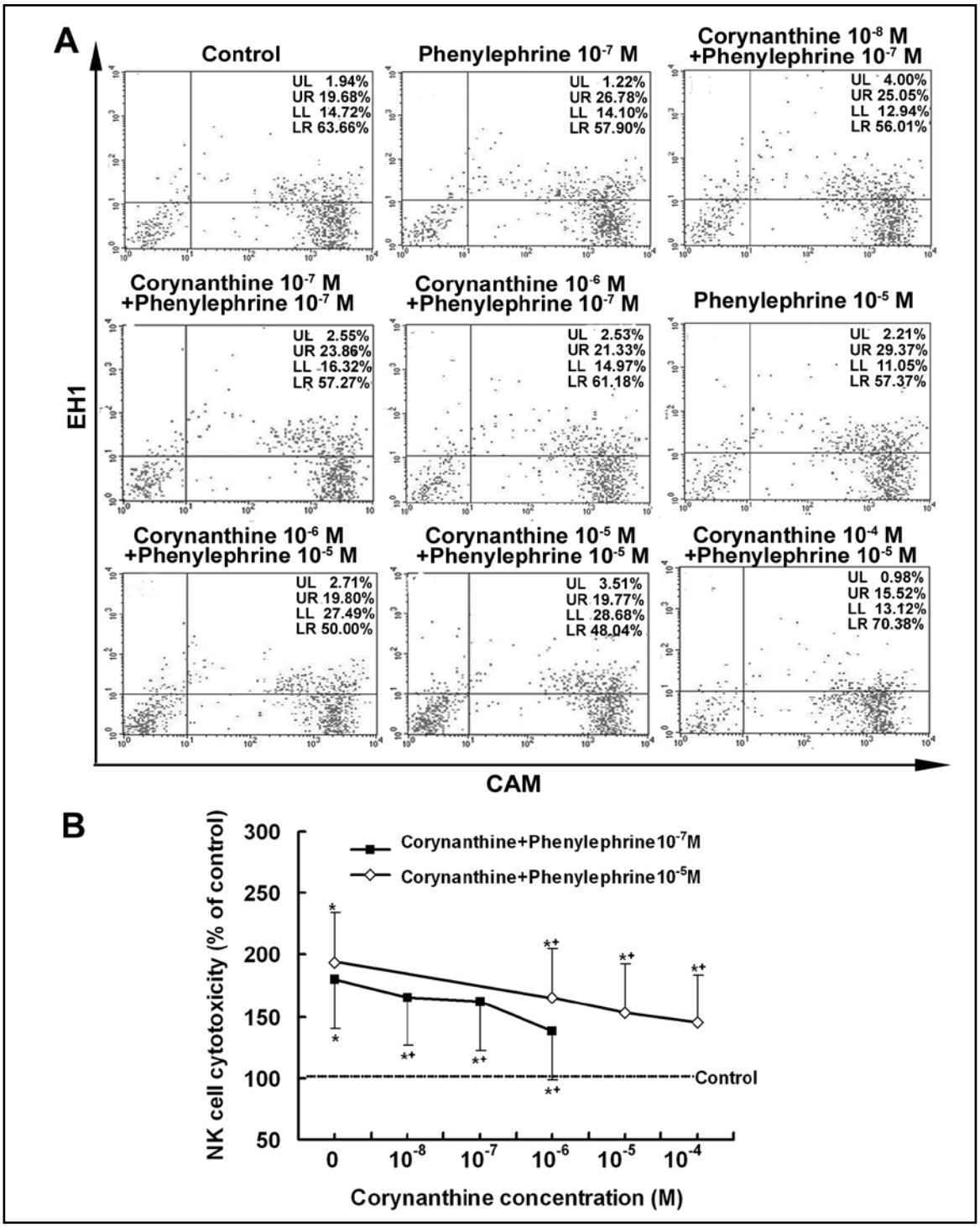

Fig. 3. Effects of α_1-AR agonist and antagonist on the cytotoxicity of NK cells. NK cells were pretreated with α_1-AR agonist phenylephrine or antagonist corynanthine plus phenylephrine. In the procedure, two doses of phenylephrine (10^{-7} and 10^{-5} M) were used and for each dose of phenylephrine, three concentrations of corynanthine acted with it on the NK cells. The enhanced cytotoxicity of NK cells induced by phenylephrine was reduced by corynanthine. (A) is a representative diagram of flow cytometry showing fluorescent dot images reflecting cytotoxicity of NK cells against YAC-1; (B) is a statistical graph of eight separate experiments. The design and calculation is the same as that of Fig. 2. *p<0.05, compared with the control lacking drug exposure; +p<0.05, compared with the zero point of corynanthine (that is, the point of phenylephrine treatment alone).

