Comparison of Terbutaline and Dobutamine in Rats with Endotoxemia

Jeng-Yuan Wu[1], Wen-Jinn Liaw[2], Chin Tzao[3], Shiu-Jen Chen[4], Jih-Hsin Wang[5] and Chin-Chen Wu[5]

[1]*Division of Thoracic Surgery*
Taichung Army General Hospital
Taichung
[2]*Department of Anesthesiology and* [3]*Division of Thoracic Surgery*
Tri-Service General Hospital
Taipei
Departments of [4]*Physiology and* [5]*Pharmacology*
National Defense Medical Center
Taipei, Taiwan, R.O.C.

Abstract

It is generally accepted that bacterial endotoxin (lipopolysaccharide, LPS) acts via endogenous mediators leading to endotoxicity. Among these endogenous mediators, tumor necrosis factor-α (TNF-α) seems to induce all characteristics for endotoxemia. Inhibition of TNF-α production by cAMP-elevating agents has been well documented. Terbutaline (an agonist of β_2-adrenoceptor) and dobutamine (an agonist of β_1-adrenoceptor), both are able to increase intracellular cAMP via activation of adenylate cyclase, were examined in the anesthetized rat with endotoxemia. Terbutaline or dobutamine was administered to the rat at 30 min after LPS injection. Hemodynamic changes and plasma TNF-α and nitrate (the end product of nitric oxide [NO]) levels as well as superoxide anion ($O_2\cdot^-$) production in the aorta were examined in this study. Results showed that terbutaline, but not dobutamine, improved the circulatory failure (e.g. hypotension and vascular hyporeactivity) in rats with endotoxemia. In addition, both terbutaline and dobutamine reduced the plasma TNF-α level, but only terbutaline attenuated the aortic $O_2\cdot^-$ production in these endotoxemic rats. The beneficial effect of terbutaline in endotoxemic animals was associated with a reduction in plasma TNF-α and aortic $O_2\cdot^-$, but not in plasma NO.

Key Words: tumor necrosis factor-α, nitric oxide, superoxide anion, terbutaline, dobutamine, lipopolysaccharide

Introduction

The sepsis induced by Gram-negative bacteria is associated with hypotension, vascular hyporeactivity to vasoconstrictor agents, myocardial dysfunction, maldistribution of organ blood flow, and in severe cases, may lead to disseminated intravascular coagulation, septic shock, and ARDS (1, 18, 25, 29). These pathophysiological effects can also be observed by injection of killed bacteria, since their active principle has been identified as endotoxin (lipopolysaccharide, LPS). Therefore, experimental endotoxemia has become a valuable experimental model for septicemia and has extensively been studied in laboratory animals. It is generally accepted that LPS acts via endogenous mediators, mainly produced by mononuclear phagocytes (22). Among these endogenous mediators, tumor necrosis factor-α (TNF-α) seems to be of particular importance for endotoxic effects (41), since it has been shown to induce all characteristics for endotoxic shock and antisera or antibody against TNF-α attenuated lethality or improved hemodynamic functions provoked by sepsis or endotoxin (7, 40).

The delayed hypotension induced by LPS is mainly attributed to the overproduction of nitric oxide

Corresponding author: Dr. Chin-Chen Wu, Department of Pharmacology, National Defense Medical Center, P.O. Box 90048-504, Neihu, Taipei, Taiwan 114, R.O.C. Tel & Fax: 886-2-8792-4858, E-mail: ccwu@ndmctsgh.edu.tw
Received: August 16, 2002; Revised: October 2, 2002; Accepted: October 20, 2002.

