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RESEARCH ARTICLE

The inotropic effects of ammonia on isolated perfused rat hearts and the mechanisms involved

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SUMMARY

Ammonia (NH_3) is a common exogenous gas in the atmosphere, as well as an endogenous chemical produced by amino acid catabolism and other pathways *in vivo*. Physiological and pathophysiological roles of NH_3 in the nervous system have been studied. Recently, endogenous NH_3 has been suggested to be a gas transmitter. However, so far the role of NH_3 in cardiovascular functions has not been reported. The present study was designed to investigate the inotropic effects of NH_3 on isolated perfused rat hearts and the possible mechanisms involved in these effects. The results showed that NH_3 had a positive inotropic effect in a concentration-dependent manner and produced a higher positive effect than NaOH and NH_4Cl. At low concentrations, the effect of NH_3 on cardiac function was caused by NH_3 molecules; at high concentrations, the effect of NH_3 on hearts may be partly correlated with a change of pH value, but was mainly caused by NH_3 molecules. The mechanisms involved in the NH_3-induced positive inotropic effect may be related to the ATP-sensitive K^+ (K_{ATP}) channel and the nitric oxide (NO)–cyclic GMP (cGMP) signaling pathway. In addition, at a concentration of 1.5 mmol l^{-1}, NH_3 significantly increased the activity of creatine kinase (CK) and lactate dehydrogenase (LDH) in the coronary perfusate and decreased the activity of Na^+,K^+-ATPase and Ca^{2+},Mg^{2+}-ATPase in the hearts. These results indicate that NH_3 at physiological or low concentrations may play a modulatory role in heart function, but at high concentrations had a damaging effect on isolated rat hearts.

Key words: ammonia, inotropic effect, signaling pathways, NaOH, NH_4Cl, rat.

INTRODUCTION

Ammonia (NH_3) is a naturally occurring chemical in the atmosphere, as well as an essential man-made chemical. At room temperature, NH_3 is a colorless, pungent-smelling gas and is lighter than air. NH_3 is a major product of amino acid catabolism and profoundly affects the function of neurons and the vasculature (Li et al., 2009). With the exception of developing conceptuses and growing animals (Wu et al., 2008; Wu et al., 2009), amino acids are not stored in tissues and must be degraded to yield NH_3 in a cell-specific manner. NH_3 is also produced in the human gut by bacteria. In addition, degradation of purines and pyrimidines generates NH_3, particularly in exercising skeletal muscle (Suryawan et al., 2009).

NH_3 occurs in micromolar concentrations in human blood. It is important to note that individual blood NH_3 levels increase considerably as a result of dietary fluctuations or exercise (Dimski, 1994; Greenhaff et al., 1991). Pathophysiologically, high concentrations ($\geq$3 mol l^{-1}) of NH_3 dilate human cerebrovascular smooth muscle (Andersson et al., 1981). Hyperammonemia is an important factor in the development of hepatic encephalopathy and has been suggested to trigger expression of inducible nitric oxide synthase (iNOS) in astroglial cells (Butterworth, 2002; Suárez et al., 2002) and to have dose-dependent effects on monoamine synthesis. NH_3 reduction is the target for therapy of hepatic encephalopathy (Olde Damink et al., 2002; Rudman et al., 1973; Ytrebø et al., 2006). High NH_3 levels can be lethal to the central nervous system (Wu and Morris, 1998). High NH_3 levels also induce changes in *N*-methyl D-aspartate (NMDA) and γ-amino butyric acid (GABA) receptors and causes downregulation in astroglial glutamate transporter molecules (Fan and Szerhb, 1993; Bender and Norenberg, 2000). In addition, like nitric oxide (NO) and hydrogen sulfide (H_2S), NH_3 may be a contributing factor in Alzheimer's disease (Seiler, 2002). A correlation between arterial NH_3 concentration and brain glutamine content in humans has been described. Moreover, brain content of glutamine is correlated with intracranial pressure. Emerging evidence shows that NH_3 itself is not harmful to the brain, but its conversion to glutamine by glutamine synthetase inhibits endothelial NOS and, therefore, blood flow and oxygen supply to the brain (Lee et al., 1996; Kawaguchi et al., 2005). In contrast, glutamine is required for production of iNOS protein by macrophages, thereby stimulating NO production under conditions of immunological activation to kill pathogenic micro-organisms (Li et al., 2007). So glutamine produced by NH_3 has different effects on NO production in different organs.

NO, H_2S, carbon monoxide (CO) and sulfur dioxide (SO_2) are endogenous gas transmitters, which affect a variety of vital functions including vasorelaxation and suppression of cell proliferation (O'Sullivan et al., 2006; Ali et al., 2006; Li and Meng, 2009; Li and Meng, 2010; Li and Moore, 2007; Zhang and Meng, 2009; Zhang and Meng, 2010). In contrast to these toxic gaseous molecules, we hypothesize that NH_3 may have a physiological role in the regulation of cardiovascular function. Currently, however, the mechanisms underlying the inotropic effects of NH_3 on isolated perfused hearts in rats are not understood. Therefore, the purpose

of this study was to investigate the inotropic effects of NH_3 and its mechanisms in isolated hearts in rats.

MATERIALS AND METHODS

Chemicals

Glibenclamide, 4-aminopyridine (4-AP), nifedipine, iberiotoxin, N^G-nitro-L-arginine methyl ester (L-NAME), 4*H*-8-bromo-1,2,4-oxadiazolo(3,4-d)benz(b)(1,4)-oxazin-1-one (NS-2028), staurosporine, indomethacin, propranolol and apamin were purchased from Sigma (St Louis, MO, USA). A radioimmunoassay kit for measuring cGMP was obtained from Shanghai University of Traditional Chinese Medicine (Shanghai, China). Assay kits for lactate dehydrogenase (LDH), creatine kinase (CK), NO, NOS and adenosine triphosphatase (ATPase) were purchased from Nan Jing Jian Cheng Company of Biological Technology (Nanjing, China). Other chemical reagents were of analytical grade.

Preparation of isolated rat hearts

Animal care and experimental protocols complied with the Animal Management Rule of the Ministry of Health, People's Republic of China (Documentation 55, 2001) and the Animal Care Committee of Shanxi University. Male Wistar rats [*Rattus norvegicus* (Berkenhout 1769)] weighing 220–250 g were obtained from Heibei Medical University (Shijiazhuang, China).