trol without treatment. The enhanced percentages than the control were 42.22%, 41.13% and 40.54% for H-89 of 10^{-8}, 10^{-7} and 10^{-6} M, respectively, plus phenylephrine. These data were not significantly different from that of phenylephrine exposure alone.

α_2-AR agonist clonidine increases cytotoxicity of NK cells, and this effect can be blocked by α_2-AR antagonist yohimbine

The treatment of NK cells with clonidine (10^{-8}, 10^{-7}, 10^{-6} and 10^{-5} M) evoked an increase in the cytotoxicity

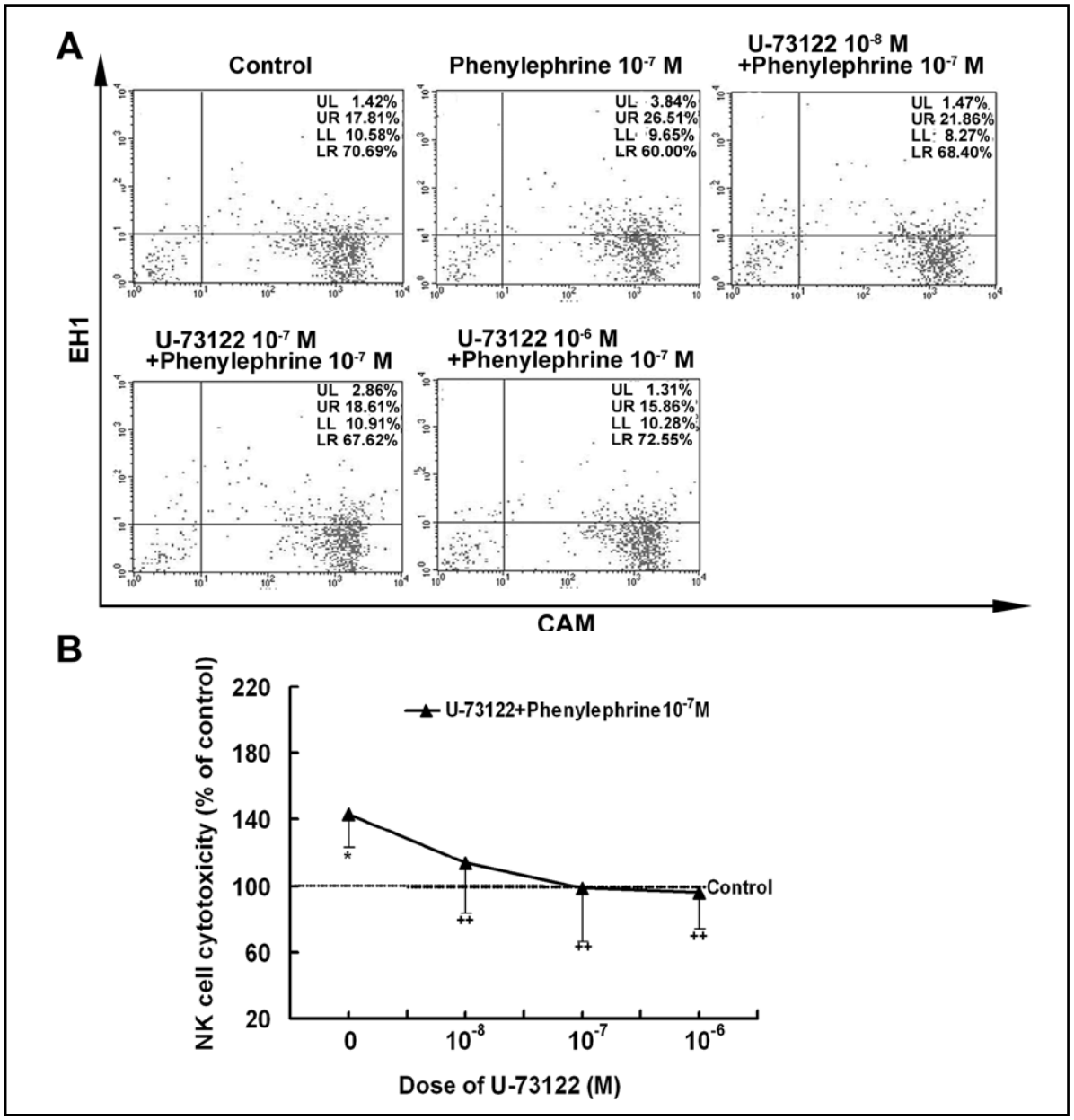

Fig. 4. PLC inhibitor U-73122 blockes the enhanced cytotoxicity of NK cells induced by phenylephrine. In this experiment, three doses of U-73122 acted with phenylephrine 10^{-7} M on the NK cells. (A) is a representative diagram of flow cytometry showing fluorescent dot images reflecting cytotoxicity of NK cells against YAC-1; (B) is a statistical graph of eight separate experiments. The arrangement and calculation is similar to that of Fig. 2. *p<0.05, compared with the control group lacking any drug treatment; ++p<0.01, compared with the zero point of U-73122 (that is, the point of phenylephrine treatment alone).

of NK cells, no matter which concentration of clonidine mentioned above was used (Figure 5).