(NO), an important endogenous vasodilator, in this endotoxin model (33, 39). NO has now been recognized to be present in many tissues, acting as ubiquitous intracellular signaling molecule in diverse mammalian cells. For instance, in the coronary vessels NO plays an important role in the regulation of vascular tone and myocardial blood flow both *in vivo* and *in vitro* (2, 8). The overproduction of NO is, however, suggested to be responsible for the vascular relaxation and hypotension seen in states of sepsis and endotoxemia (21). The discrepancy between beneficial and deleterious effects of NO is because of the synthesis of NO via different isoforms of NO synthase (NOS). It has been identified that NO synthesized from L-arginine is via three isoenzymes expressed either constitutively (neuronal NOS; endothelial NOS) or following stimulation by cytokines (inducible NOS, iNOS) (21, 23). The induction of iNOS has been identified in endotoxin- and cytokine-treated macrophages, hepatocytes, endothelial cells or myocardium (21), and in some organs from animals treated with endotoxin (2). In addition, NO can react with superoxide anion ($O_2 \cdot^-$), leading to formation of the peroxynitrite anion (ONOO-) (16), which oxidizes sulfhydryl groups and generates hydroxyl radical (·OH) (6). It is assumed that LPS may act with macrophages to cause the generation of free radicals, including hydrogen peroxide (H_2O_2), $O_2 \cdot^-$ and ·OH, leading to oxidative damage in many tissues such as liver (35). This progression of circulatory failure to a multiple organ dysfunction syndrome is associated with a substantial increase in mortality (9).

Terbutaline is a β_2-selective agonist and has been clinically used for the long-term treatment of obstructive airway diseases and for treatment of acute bronchospasm (13). Dobutamine is a β_1-selective agonist and has been used to increase oxygen transport and thereby improve tissue perfusion in critically ill patients (31). In addition, stimulation of β-adrenoceptors has been shown to inhibit the release of inflammatory mediators (e.g. TNF-α) from monocytes or marcophages (30, 38). In order to examine the hypothesis that cAMP-elevating agents are able to regulate the production of cytokines in sepsis, we compared effects of terbutaline and dobutamine on animals with endotoxemia. Here, we show that terbutaline inhibits TNF-α and $O_2 \cdot^-$ production in plasma and aortas, respectively, but has no effect on the production of NO, and thus, mitigates the development of detrimental effects of endotoxin (e.g. circulatory failure) in animals. In contrast, dobutamine further enhances the production of NO and $O_2 \cdot^-$ production in plasma and aortas, respectively, although reduces the plasma TNF-α content, and thus, has no beneficial effect on animals with endotoxemia. These results indicate that terbutaline has therapeutic effects, which could be taken into consideration in the treatment of patients with sepsis.

Materials and Methods

In Vivo Experiments

Ten-week-old male Wistar-Kyoto (WKY) rats, whose stock originated from the Charles River Breeding Laboratories in Japan, were purchased from the Department of Laboratory Animal Science of the National Defense Medical Center. This study was approved by the local Institutional Review Board according to the recommendations from Helsinki and the internationally accepted principles in the care and the use of experimental animals. Rats were anesthetized by intraperitoneal injection of urethane (1.2 g/kg). The trachea was cannulated to facilitate respiration and environmental temperature was maintained at 24°C with an air-conditioning system. The right carotid artery was cannulated and connected to a pressure transducer (P23ID, Statham, Oxnard, CA, USA) for the measurement of phasic blood pressure and mean arterial blood pressure (MAP) as well as heart rate (HR) which were displayed on a Gould model TA5000 polygraph recorder (Gould Inc., Valley View, Ohio, USA). The left jugular vein was cannulated for the administration of drugs. Upon completion of the surgical procedure, cardiovascular parameters were allowed to stabilize for 20 min.

After recording baseline hemodynamic parameters, animals were given norepinephrine (NE, 1 μg/kg i.v.), and 10 min later animals received vehicle (saline) or *Escherichia coli* LPS (10 mg/kg i.v.) and were monitored for 360 min. The pressor responses to NE were reassessed at every hour after vehicle or LPS injection. Prior to (i.e. at time 0) and at 1 h or 6 h after vehicle or LPS, 0.3-0.5 ml of blood was taken to measure the changes in TNF-α and nitrate (an indicator of NO). Any blood withdrawn was immediately replaced by the injection of an equal volume of saline (i.v.) in order to maintain the blood volume. The doses of terbutaline (10 μg/kg/min for 30 min) and dobutamine (3 μg/kg/min for 330 min) used in this study were according to our previous study (15, 44), but these drugs were intravenously infused at 30 min after the injection of LPS. All hemodynamic and biochemical parameters were recorded for 6 h in all animal groups.

Measurement of TNF-α in Plasma Levels

Blood samples (0.3 ml) for the measurement of TNF-α level in the plasma were obtained at 0 and 1 h after the injection of saline or LPS. These samples

were collected from a catheter placed in the carotid artery and were centrifuged at 7,200 *g* for 3 min in order to obtain the plasma for measuring the levels of TNF-α and nitrate (as described bellow). The plasma samples (100 μl) were diluted 1:2 and TNF-α was measured in duplicate with an enzyme linked immunoadsorbent assay (ELISA) kit (Genzyme Co., Cambridge, MA, USA) as previously described (15, 44).