Rats were injected intravenously with anticoagulant heparin under the terminal anesthesia (pentobarbital sodium 30 mg kg^{-1}, i.p.). The hearts were quickly excised and perfused on a Langendorff apparatus with a modified Krebs–Henseleit (K–H) buffer (mmol l^{-1}: 118.0 NaCl, 4.7 KCl, 25.0 $NaHCO_3$, 1.2 KH_2PO_4, 1.2 $MgCl_2$, 1.25 $CaCl_2$ and 11.0 glucose, pH 7.3) under a 100 cm H_2O pressure at 37°C, gassed with 95% O_2–5% CO_2. A saline-filled latex balloon was inserted into the left ventricle through the left atrium and connected to a pressure transducer for measurement of left ventricular pressure. Heart rate, coronary flow, left ventricular developed pressure (LVDP=left ventricular systolic pressure–left ventricular diastolic pressure) and maximum left ventricular pressure development ($\pm LV\,dP/dt_{max}$) were measured by a MedLab Biological Signal Collection System (Medease Science and Technology, Nanjing, China) with the left ventricular diastolic pressure pro-stabilized at 10 mmHg (Suzuki et al., 2000). The whole system was water jacketed and maintained at 37°C. The hearts were perfused for 20–30 min to establish equilibrium hemodynamics. The equilibrium phase was terminated when LVDP, heart rate and coronary flow were maintained at the same level for three continuous periods of measurement, 5 min apart. The baseline measurements were recorded at the end of this time. Hearts not meeting these criteria were not used in the study.

Inotropic effects of NH_3 in rat hearts

To study the positive inotropic effect of NH_3 on the isolated perfused rat hearts, NH_3 (0–4 mmol l^{-1}) was added to the perfused fluid. The parameters of cardiac function under various conditions were measured. In order to study whether the impact of NH_3 on heart function was related to the change in pH value of the perfusate or to NH_4^+, we also studied the effects of NaOH and NH_4Cl (0–4 mmol l^{-1}) on heart function.

Effect of inhibitors of signaling pathways on inotropic effects induced by NH_3

To investigate the roles of Ca^{2+} and K^+ channels and the various cellular signaling pathways in the effect of NH_3 on the isolated rat hearts, various specific inhibitors (nifedipine 30 nmol l^{-1}, glibenclamide 10 mmol l^{-1}, 4-AP 2 mmol l^{-1}, iberiotoxin 100 nmol l^{-1}, L-NAME 100 mmol l^{-1}, NS-2028 10 mmol l^{-1}, propranolol 10 mmol l^{-1}, indomethacin 10 mmol l^{-1}, staurosporine 30 nmol l^{-1}, apamin 50 nmol l^{-1} and saline control) were applied in this study. Hearts were allowed to stabilize for 20–30 min, and then inhibitors were administered for a 10 min pre-dosing period before the addition of NH_3. Inhibitors and NH_3 (0.5 or 1.5 mmol l^{-1}) were then administered together for a 10 min period. Heart rate, coronary flow, LVDP and $\pm LV\,dP/dt_{max}$ were measured throughout the experiments. The data for this set were calculated as the percentage of pre-NH_3 values.

Measurement of LDH and CK activity in perfusate

Perfusate was collected during the NH_3 perfusion period. The activities of LDH and CK in the perfusate were assayed using a kit according to the manufacturer's instructions.

Preparation of myocardial tissue

Rat hearts were allowed to stabilize for 20–30 min, and then NH_3 (0.5 or 1.5 mmol l^{-1}) was added to the perfused fluid for 10 min. The hearts were quickly frozen in liquid nitrogen, and subsequently used for measuring the activity of ATPase and NOS and the level of NO and cGMP.

Assay of NOS and ATPase activity

Total NOS (tNOS) and inducible NOS (iNOS) activity was measured by using a NOS assay kit according to the manufacturer's protocol. The resulting absorbance was determined at 530 nm with a spectrophotometer.

Ca^{2+},Mg^{2+}-ATPase and Na^+,K^+-ATPase activity was estimated by quantifying the release of inorganic phosphorus from adenosine triphosphate (ATP) according to the kit instructions. One activity unit of ATPase is expressed as 1 μmol phosphate mg^{-1} protein h^{-1}.

Estimation of cGMP and NO levels

To measure the content of cGMP, the perfused hearts were homogenized in a glass homogenizer. The homogenates were centrifuged at 1000 ***g*** for 10 min at 4°C and the supernatants were extracted with two volumes of water-saturated ethanol and 75% ethanol. The supernatant was taken for cGMP measurement using a radioimmunoassay kit. The radioactivity present in the bound fraction was measured in terms of counts per minute (c.p.m.) by a Gamma Counter (FT-646A, Beijing, China). The concentrations of cGMP were calculated by interpolation of $\%B/B_0$ (where B represents the c.p.m. value of the sample tube and B_0 is the c.p.m. value of the blank tube) from a standard curve and are expressed as pmol g^{-1}.

NO levels were measured using a NO assay kit according to the manufacturer's protocol. The resulting absorbance was determined at 550 nm with a spectrophotometer.

Protein assay

Protein content of the myocardial homogenate was evaluated by the Bradford method (Bradford, 1976), with bovine serum albumin used as a standard.

Statistical analysis

All values are expressed as means ± s.d. Student's *t*-test for unpaired samples was used to compare the mean values between the control and test groups. Multiple comparisons were made with one-way ANOVA followed by *post hoc* analysis (Tukey's test). Statistical significance was set at $P<0.05$.