Two concentrations of clonidine (10^{-7} and 10^{-5} M) were used and for each concentration of clonidine, three concentrations of yohimbine (10^{-8}, 10^{-7} or 10^{-6} M and 10^{-6}, 10^{-5} or 10^{-4} M, respectively) acted with it on the NK cells. As shown above, treatment with clonidine alone led to an enhancement in NK cell cytotoxicity (Figure 6). However, after the co-exposure to yohimbine and clonidine, the NK cell cytotoxicity was decreased relative to that of clonidine treatment alone and returned to the level of control lacking any treatment (Figure 6).

PLC inhibitor U-73122 does not alter the increased NK cell cytotoxicity by α_2-AR agonist clonidine

NK cells were co-treated with PLC inhibitor U-73122 (10^{-8}, 10^{-7} or 10^{-6} M) and clonidine (10^{-7} M). Clonidine elevated the NK cell cytotoxicity by 45.51% in comparison to the control lacking any treatment, with a significant difference. The NK cell cytotoxicity with the co-exposure to U-73122 and clonidine was still increased compared with the control without treatment. The increased percentages than the control were 41.97%, 37.91% and 41.70% for U-73122 of 10^{-8}, 10^{-7} and 10^{-6} M, respectively, and clonidine. These data did not remarkably differ from that of clonidine exposure alone.