Determination of Plasma Nitrate

Fifty microliters plasma, which was kept in -20 °C freezer, was thawed and de-proteinized by incubating them with 95% ethanol (4 °C) for 30 min. The samples were subsequently centrifuged for a further 5 min at 14,000 *g*. It is noted that the nitrate concentration in plasma depicted in the study is actually the total nitrite and nitrate concentration in plasma. In this method nitrate is reduced to NO via nitrite. The amounts of nitrate in the plasma (6 μl) were measured by adding a reducing agent (0.8% VCl_3 in 1N HCl) to the purge vessel to convert nitrate to NO, which was stripped from the plasma by using a helium purge gas. The NO is then drawn into the Sievers Nitric Oxide Analyzer (Sievers 280 NOA, Sievers Inc., Boulder, CO, USA). Nitrate concentrations were calculated by comparison with standard solutions of sodium nitrate (Sigma Chemical Co., St. Louis, MO, USA).

Measurement of $O_2\cdot^-$ Production in the Aorta

To assess aortic $O_2\cdot^-$ production, thoracic aortas were obtained from sham-treated controls as well as from endotoxemic rats treated with vehicle or drugs at 6 h after the injection of saline or LPS. The vessels were cleared of adhering periadventitial fat, taking care not to damage the endothelium. The thoracic aortas were cut into rings of 5 mm width and incubated with warmed (37 °C), oxygenated (95% O_2/5% CO_2) Krebs-Hepes buffer for 30 min and then gently transferred to scintillation plates. These scintillation plates containing Krebs-Hepes buffer with 1.25 mM lucigenin (final volume of 250 μl) were placed into a microplate luminometer (LB96V, Berthold, Germany). Counts were obtained at 90-sec intervals at room temperature. Plates containing all components with the exception of aortic rings were counted and these blank values subtracted from the chemiluminescence signals obtained from the aortic rings. It is noted that reaction mixtures without aortic rings did not generate signals above background when observed for times of ≤15 min. In addition, we found that removal of vessel segments from the reaction mixture after 15 min resulted in a rapid decline in signal to background levels. All vessels were dried in a 90 °C oven for 24 hr. The results were expressed as relative unit of luminescence (RUL) per 15 minutes per milligram dry weight vessel (i.e. RUL/15 min/mg) and analyzed as previously described (43).

Chemicals

Bacterial LPS (*E. coli* serotype 0127:B8), NE bitartrate, urethane and terbutaline were purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.). Dobutamine hydrochloride was obtained from Eli Lilly Co. (Sin-tu, Taiwan, R.O.C.). All solutions were made in saline.

Statistics

All values in the figures and text are expressed as mean ± S.E.M. of *n* observations, where *n* represents the number of animals studied. Statistical evaluation was performed by analysis of variance (ANOVA) followed by a multiple comparison test (Scheffe's test). A *P* value of less than 0.05 was considered to be statistically significant.

Results

Plasma TNF-α

The basal plasma levels of TNF-α and nitrate were not significantly different between any of the experimental groups studied. The injection of LPS caused a significant increase in the plasma levels of TNF-α, which reached a peak at 60-90 min after LPS injection. Thus, we chose 1 h as a time point of TNF-α measurement (Fig. 1). In the sham-operated group, no significant amounts of TNF-α were detectable during the experimental period, indicating that the surgical procedure alone did not cause an increase in plasma TNF-α levels.

Treatment of LPS rats with terbutaline (10 μg/kg/min for 30 min, at 30 min after LPS) or dobutamine (3 μg/kg/min for 330 min, at 30 min after LPS) significantly inhibited the TNF-α level in plasma induced by LPS (10 mg/kg i.v.) (Fig. 1). However, injection of normal control rats with terbutaline (68 ± 33 and 51 ± 20 pg/ml at 0 and 1 h, respectively, $P > 0.05$, n=4) or dobutamine (79 ± 31 and 62 ± 40 pg/ml at 0 and 1 h, respectively, $P > 0.05$, n=4) alone had no significant effects on the TNF-α level in plasma.