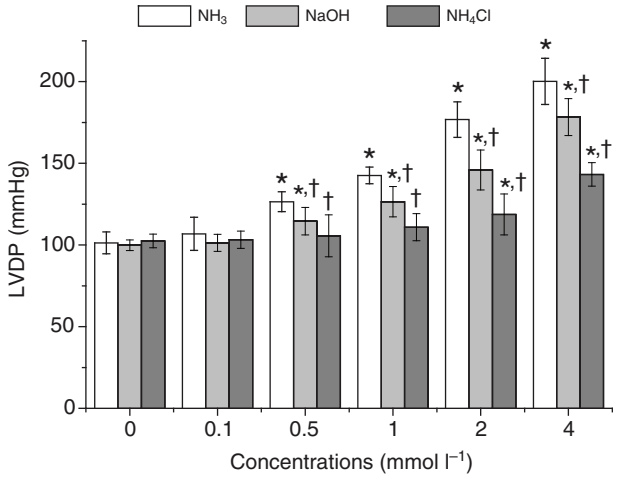

Fig. 1. Effect of NH_3 on the left ventricular developed pressure (LVDP) in isolated perfused rats hearts. *P<0.05 compared with the corresponding control (0 concentration) group; †P<0.05 compared with the NH_3 group at the same concentration.

RESULTS

The positive inotropic effects of NH_3

Fig. 1 and Table 1 show the changes in $\pm LV\,dP/dt_{max}$, LVDP, heart rate and coronary flow of isolated hearts exposed to the different concentrations of NH_3, NaOH and NH_4Cl. These three substances all elicited positive inotropic effects in a concentration-dependent manner, with the maximum response shown at the highest concentration tested (4 mmol l^{-1}). NH_3 produced a higher positive inotropic effect than NaOH and NH_4Cl at the same concentration. From 0.5 to 4 mmol l^{-1}, the increases in LVDP induced by NH_3 were significantly greater than those induced by NaOH and NH_4Cl (Fig. 1).

In addition, the heart rate and coronary flow were significantly increased by NH_3 at concentrations from 0.5 to 4 mmol l^{-1}, but they only were significantly increased by NaOH at the highest concentration tested (4 mmol l^{-1}). A concentration of 0.1 mmol l^{-1} NH_3 was infused for 10 min resulting in a statistically significant increase in $+LV\,dP/dt_{max}$ and $-LV\,dP/dt_{max}$, by 10.14–89.97% and 12.82–95.68%, respectively (Table 1). In contrast, no significant changes in the $\pm LV\,dP/dt_{max}$ were observed with 0.1 mmol l^{-1} NaOH or NH_4Cl. Table 2 presents changes of pH value of K–H buffer caused by NH_3 and NaOH at different concentrations at 37°C. At a concentration of 0.5 mmol l^{-1}, NH_3 did not change the pH value of K–H buffer. At higher concentrations (2 and 4 mmol l^{-1}), the increases in the pH value of the K–H buffer induced by NH_3 were significantly lower than those induced by NaOH at the same concentration.

Involvement of ATP-sensitive K^+ channel in the inotropic effects of NH_3

The role of the ATP-sensitive K^+ (K_{ATP}) channel in the NH_3-mediated inotropic effect was examined by administering glibenclamide (10 mmol l^{-1}) before and during NH_3 infusion. Glibenclamide alone significantly decreased coronary flow by around 15% over the baseline value but did not affect LVDP and heart rate. In the presence of glibenclamide, the increases in LVDP induced by NH_3 (0.5 and 1.5 mmol l^{-1}) were partially inhibited (Fig. 2).

Involvement of soluble NO synthase and guanylate cyclase in the positive inotropic effects of NH_3

The role of NO synthase in the NH_3-mediated inotropic action was examined by administering L-NAME (100 mmol l^{-1}) before and during NH_3 infusion. L-NAME alone significantly decreased LVDP by around 15% over the baseline value but did not have a significant effect on the coronary flow and heart rate. In the presence of L-NAME, the increases in LVDP induced by NH_3 (0.5 and 1.5 mmol l^{-1}) were partially inhibited (Fig. 3).

Table1. Alterations of functional parameters before and after perfusion of different concentrations of NH_3, NaOH and NH_4Cl into rat hearts

	0	0.1 mmol l^{-1}	0.5 mmol l^{-1}	1 mmol l^{-1}	2 mmol l^{-1}	4 mmol l^{-1}
NH_3						
HR	241.65±15.23	249.7±13.77	260.28±12.13*	267.45±11.07*	280.16±14.87*	289.74±10.87**
CF	11.89±1.23	12.38±1.08	13.61±0.86*	13.98±1.13*	14.86±0.98**	16.05±1.27**
$+dP/dt_{max}$	2387.3±126.8	2629.6±114.6*	2960.7±152.3*	3342.2±173.7**	3819.2±215.6***	4535.8±229.6***
$-dP/dt_{max}$	–1389.1±118.9	–1567.2±123.6*	–1736.2±132.0*	–1944.4±119.6**	–2297.7±184.6***	–2718.4±206.4***
NaOH						
HR	239.88±12.17	234.22±15.21	239.68±16.33	242.86±17.45	252.15±12.33	265.47±14.18*
CF	12.34±1.11	11.88±1.23	12.07±1.19	11.89±1.39	12.83±1.47	13.98±1.55*
$+dP/dt_{max}$	2325.6±127.9	2458.1±115.9	2647.2±145.2*	2859.6±152.3*	3297.4±143.5**	3962.9±197.5***
$-dP/dt_{max}$	–1424.3±141.1	–1478.2±143.7	–1607.4±126.5*	–1723.1±124.2*	–1952.0±118.3*	–2237.8±143.7***
NH_4Cl						
HR	245.34±16.37	244.22±17.15	249.77±14.59	251.76±15.27	257.39±18.67	263.59±12.55*
CF	12.12±1.67	12.15±1.34	12.46±1.28	12.49±1.47	12.98±1.52	13.37±1.79
$+dP/dt_{max}$	2366.7±134.7	2358.1±123.4	2447.2±161.6	2539.6±153.8	2697.4±129.8*	3162.9±185.3**
$-dP/dt_{max}$	–1409.7±123.6	–1446.3±112.8	–1538.4±139.6	–1603.1±174.1	–1652.0±127.4*	–1937.8±177.2**

Values are means ± s.d. (N=6); *P<0.05, **P<0.01, ***P<0.001 compared with control (0 concentration).
HR, heart rate; CF, coronary flow; $\pm dP/dt_{max}$, maximal rise/fall rate of left ventricular pressure.