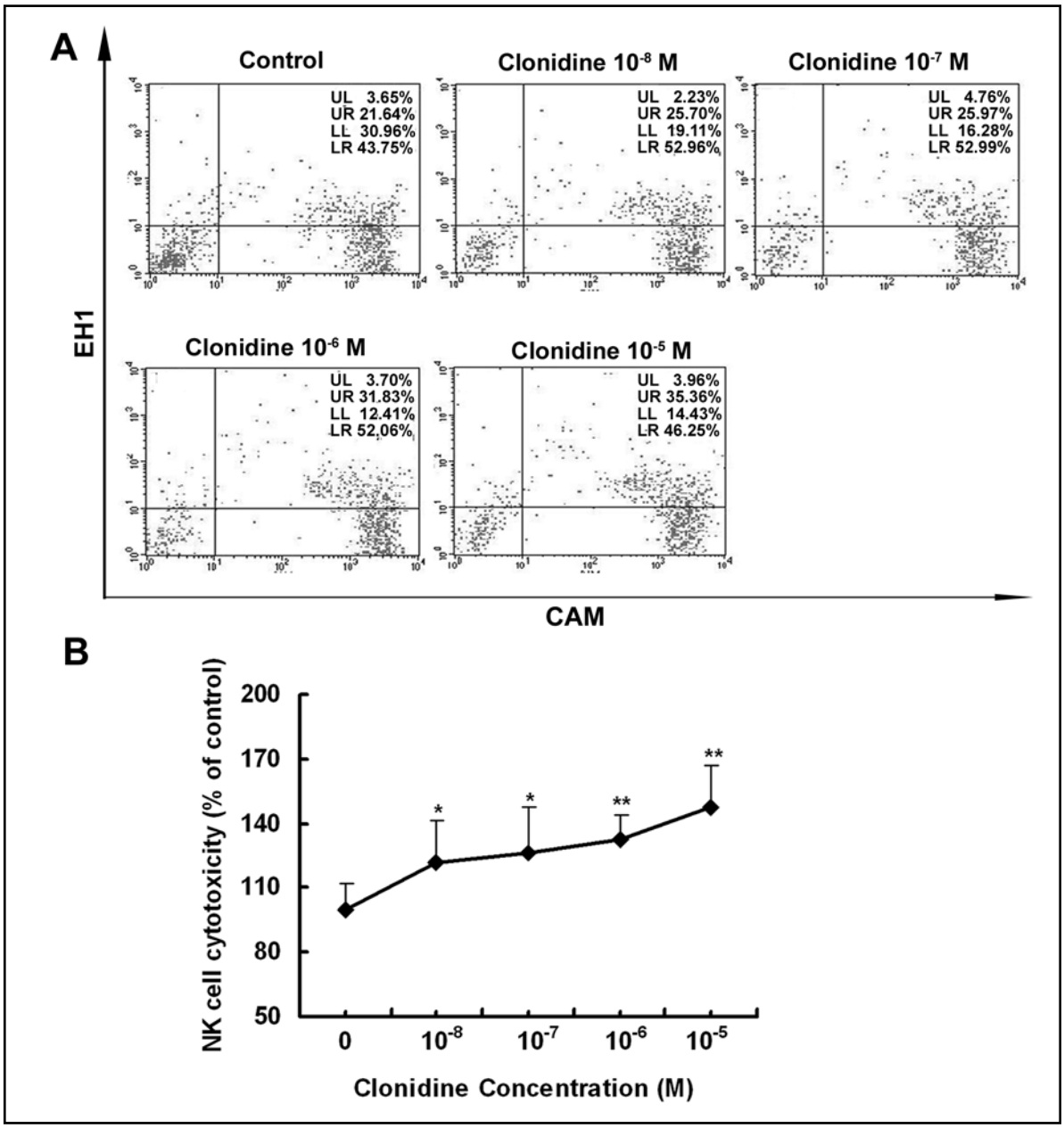

Fig. 5. Stimulation of α_2-AR increases the cytotoxicity of NK cells. Purified NK cells were exposed to α_2-AR agonist clonidine. Flow cytometric assay was employed to detect the cytotoxicity of NK cells against YAC-1. (A) is a representative diagram showing fluorescent dot images reflecting cytotoxicity of NK cells; (B) is a statistical graph of eight separate experiments. The panel and calculation is the same as that of Fig. 2. *p<0.05, **p<0.01, compared with the zero point (lacking clonidine treatment).

PKA inhibitor H-89 abolishes the increased NK cell cytotoxicity by α_2-AR agonist clonidine

As exhibited above, clonidine (10^{-7} M) alone elicited an increase in the cytotoxicity of NK cell relative to those of control lacking any treatment (Figure 7). After the co-exposure to H-89 (10^{-8}, 10^{-7} and 10^{-6} M) and clonidine (10^{-7} M), NK cell cytotoxicity was reduced compared with that of clonidine exposure alone and returned to the level of control without treatment (Figure 7), indicating that H-89 can abolish the increased NK cell cytotoxicity induced by clonidine.

DISCUSSION

Jetschmann *et al.* (1997) have reported the expression of α_1-ARs and α_2-ARs in human NK (CD16+) cells. However, no direct evidence has indicated the expression of α-ARs in murine NK cells. The present study showed that NK cells expressed the mRNA of α_1- and α_2-AR, demonstrating the presence of α_1-ARs and α_2-ARs on murine NK cells. It not only supports the observation mentioned above (Jetschmann *et al.* 1997), but also extends the fact of NK cells expressing α-AR subtypes.