Hemodynamic Parameters

As results shown in Fig. 2, the mean baseline values for MAP were between 108 ± 5 to 113 ± 2

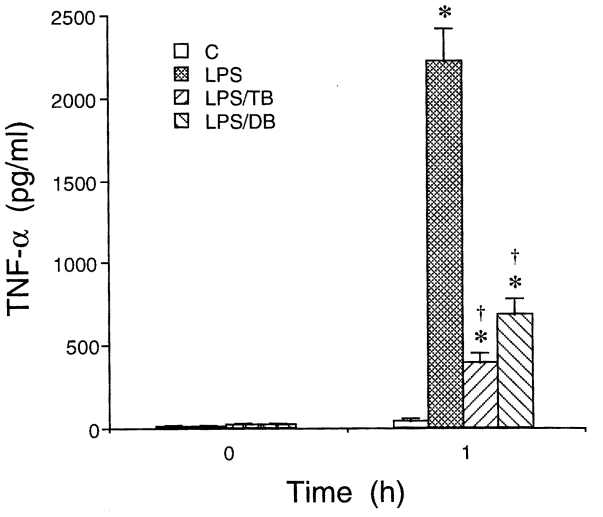

Fig. 1. Effects of terbutaline and dobutamine on plasma levels of TNF-α in rats treated with endotoxin. Depicted are the changes in plasma TNF-α levels during the experimental period in different groups of animals which received injection of vehicle (C; n=3), vehicle plus lipopolysaccharide (LPS; 10 mg/kg; n=6), terbutaline (10 μ/kg/min for 30 min, at 30 min after LPS) plus LPS (LPS/TB; n=5), or dobutamine (3 μg/kg/min for 330 min, at 30 min after LPS) plus LPS (LPS/DB; n=5). Note that each value of TNF-α is the mean of duplicate plasma samples from the same animal. Data are expressed as mean±S.E.M. $*P < 0.05$ represents significant differences when compared with the control group. $^{\dagger}P < 0.05$ represents significant differences between endotoxemic rats pretreated with and without terbutaline or dobutamine.

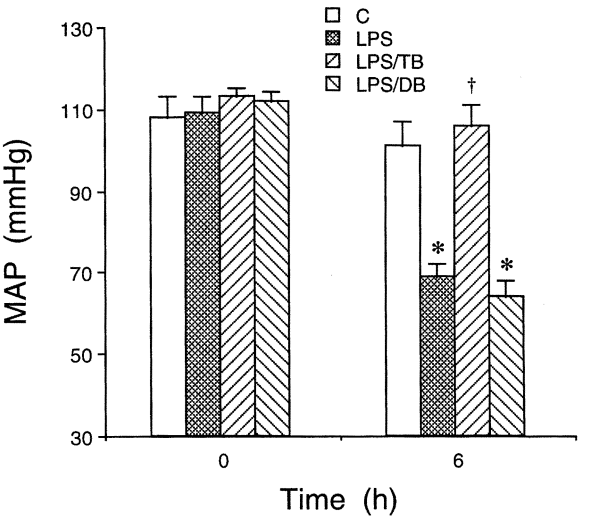

Fig. 2. Effects of terbutaline and dobutamine on mean arterial blood pressure (MAP) in rats treated with endotoxin. Depicted are the changes in MAP during the experimental period in different groups of animals which received injection of vehicle (C; n=6), vehicle plus LPS (LPS; 10 mg/kg; n=10), terbutaline (10 μg/kg/min for 30 min, at 30 min after LPS) plus LPS (LPS/TB; n=8), or dobutamine (3 mg/kg/min for 330 min, at 30 min after LPS) plus LPS (LPS/DB; n=6). Data are expressed as mean±S.E.M. $*P < 0.05$ represents significant differences when compared with the control group. $^{\dagger}P < 0.05$ represents significant differences between endotoxemic rats pretreated with and without terbutaline.

mmHg in all animal groups studied and were not significantly different between groups. The injection of LPS resulted in a decrease in MAP from 109 ± 4 mmHg at time 0 to 69 ± 3 mmHg at 6 h. In the sham-operated group, there was no significant change of MAP during the experimental period (i.e. from 108 ± 5 mmHg at time 0 to 103 ± 6 mmHg at 6 h). The infusion of terbutaline, but not dobutamine, at 30 min after LPS-injection caused a complete restoration in MAP.