Table 2. Changes of pH value of Krebs–Henseleit buffer caused by NH_3 and NaOH at different concentrations at 37°C

	0	0.5 mmol l^{-1}	1 mmol l^{-1}	2 mmol l^{-1}	4 mmol l^{-1}
NH_3	7.40±0.04	7.46±0.06	7.68±0.03*	8.02±0.04*,†	8.49±0.05*,†
NaOH	7.40±0.05	7.60±0.04*	7.81±0.05*	8.29±0.06	8.76±0.05*

Values are means ± s.d. (N=9); *P<0.05 compared with control; †P<0.05 compared with NaOH group at the same concentration.

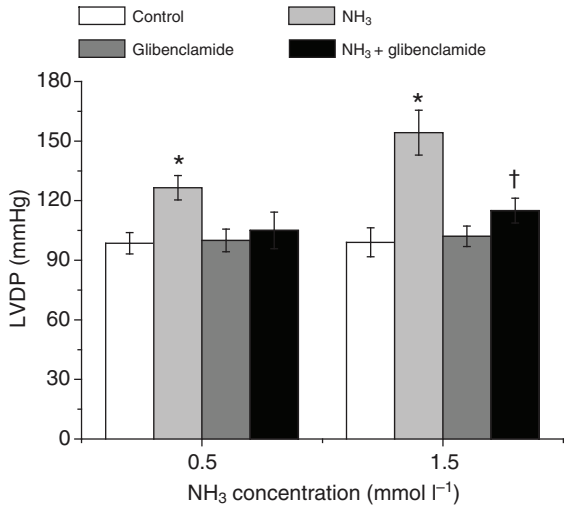

Fig. 2. Inhibitory effect of glibenclamide on the rise of LVDP mediated by NH_3 in the perfused rat hearts. *P<0.05 compared with the control group; †P<0.05 compared with the glibenclamide alone group. There was a significant difference between the inhibition by the NH_3 group compared with the control and the inhibition by the NH_3 + glibenclamide group compared with the glibenclamide group (P<0.05).

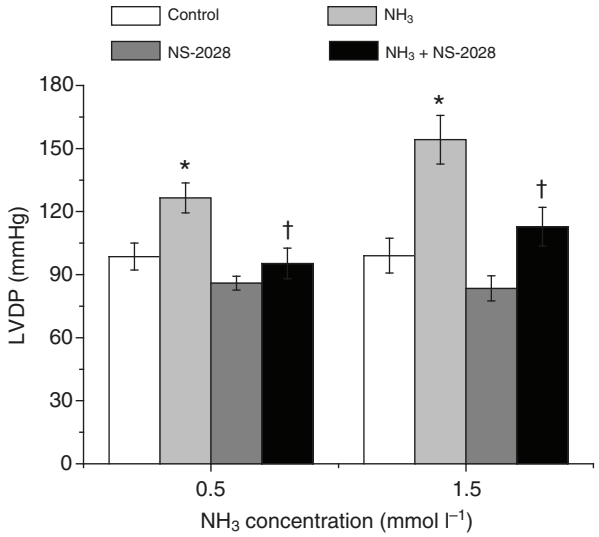

Fig. 4. Inhibitory effect of NS-2028 on the rise of LVDP mediated by NH_3 in perfused rat hearts. *P<0.05 compared with the control group; †P<0.05 compared with the NS-2028 alone group. There was a significant difference between the inhibition by the NH_3 group compared with the control and the inhibition by the NH_3 + NS-2028 group compared with the NS-2028 group (P<0.05).

NS-2028 has been shown to specifically inhibit soluble guanylate cyclase and reduce the production of cGMP (Olesen et al., 1998). In this study, NS-2028 (10 mmol l^{-1}) was used to treat isolated hearts 10 min before the application of NH_3. NS-2028 alone significantly decreased LVDP and coronary flow by around 15% over the baseline value but did not have any effect on the heart rate. The increases in LVDP induced by NH_3 (0.5 and 1.5 mmol l^{-1}) were partially inhibited by NS-2028 (Fig. 4).

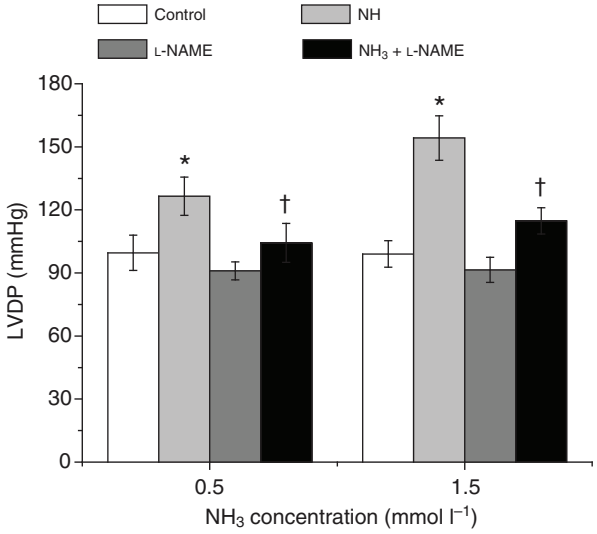

Fig. 3. Inhibitory effect of L-NAME on the rise of LVDP mediated by NH_3 in perfused rat hearts. *P<0.05 compared with the control group; †P<0.05 compared with the L-NAME alone group. There was a significant difference between the inhibition by the NH_3 group compared with the control and the inhibition by the NH_3 + L-NAME group compared with the L-NAME group (P<0.05).

Non-involvement of other ion channels and signaling pathways in the positive inotropic effects of NH_3

To determine the roles of the BK_{Ca} (big Ca^{2+}-activated K^+) channel, SK_{Ca} (small Ca^{2+}-activated K^+) channel, K_v (voltage-dependent K^+) channel, L-type calcium channel, protein kinase C (PKC), cycloxygenase and β-adrenoceptor in the positive inotropic effect of NH_3, the hearts received iberiotoxin, apamin, 4-AP, nifedipine, staurosporine, indomethacin and propranolol, respectively, before and during NH_3 infusion. However, the positive inotropic effect of NH_3 in the hearts was not affected by these drugs (data not shown).

Involvement of the K_{ATP} channel and SK_{Ca} channel in the inotropic effects of NaOH

The role of the K_{ATP} channel and SK_{Ca} channel in the NaOH-mediated inotropic action was examined by administering either glibenclamide (10 mmol l^{-1}) or apamin (50 nmol l^{-1}) before and during the NaOH infusion. Glibenclamide alone significantly decreased coronary flow by around 15% over the baseline value but did not have any effect on LVDP and heart rate. Apamin alone did not have any effect on LVDP, coronary flow and heart rate. In the presence of glibenclamide or apamin, the increases of LVDP induced by NaOH at 1 mmol l^{-1} were partially inhibited (Fig. 5).