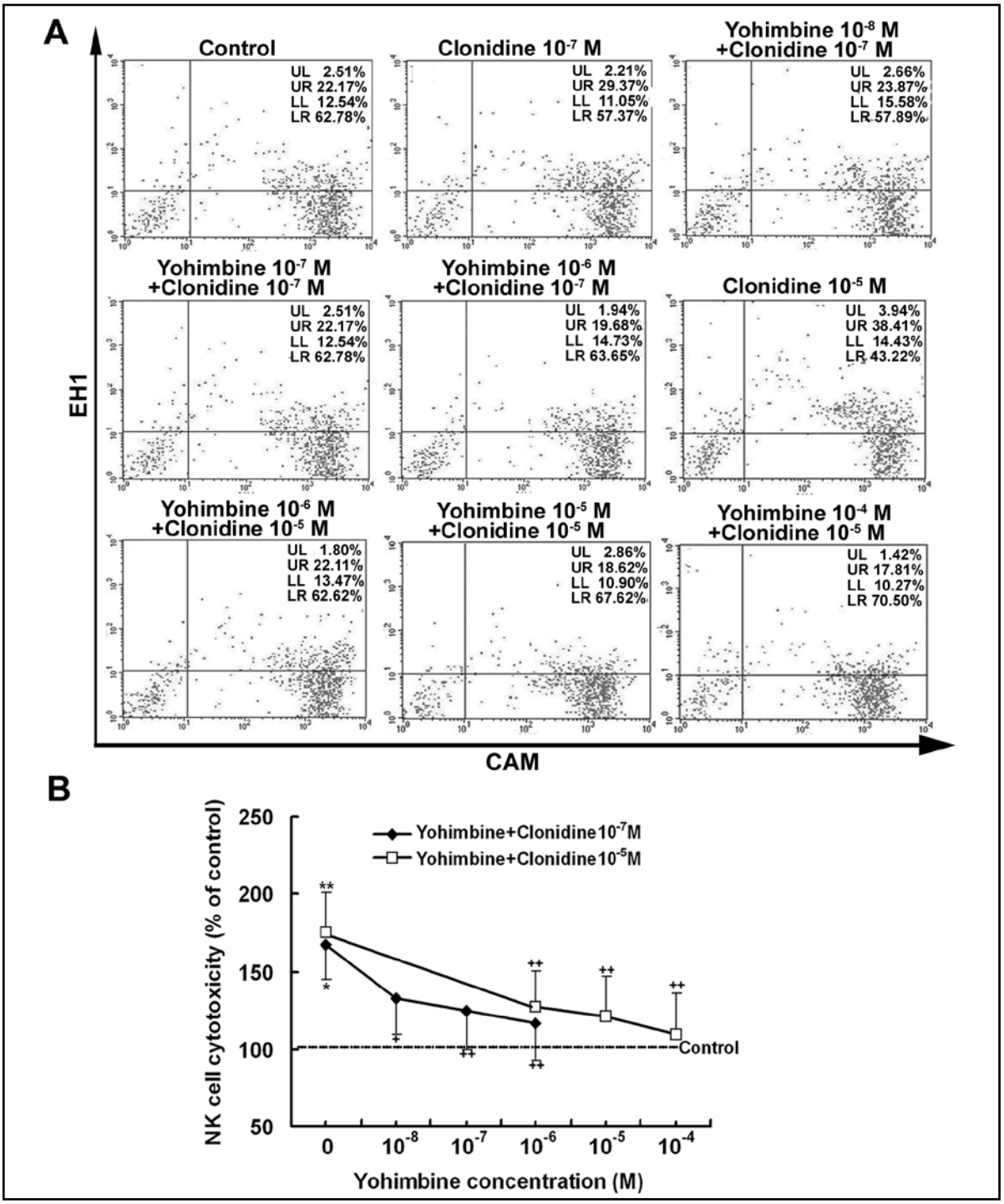

Fig. 6. Effects of α_2-AR agonist and antagonist on the cytotoxicity of NK cells. NK cells were pretreated with α_2-AR agonist clonidine or antagonist yohimbine plus clonidine. In the procedure, two doses of clonidine (10^{-7} and 10^{-5} M) were used and for each dose of clonidine, three concentrations of yohimbine acted with it on the NK cells. Yohimbine blocked the increased cytotoxicity of NK cells induced by clonidine. (A) is a representative diagram of flow cytometry showing fluorescent dot images reflecting cytotoxicity of NK cells against YAC-1; (B) is a statistical graph of eight independent experiments. The design and calculation is similar to that of Fig. 2. *p<0.05, **p<0.01, compared with the control without any drug treatment; +p<0.05, ++p<0.01, compared with the zero point of yohimbine (that is, the point of clonidine treatment alone).