As results shown in Fig. 3, the baseline mean values for HR were between 307 ± 11 to 317 ± 10 beats/min in all animal groups studied and were not significantly different between groups. Figure 3 demonstrates that administration of LPS causes a significant increase in HR at the end of the experimental period (i.e. from 317 ± 11 beats/min at time 0 to 409 ± 8 beats/min at 6 h). The infusion of terbutaline caused a reduced tachycardia while that of dobutamine significantly enhanced the tachycardia elicited by LPS.

It is noted that injection of normal control rats with terbutaline alone had no significant effects on MAP except causing a transient hypotension which was accompanied with a reflex tachycardia within 30 min, whereas dobutamine alone significantly increased

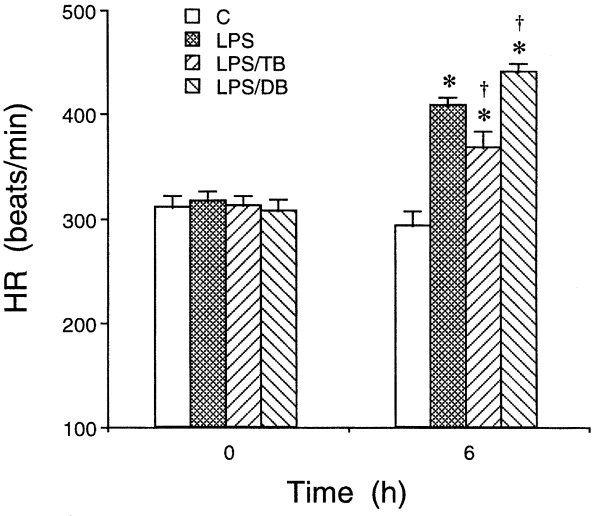

Fig. 3. Effects of terbutaline and dobutamine on heart rate (HR) in rats treated with endotoxin. Depicted are the changes in HR during the experimental period in different groups of animals which received injection of vehicle (C; n=6), vehicle plus LPS (LPS; 10 mg/kg; n=10), terbutaline (10 μg/kg/min for 30 min, at 30 min after LPS) plus LPS (LPS/TB; n=8), or dobutamine (3 μg/kg/min for 330 min, at 30 min after LPS) plus LPS (LPS/DB; n=6). Data are expressed as mean±S.E.M. $*P < 0.05$ represents significant differences when compared with the control group. $^{\dagger}P < 0.05$ represents significant differences between endotoxemic rats pretreated with and without terbutaline or dobutamine.

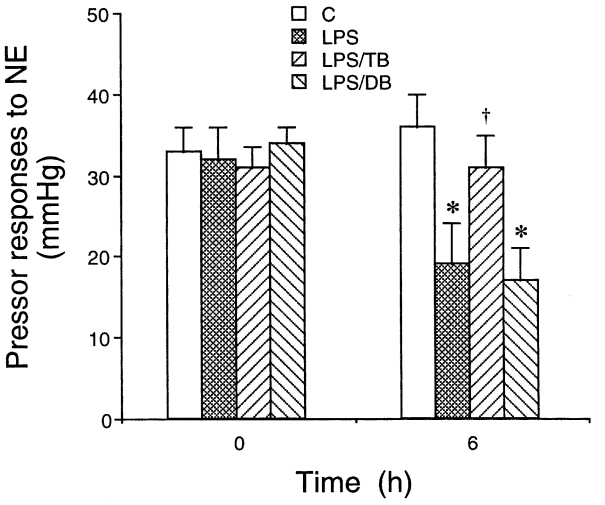

Fig. 4. Effects of terbutaline and dobutamine on pressor responses to NE *in vivo* in rats treated with endotoxin. Depicted are the changes in pressor responses to NE in different groups of animals which received injection of vehicle (C; n=6), vehicle plus LPS (LPS; 10 mg/kg; n=10), terbutaline (10 μg/kg/min for 30 min, at 30 min after LPS) plus LPS (LPS/TB; n=8), or dobutamine (3 μg/kg/min for 330 min, at 30 min after LPS) plus LPS (LPS/DB; n=6). Data are expressed as mean ± S.E.M. $*P < 0.05$ represents significant differences when compared with the control group. $^{\dagger}P < 0.05$ represents significant differences between endotoxemic rats pretreated with and without terbutaline.