However, the positive inotropic effect of NaOH on the hearts was not affected by other drugs used in our paper (data not shown).

Effect of NH_3 on LDH and CK activity in coronary perfusates

From Fig. 6 we can see that the activity of LDH and of CK in the coronary perfusate was significantly higher in the 1.5 mmol l^{-1} NH_3 group than in the control group.

Effect of NH_3 on NOS and ATPase activity in perfused hearts

Fig. 7 presents the activity of NOS and ATPase in rat hearts perfused with 0.5 and 1.5 mmol l^{-1} NH_3. Both tNOS activity and iNOS activity in the hearts were significantly increased by 0.5 and 1.5 mmol l^{-1}

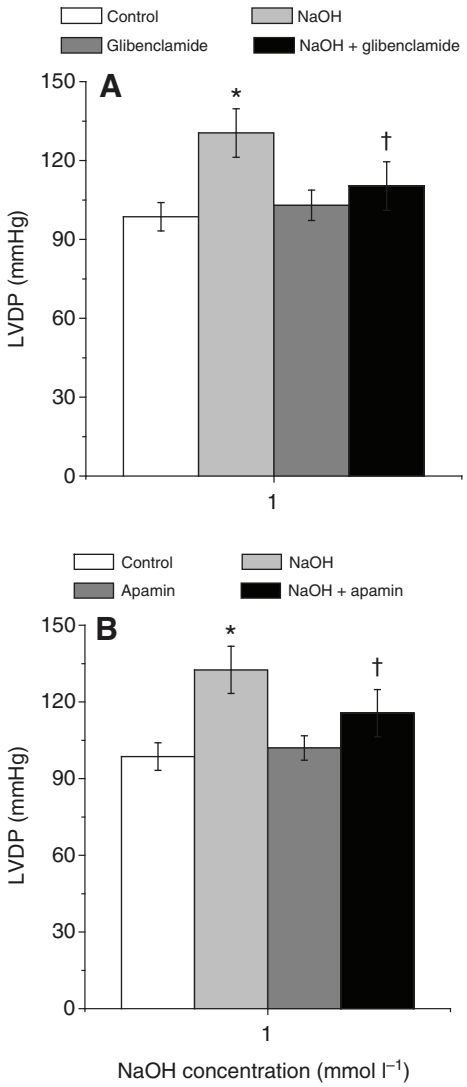

Fig. 5. Inhibitory effect of glibenclamide (A) and apamin (B) on the rise of LVDP mediated by NaOH in perfused rat hearts. *P<0.05 compared with the control group; †P<0.05 compared with the glibenclamide (A) or the apamin (B) alone group. There was a significant difference between the inhibition by the NaOH group compared with the control and the inhibition by the NaOH + glibenclamide (A) compared with the glibenclamide group or the NaOH + apamin (B) group (P<0.05) compared with the apamin group.

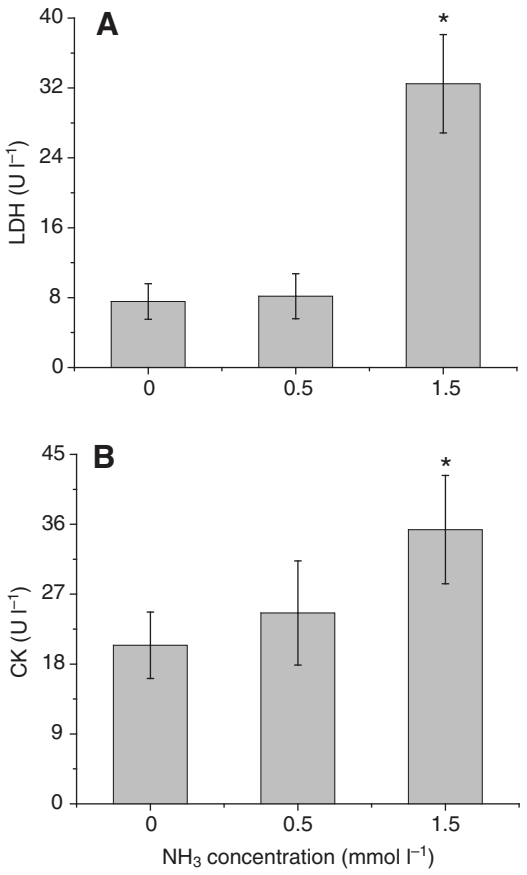

Fig. 6. Effect of NH_3 on the activity of lactate dehydrogenase (LDH, A) and creatine kinase (CK, B) in the coronary perfusate. *P<0.05 compared with the control (0 NH_3) group.

NH_3. In addition, at a concentration of 1.5 mmol l^{-1}, NH_3 caused significant decreases in Na^+,K^+-ATPase and Ca^{2+},Mg^{2+}-ATPase activity in the hearts.

Effect of NH_3 on the level of NO and cGMP in perfused hearts

NO and cGMP levels were assessed in rat hearts perfused with NH_3 (Fig. 8). Levels of both NO and cGMP in the hearts were significantly increased by 0.5 and 1.5 mmol l^{-1} NH_3.