NK cells are one of subpopulations of lymphocytes and they are believed to play a significant role in cellular resistance to viral disease and malignancy (Pross & Lotzova, 1993), which are named as the cytotoxicity of NK cells. Some evidences including the previous work in our laboratory have revealed that norepinephrine attenuates the cytotoxicity of NK cells and this depressive effect is abolished by antagonist of β-ARs (Jiang *et al.* 2004; Peng *et al.* 2004; Benish *et al.* 2008; Glasner *et al.* 2010). These results suggest that the inhibition of the NK cell cytotoxicity by NE is mediated mainly via β-ARs. Nevertheless, the functional significance of

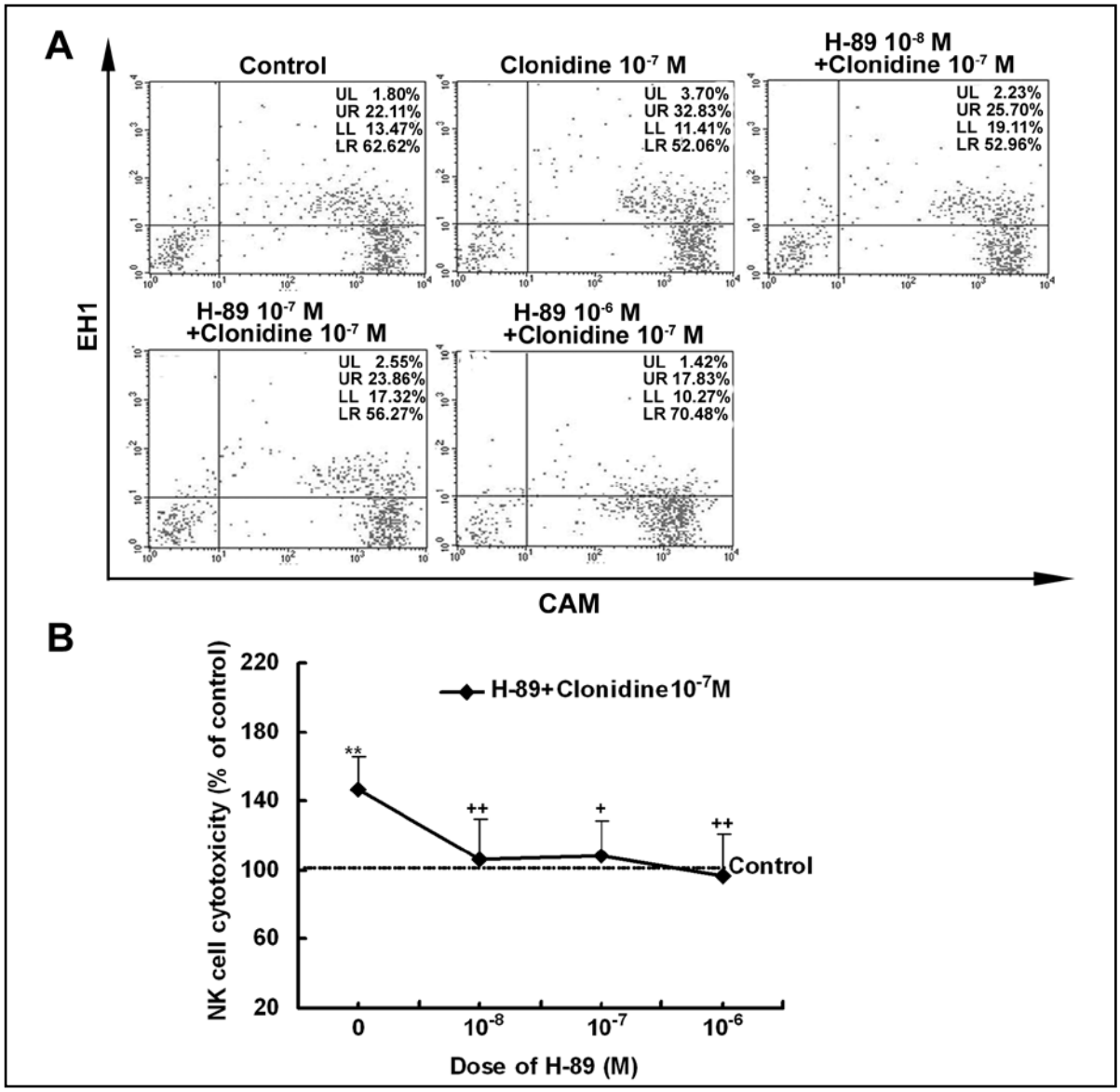

Fig. 7. Influences of PKA inhibitor H-89 on the increased cytotoxicity of NK cells induced by clonidine. In this experiment, three concentrations of H-89 acted with clonidine 10^{-7} M on the NK cells. (A) is a representative diagram of flow cytometry showing fluorescent dot images reflecting cytotoxicity of NK cells against YAC-1; (B) is a statistical graph of eight separate experiments. The arrangement and calculation is the same as that of Fig. 2. $^{**}p<0.01$, compared with the control group without any drug treatment; $+p<0.05$, $++p<0.01$, compared with the zero point of H-89 (that is, the point of clonidine treatment alone).

α-ARs on NK cells is studied much less than that of β-ARs and still remains obscure.

In the present study, phenylephrine (10^{-8} to 10^{-5} M), a selective α_1-AR agonist, and clonidine (10^{-8} to 10^{-5} M), a selective α_2-AR agonist, could both increase the cytotoxicity of NK cells. These results strongly demonstrate that stimulation of either α_1-AR or α_2-AR increases the cytotoxicity of NK cells. We observed that the enhanced cytotoxicity of NK cells induced by phenylephrine was reduced by corynanthine, an antagonist of α_1-AR. Moreover, the increased cytotoxicity of NK cells induced by clonidine was completely abolished by the preincubation of NK cells with α_2-AR antagonist yohimbine. These results demonstrate that both α_1-AR and α_2-AR on NK cells are potently implicated in the enhanced modulation of NK cell cytotoxicity. A bidirectional cross-talk has been found between α_1-AR and β_2-AR and CAs upregulate the expression of α_1-AR in the immune system via binding to β_2-AR (Kavelaars 2002; Copik *et al.