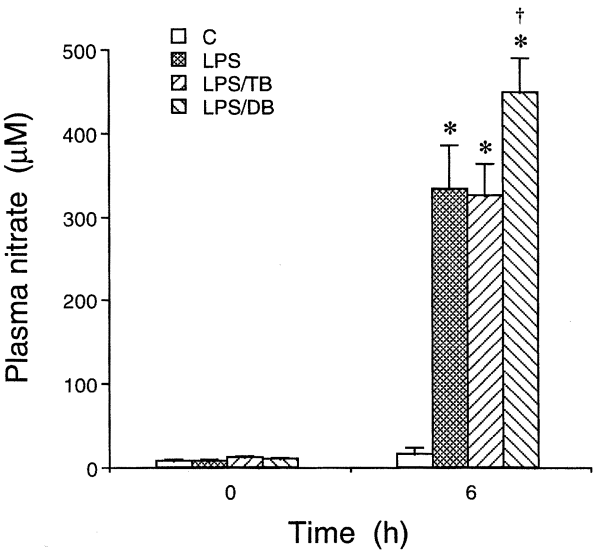

Fig. 5. Effects of terbutaline and dobutamine on plasma levels of nitrate in rats treated with endotoxin. Depicted are the changes in plasma nitrate levels during the experimental period in different groups of animals which received injection of vehicle (C; n=6), vehicle plus LPS (LPS; 10 mg/kg; n=10), terbutaline (10 μg/kg/min for 30 min, at 30 min after LPS) plus LPS (LPS/TB; n=8), or dobutamine (3 μg/kg/min for 330 min, at 30 min after LPS) plus LPS (LPS/DB; n=6). Data are expressed as mean ± S.E.M. $*P < 0.05$ represents significant differences when compared with the control group. $^{\dagger}P < 0.05$ represents significant differences between endotoxemic rats pretreated with and without dobutamine.

HR (311 ± 10 and 403 ± 14 beats/min at 0 and 6 h, respectively, $P < 0.05$, n=6).

Vascular Reactivity to NE In Vivo

The mean baseline values for the pressor responses to NE ranged from 31 ± 2 to 34 ± 2 mmHg and were not significantly different between any of the experimental groups studied. Injection of LPS resulted in a substantial attenuation of the pressor responses elicited by NE (Fig. 4). In contrast, in the sham-operated group, injection of saline had no significant effect on the NE-induced pressor responses at the 6-h experimental period.

Treatment of rats with terbutaline, but not dobutamine, significantly ameliorated the vascular hyporeactivity to NE at 6 h after LPS-injection (Fig. 4). However, the injection of normal control rats with terbutaline (31 ± 4 and 32 ± 3 mmHg at 0 and 6 h, respectively, $P > 0.05$, n=6) or dobutamine (33 ± 3 and 32 ± 4 mmHg at 0 and 6 h, respectively, $P > 0.05$, n=6) alone had no significant effects on the pressor responses to NE *in vivo*.

Plasma Nitrate

The basal plasma levels of nitrate were not significantly different between any of the experimental groups studied. The injection of LPS caused a significant increase in the plasma level of nitrate and endotoxemia for 6 h was associated with a 40-fold rise (Fig. 5). In the sham-operated group, there was no significant change of plasma nitrate during the experimental period.

Treatment of LPS rats with terbutaline had no effect on the plasma nitrate, whereas dobutamine further increased the nitrate level in plasma. However, injection of normal control rats with terbutaline (16±6 and 13 ± 4 μM at 0 and 6 h, respectively, $P > 0.05$, n=6) or dobutamine (18 ± 5 and 21 ± 5 μM at 0 and 6 h, respectively, $P > 0.05$, n=6) alone had no significant effects on the nitrate level in plasma.

Aortic $O_2\cdot^-$

The basal production of $O_2\cdot^-$ was detectable in thoracic aortas obtained from sham-treated rats (2374 ± 121 RUL/15 min/mg, n=6). The injection of LPS caused about a three times increase in the aortic $O_2\cdot^-$ level (Fig. 6). Treatment of LPS-rats with terbutaline significantly inhibited the production of $O_2\cdot^-$ in the aorta, whereas dobutamine slightly, but significantly, further enhanced the production of aortic $O_2\cdot^-$ (Fig. 5).