DISCUSSION

In this study, we utilized the isolated perfused heart model to elucidate the effect of NH_3 on myocardial contractility and hemodynamic parameters. The results show that NH_3 elicits a positive inotropic effect in a concentration-dependent manner. This effect cannot be explained by changes in pH or NH_4^+ alone as NH_3 produced a higher positive effect than NaOH and NH_4Cl at the same concentration. In particular, at a concentration of 0.5 mmol l^{-1}, NH_3 did not change the pH value of K–H buffer at 37°C (Table 2), but NH_3 elicited a significant positive inotropic effect. From 0.5 to 4 mmol l^{-1}, the increases in LVDP induced by NH_3 were significantly higher than those induced by NaOH and NH_4Cl (Fig. 1). In addition, heart rate and coronary flow were significantly increased by NH_3 at concentrations from 0.5 to 4 mmol l^{-1}, but were only significantly increased by NaOH at the highest concentration tested (4 mmol l^{-1}). A concentration of 0.1 mmol l^{-1} NH_3 infused for 10 min resulted in a statistically significant increase in the $\pm LV\,dP/dt_{max}$. In contrast, no significant changes in the $\pm LV\,dP/dt_{max}$ were observed with 0.1 mmol l^{-1} NaOH or NH_4Cl (Table 1). These findings suggest that, at low concentrations, the impact of NH_3 on cardiac function was the result of NH_3 molecules; at high concentrations, the effect of NH_3 on the hearts might be partly correlative with the change of pH value, but mainly caused by NH_3 molecules. One possible reason is that NH_3 molecules pass membrane barriers by diffusion more easily than NH_4^+ and OH^- (Cooper, 1994; Kvamme, 1983). In addition, another study from our laboratory indicated that NH_3 at low concentrations could promote a concentration-dependent vasoconstriction in the isolated aorta in rats (Z.M., unpublished).

The opening of the K_{ATP} channel is associated with potassium efflux, polarization of the cell membrane and shortening of action potential duration (Noma, 1983; Cole et al., 1991). These effects reduce Ca^{2+} influx *via* L-type Ca^{2+} channels and decrease the time for Na^+–Ca^{2+} exchange operating in the reverse mode. The resulting

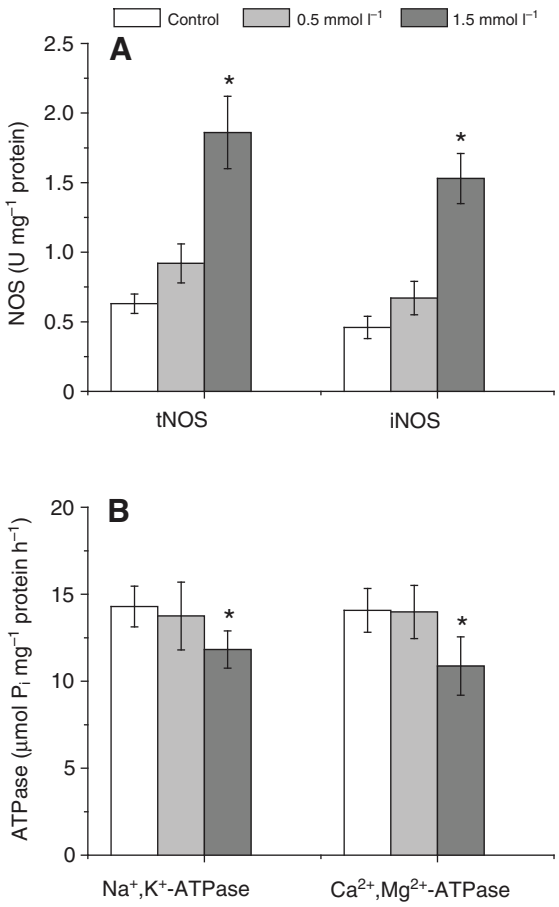

Fig. 7. Effect of NH_3 on the activity of nitric oxide synthase [NOS, total (t) or inducible (i); A] and ATPase (B) in perfused rat hearts. P_i, inorganic phosphate. *P<0.05 compared with the control group.

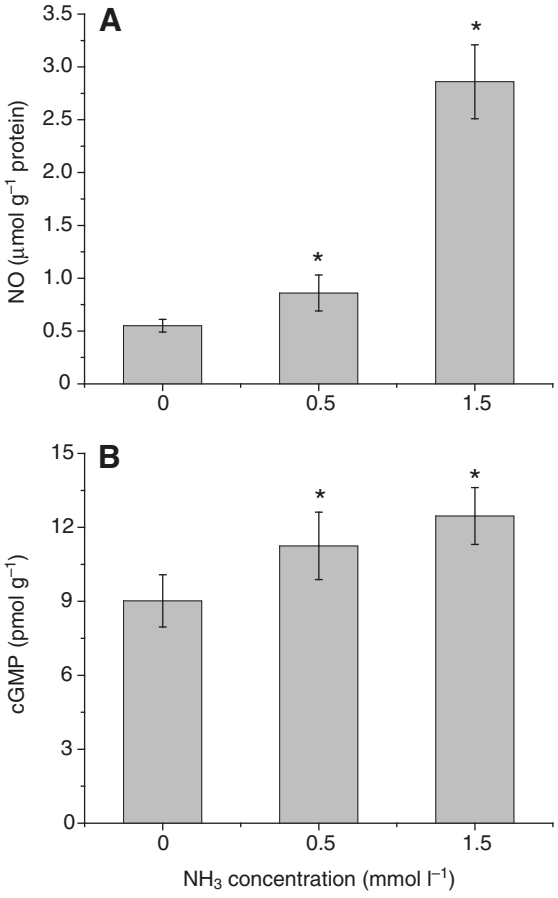

Fig. 8. Effect of NH_3 on the level of nitric oxide (NO, A) and cyclic GMP (cGMP, B) in perfused rat hearts. *P<0.05 compared with the control (0 NH_3) group.

decrease in Ca^{2+} influx would be expected to lead to a reduction of cardiomyocyte contraction. We determined whether the K_{ATP} channels were involved in the positive inotropic effects of NH_3. Glibenclamide, a K_{ATP} channel inhibitor, partially inhibited the positive effects induced by NH_3 (0.5 and 1.5 mmol l^{-1}). These results suggest that the positive inotropic effects of NH_3 might be related to K_{ATP} channels. The mechanism behind this observation is unclear and further research is needed.

In the present study, L-NAME and NS-2028 partially attenuated the positive inotropic effects induced by the different concentrations of NH_3. In addition, NO and cGMP levels in the heart were significantly increased by different concentrations of NH_3. The endogenously produced small increase in NO and cGMP can elicit a positive inotropic effect in isolated rat hearts (Kojda et al., 1997; Massion et al., 2003). These results suggest that NO and cGMP play a significant role in the NH_3-mediated positive inotropic effect. Our results are consistent with those of a previous report by Faff et al. (Faff et al., 1996), who proposed that NH_3 might act in cell signaling through activation of cAMP-dependent signaling pathways in which mildly increased cGMP inhibits cAMP hydrolysis through inhibition of phosphodiesterase.