* 2009). The phenomena that α_1-AR antagonists did not completely block the increased NK cell cytotoxicity by phenylephrine propose that an interaction may exist between the AR subtypes in accomplishing their modulation of NK cell function. Previous studies suggest that the suppressive effect of NE on the NK cell cytotoxicity is mediated mainly via β-ARs (Peng *et al.* 2004; Jiang *et al.* 2004; Benish *et al.* 2008; Glasner *et al.* 2010). On the basis of the current findings, we propose that α_1-AR- or α_2-AR-mediated immunoenhancement, no matter what mechanisms

are involved, is important as a positive modulation of the immune system to maintain the homeostasis of immune response.

In general, α_1-ARs couple to Gq proteins to activate PLC that increases IP3 and DAG, a promoter of PKC; α_2-ARs activate inhibitory G proteins and therefore suppress AC and diminish intracellular cAMP (Elenkov *et al.* 2000). In the current study, we found that the PLC inhibitor U-73122 blocked the enhanced cytotoxicity of NK cells by phenylephrine, but did not alter the increased NK cell cytotoxicity by clonidine. The reverse effect of U-73122 on phenylephrine action could be achieved through suppression of the phenylephrine-stimulated α_1-ARs-PLC pathway. The findings suggest that a classical signal transduction pathway coupled with α_1-ARs via PLC participates in the α_1-AR-mediated regulation of NK cell cytotoxicity. However, PKA inhibitor H-89 abolished the increased NK cell cytotoxicity by clonidine, but did not alter the enhanced cytotoxicity of NK cells by phenylephrine. Our current results demonstrate that a classical signal transduction pathway coupled with α_2-ARs via PKA is involved in the α_2-AR-mediated modulation of NK cell cytotoxicity.

In summary, NK cells express two kinds of subtypes of α-ARs, α_1-ARs and α_2-ARs. Either stimulation of α_1-ARs with phenylephrine or activation of α_2-ARs with clonidine augments NK cell function. These enhanced effects on the NK cell function are reduced by corynanthine and blocked by yohimbine respectively. The modulation of NK cell function by α_1-AR activation is mediated via intracellular PLC signal transduction pathways in NK cells, and the regulation of NK cell function by α_2-AR activation is realized through PKA pathways in NK cells. These findings suggest that both the intracellular signal-transduction pathways of α_1-ARs-PLC and α_2-ARs-PKA participate in mediating the modulation of NK cell function.

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