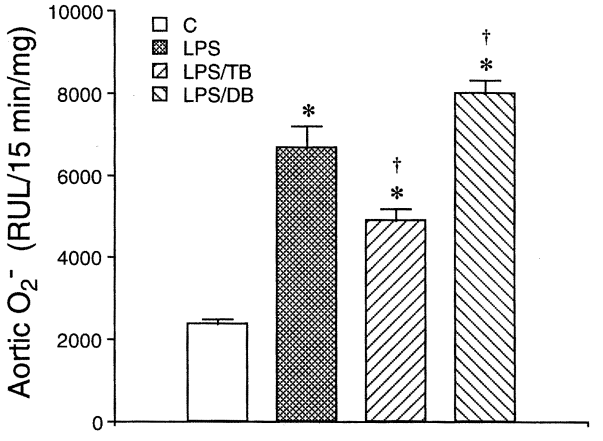

Fig. 6. Effects of terbutaline and dobutamine on the production of superoxide anion ($O_2\cdot^-$) in aortas from rats treated with endotoxin. Depicted are the changes in aortic $O_2\cdot^-$ levels during the experimental period in different groups of animals which received injections of vehicle (C; n=6), vehicle plus LPS (LPS; 10 mg/kg; n=10), terbutaline (10 μg/kg/min for 30 min, at 30 min after LPS) plus LPS (LPS/TB; n=8), or dobutamine (3 μg/kg/min for 330 min, at 30 min after LPS) plus LPS (LPS/DB; n=6). Data are expressed as mean ± S.E.M. $^*P < 0.05$ represents significant differences when compared with the control group. $^\dagger P < 0.05$ represents significant differences between endotoxemic rats pretreated with and without terbutaline or dobutamine.

It is noted that injection of normal control rats with terbutaline (2210 ± 101 RUL/15 min/mg, n=6) or dobutamine (2457 ± 137 RUL/15 min/mg, n=6) alone had no significant effects on the $O_2\cdot^-$ level in the aorta.

Discussion

In this study, we demonstrated that cAMP-elevating agents, terbutaline and dobutamine, had different effects on animals with endotoxemia, although they all inhibited the production of TNF-α. Terbutaline is a selective β_2-agonist with very low affinity for α-adrenoceptors, whereas dobutamine is selective stimulator for β_1-adrenoceptor. Occupation of β_1- and β_2-adrenoceptor by dobutamine and terbutaline, respectively, results in a conformational change that leads to G protein activation. This in turn activates adenylate cyclase, which results in the conversion of ATP to cAMP, the second messenger of β_1- and β_2-adrenoceptor function. Based on our present data, this study is the first to demonstrate that terbutaline therapeutically (i) reduces TNF-α levels in the plasma and $O_2\cdot^-$ levels in the aorta, and (ii) attenuates the delayed circulatory failure and vascular hyporeactivity to NE in rats with endotoxic shock. In contrast, dobutamine (i) enhances NO levels in the plasma and $O_2\cdot^-$ levels in the aorta, and (ii) did not improve the fall in MAP and the vascular hyporeactivity to NE. These results indicate that terbutaline, but not dobutamine, has therapeutical effects in animals with endotoxemia.

A number of studies suggest that the suppression of LPS-induced TNF-α plasma levels is associated with a parallel increase in interleukin-10 (IL-10). For instance, this is observed with prostaglandin E (34), with epinephrine or isoproterenol (37, 38), and with adenosine receptor agonists (10). The inhibition of TNF-α production by all of these agents has been linked to their stimulating effect on adenylate cyclase, leading to an increase in intracellular cAMP levels. Prostaglandin E_2 enhances production of LPS-induced IL-10 and inhibits release of LPS-induced TNF-α *in vivo* and *in vitro*, and that the effect of prostaglandin E_2 can be mimicked by agents that similarly elevate intracellular cAMP levels (37, 45). Similar findings were observed with dibutyryl cAMP, the stable analogue of cAMP *in vitro* and *in vivo* (3, 27). Thus, it is generally assumed that increased intracellular cAMP levels are responsible for both increased IL-10 production and decreased TNF-α release reported with the administration of these agents. However, the inhibition of TNF-α release by dobutamine did not show any beneficial effect on animals with endotoxemia. Thus, it was not meaningful to determine the IL-10 level in this study.