Furthermore, we investigated the mechanism of the positive inotropic effect induced by NaOH on rat hearts. The positive inotropic effect of NaOH at 1 mmol l^{-1} was partially inhibited by glibenclamide and apamin. This result suggests that the effect was partially related to the K_{ATP} channel and SK_{Ca} channel. Although the positive inotropic effect of NH_3 was also partially related to K_{ATP} channels, the mechanism differed from that for NaOH. These results further indicate that the positive inotropic effect induced by NH_3 was mainly caused by NH_3 molecules and was partly correlated with the change of pH value of the heart perfusate. Similarly, Andersson and colleagues (Andersson et al., 1981) reported that the relaxation of cerebrovascular smooth muscle caused by NH_3 was independent of NH_3-induced extracellular changes in pH. Wakabayashi and colleagues (Wakabayashi et al., 1992) reported that ammonium compounds increased vascular tone by causing influx of extracellular calcium through the voltage-dependent calcium channel, and intracellular alkalinization was involved in this process.

CK is an enzyme (EC 2.7.3.2) expressed by various tissues and cell types. CK (using ATP) catalyzes the conversion of creatine to phosphocreatine and adenosine diphosphate (ADP). Thus CK is an important enzyme in many tissues (Wallimann and Hemmer, 1994). Increased CK levels may signal muscular dystrophy, polymyositis, dermatomyositis and sometimes myositis, as well as heart attacks. An increase of extracellular LDH enzyme activity reflects an increase in the number of membrane-damaged cells (Lobner, 2000). In addition, the plasma membrane Ca^{2+},Mg^{2+}-ATPase and Na^+,K^+-ATPase play important roles in maintaining the normal active transport for positive ions such as Na^+, K^+ and Ca^{2+}. Inhibition of

these enzymes may lead to the elevation of plasma membrane permeability for positive ions, possibly resulting in a drop in intracellular K^+ content and an abnormal rise of cytosolic Ca^{2+} concentration (Jurma et al., 1997). In the present study, at a concentration of 1.5 mmol l^{-1}, NH_3 significantly increased CK and LDH activity in the coronary perfusate and significantly decreased the activity of Na^+,K^+-ATPase and Ca^{2+},Mg^{2+}-ATPase in the heart. These results suggested that NH_3 at a concentration of 1.5 mmol l^{-1} has a damaging effect on the isolated hearts.

CONCLUSIONS

In conclusion, we have shown that NH_3 produces a positive inotropic effect in a concentration-dependent manner. At low concentrations, the impact of NH_3 on cardiac function was *via* NH_3 molecules; at high concentrations, the effect of NH_3 on the hearts might be partly correlated with the change of pH value, but mainly caused by NH_3 molecules. The mechanism of the positive inotropic effect induced by NH_3 on the hearts might be related to the K_{ATP} channel and activation of the NO–cGMP pathway. These results suggest that NH_3 at physiological or low concentrations might play a modulatory role in heart function, but at high concentrations has a damaging effect on isolated rat hearts.

LIST OF SYMBOLS AND ABBREVIATIONS

$\pm LV\,dP/dt_{max}$	maximum left ventricular pressure development
4-AP	4-aminopyridine
ATPase	adenosine triphosphatase
BK_{Ca}	big Ca^{2+}-activated K^+ channel
CK	creatine kinase
GABA	γ-amino butyric acid
H_2S	hydrogen sulfide
K_{ATP}	ATP-sensitive K^+ channel
K_v	voltage-dependent K^+ channel
LDH	lactate dehydrogenase
L-NAME	N^G-nitro-L-arginine methyl ester
LVDP	left ventricular developed pressure
NH_3	ammonia
NMDA	*N*-methyl D-aspartate
NOS	nitric oxide synthase
NS-2028	4*H*-8-bromo-1,2,4-oxadiazolo(3,4-d)benz(b)(1,4)-oxazin-1-one
SK_{Ca}	small Ca^{2+}-activated K^+ channel

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REFERENCES

Ali, M. Y., Ping, C. Y., Mok, Y. Y., Ling, L., Whiteman, M., Bhatia, M. and Moore, P. K. (2006). Regulation of vascular nitric oxide in vitro and in vivo: a new role for endogenous hydrogen sulphide? *Br. J. Pharmacol.* **149**, 625-634.

Andersson, K. E., Brandt, L., Hindfelt, B. and Ljunggren, B. (1981). Cerebrovascular effects of ammonia in vitro. *Acta Physiol. Scand.* **113**, 349-353.

Bender, A. S. and Norenberg, M. D. (2000). Effect of ammonia on GABA uptake and release in cultured astrocytes. *Neurochem. Int.* **36**, 389-395.

Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* **72**, 248-254.

Butterworth, R. F. (2002). Pathophysiology of hepatic encephalopathy: a new look at ammonia. *Metab. Brain Dis.* **17**, 221-227.

Cole, W. C., McPherson, C. D. and Sontag, D. (1991). ATP-regulated K^+ channels protect the myocardium against ischemia/reperfusion damage. *Circ. Res.* **69**, 571-581.

Cooper, A. J. L. (1994). Ammonia metabolism in mammals: interorgan relationships. *Adv. Exp. Med. Biol.* **341**, 21-37.

Dimski, D. S. (1994). Ammonia metabolism and the urea cycle: function and clinical implications. *J. Vet. Intern. Med.* **8**, 73-78.

Faff, L., Reichenbach, A. and Albrecht, J. (1996). Ammonia-induced taurine release from cultured rabbit Muller cells is an osmoresistant process mediated by intracellular accumulation of cyclic AMP. *J. Neurosci. Res.* **46**, 231-238.

Fan, P. and Szerhb, J. C. (1993). Effects of ammonium ions on synaptic transmission and on responses to quisqualate and N-methyl-D-aspartate in hippocampal CA1 pyramidal neurons in vitro. *Brain Res.* **632**, 225-231.

Greenhaff, P. L., Leiper, J. B., Ball, D. and Maughan, R. J. (1991). The influence of dietary manipulation on plasma ammonia accumulation during incremental exercise in man. *Eur. J. Appl. Physiol. Occup. Physiol.* **63**, 338-344.

Jurma, O. P., Hom, D. G. and Andersen, J. K. (1997). Decreased glutathione results in calcium-mediated cell death in PC12. *Free Radic. Biol. Med.* **23**, 1055-1066.

Kawaguchi, T., Brusilow, S. W., Traystman, R. J. and Koehler, R. C. (2005). Glutamine-dependent inhibition of pial arteriolar dilation to acetylcholine with and without hyperammonemia in the rat. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **288**, R1612-R1619.

Kojda, G., Kottenberg, K. and Noack, E. (1997). Inhibition of nitric oxide synthase and soluble guanylate cyclase induces cardiodepressive effects in normal hearts. *Eur. J. Pharmacol.* **334**, 181-190.

Kvamme, E. (1983). Ammonia metabolism in the CNS. *Prog. Neurobiol.* **20**, 109-132.

Lee, T. J. F., Sarwinski, S., Ishine, T., Lai, C. C. and Chen, F. Y. (1996). Inhibition of cerebral neurogenic vasodilation by L-glutamine and nitric oxide synthase inhibitors and its reversal by L-citrulline. *J. Pharmacol. Exp. Ther.* **276**, 353-358.

Li, J. L. and Meng, Z. Q. (2009). The role of sulfur dioxide as an endogenous gaseous vasoactive factor in synergy with nitric oxide. *Nitric Oxide* **20**, 166-174.

Li, J. L. and Meng, Z. Q. (2010). Sulfur dioxide upregulates the aortic nitric oxide pathway in rats. *Eur. J. Pharmacol.* **645**, 143-150.

Li, L. and Moore, P. K. (2007). An overview of the biological significance of endogenous gases: new roles for old molecules. *Biochem. Soc. Trans.* **35**, 1138-1141.

Li, P., Yin, Y. L., Li, D., Kim, S. W. and Wu, G. Y. (2007). Amino acids and immune function. *Br. J. Nutr.* **98**, 237-252.

Li, X. L., Bazer, F. W., Gao, H. J., Jobgen, W. J., Johnson, G. A., Li, P., McKnight, J. R., Satterfield, M. C., Spencer, T. E. and Wu, G. Y. (2009). Amino acids and gaseous signaling. *Amino Acids* **37**, 65-78.

Lobner, D. (2000). Comparison of the LDH and MTT assays for quantifying cell death: validity for neuronal apoptosis? *J. Neurosci. Meth.* **96**, 147-152.

Massion, P. B., Feron, O., Dessy, C., and Balligand, J. L. (2003). Nitric oxide and cardiac function: ten years after, and continuing. *Circ. Res.* **93**, 388-398.

Noma, A. (1983). ATP-regulated K^+ channels in cardiac muscle. *Nature* **305**, 147-148.

Olde Damink, S. W., Deutz, N. E., Dejong, C. H., Soeters, P. B. and Jalan, R. (2002). Interorgan ammonia metabolism in liver failure. *Neurochem. Int.* **41**, 177-188.

Olesen, S. P., Drejer, J., Axelsson, O., Moldt, P., Bang, L., Nielsen-Kudsk, J. E., Busse, R. and Mülsch, A. (1998). Characterization of NS 2028 as a specific inhibitor of soluble guanylate cyclase. *Br. J. Pharmacol.* **123**, 299-309.

O'Sullivan, S. E. (2006). What is the significance of vascular hydrogen sulphide (H_2S)? *Br. J. Pharmacol.* **149**, 609-610.

Rudman, D., DiFulco, T. J., Galambos, J. T., Smith, R. B., Salam, A. A. and Warren, W. D. (1973). Maximal rates of excretion and synthesis of urea in normal and cirrhotic subjects. *J. Clin. Invest.* **52**, 2241-2249.

Seiler, N. (2002). Ammonia and Alzheimer's disease. *Neurochem. Int.* **41**, 189-207.

Suárez, I., Bodega, G. and Fernández, B. (2002). Glutamine synthetase in brain: effect of ammonia. *Neurochem. Int.* **41**, 123-142.

Suryawan, A., O'Connor, P. M. J., Bush, J. A., Bush, J. A., Nguyen, H. V. and Davis, T. A. (2009). Differential regulation of protein synthesis by amino acids and insulin in peripheral and visceral tissues of neonatal pigs. *Amino Acids* **37**, 97-104.

Suzuki, K., Brand, N. J., Smolenski, R. T., Jayakumar, J., Murtuza, B. and Yacoub, M. H. (2000). Development of a novel method for cell transplantation through the coronary artery. *Circulation* **102**, III-359-III-364.

Wakabayashi, I., Hatake, K. and Sakamoto, K. (1992). Ammonium ion increases the tone of rat portal vein. *Gen. Pharmacol.* **23**, 1189-1192.

Wallimann, T. and Hemmer, W. (1994). Creatine kinase in non-muscle tissues and cells. *Mol. Cell. Biochem.* **133**, 193-220.

Wu, G. and Morris, S. M. (1998). Arginine metabolism: nitric oxide and beyond. *Biochem. J.* **336**, 1-17.

Wu, G., Bazer, F. W., Datta, S., Johnson, G. A., Li, P., Satterfield, M. C. and Spencer, T. E. (2008). Proline metabolism in the conceptus: Implications for fetal growth and development. *Amino Acids* **35**, 691-702.

Wu, G., Bazer, F. W. and Davis, T. A. (2009). Arginine metabolism and nutrition in growth, health and disease. *Amino Acids* **37**, 153-168.

Ytrebø, L. M., Sen, S., Rose, C., Ten Have, G. A. M., Davies, N. A., Hodges, S., Nedredal, G. I., Romero-Gomez, M., Williams, R., Revhaug, A. et al. (2006). Interorgan ammonia, glutamate, and glutamine trafficking in pigs with acute liver failure. *Am. J. Physiol. Gastrointest. Liver Physiol.* **291**, 373-381.

Zhang, Q. X. and Meng, Z. Q. (2009). The vasodilator mechanism of sulfur dioxide on isolated aortic rings of rats: involvement of the K^+ and Ca^{2+} channels. *Eur. J. Pharmacol.* **602**, 117-123.

Zhang, Q. X. and Meng, Z. Q. (2010). The negative inotropic effects of gaseous sulfur dioxide and its derivatives in the isolated perfused rat heart. *Environ. Toxicol.* doi: 10.1002/tox.20628 (in press).