It is well established that overproduction of NO via iNOS contributes to hypotension and vascular hyporeactivity to vasoconstrictor agents in endotoxic shock. The expression of iNOS in animals with endotoxemia is associated with the level of cytokines such as TNF-α, IL-1 and interferon-γ (21, 39). Our data confirm that the level of TNF-α is increased in animals with endotoxemia and this increment of TNF-α may be associated with the overproduction of NO in endotoxemic animals. Indeed, NO levels in plasma were significantly increased after several hours of endotoxemia; however, the reduction of TNF-α alone (e.g. terbutaline and dobutamine in this study) may not be enough to inhibit the production of NO. Although agents that inhibit the induction NO were able to prevent or reverse the delayed hypotension and the *in vivo* or *ex vivo* vascular hyporeactivity (36), our present study shows that both terbutaline and dobutamine do not affect NO production and only terbutaline has beneficial effects against the LPS-induced hypotension and vascular hyporeactivity in this experimental model, suggesting that (i) NO only plays a partial role in this circulatory failure and (ii) the production of $O_2\cdot^-$ may be more related to the vascular hyporeactivity in septic shock. Similar findings have also been proposed by Metcalf et al. (19) and Macarthur et al. (17), respectively. Thus, one may question the relationship between NO and endotoxic shock. However, the further increase of

plasma NO by dobutamine may lead to the detrimental insult caused by endotoxin, suggesting that terbutaline inhibits the development of delayed vascular dysfunction by inhibiting other mediators (e.g. $O_2\cdot^-$) than NO in the present study. In addition, it seems that NO overproduction is associated with the hypotension induced by endotoxin, whereas vascular $O_2\cdot^-$ formation is more relevant to vascular hyporeactivity to vasoconstrictor agents in endotoxic shock. We also propose that β_1-adrenoceptor agonists alone are not suitable therapeutic agents for septic shock since they may increase the formation of $O_2\cdot^-$ in the vasculature. This discrepancy of $O_2\cdot^-$ in the vasculature between β_2- and β_1-adrenoceptor agonists has also been demonstrated by a similar finding (5), which demonstrated that dopamine, an agent possesses β_1-adrenoceptor agonist property, modulated chemokine production by an oxidative rather than antioxidant mechanism, suggesting that the discrepancy may result from the specificity of β-adrenoceptor.

Although a short-term infusion of dobutamine had been used as a prognostic method in patients with the sepsis syndrome (42), the usefulness of the dobutamine test for detecting a pathological oxygen uptake/supply dependency has been challenged (20). For instance, this may be partly due to mathematical coupling of shared variables (26). In addition, the unrestrained use of dobutamine in an attempt to achieve supranormal oxygen transport values even increased mortality in a heterogeneous group of critically ill patients (12). Moreover, The thermogenic effect of NE in the splanchnic region (4) may have blunted an additional effect of dobutamine; and, finally, reduced β-adrenoceptor responsiveness due to this treatment may have attenuated the efficacy of dobutamine (28, 32). However, the administration of NE in this study was not as a therapeutic agent, but as an indicator of vascular reactivity. In addition, terbutaline, another agonist of β-adrenoceptor, showed beneficial effect in septic animals. Thus, it is unlikely that ineffectiveness of dobutamine on animals with sepsis is because of the thermogenic effect of NE.

We conclude that our present study provides functional evidence showing that terbutaline, but not dobutamine, exerts marked anti-inflammatory and antioxidant effects by suppressing LPS-induced TNF-α and $O_2\cdot^-$ production, while has no significant effect on the NO production. Regarding the molecular mechanism of action, several possibilities should be addressed. Some of the effects may be related to the primary cAMP-increasing effect of the drug (effects on TNF-α, IL-10, and possibly, IL-12) (11, 24). Although the reduction of TNF-α production is a cAMP-mediated effect, however, this is not certain for having improvement in animals with hypotension (according to the results of dobutamine). While the mechanism and the sequence of effects of terbutaline on the LPS-induced inflammatory response require further studies, the present study coupled with other current observations (14) suggests that β_2-adrenoceptor agonists are better therapeutic agents than β_1-adrenoceptor agonists in sepsis, possibly via modulation of the production of key inflammatory mediators (e.g. TNF-α and $O_2\cdot^-$).

Acknowledgments

This work was supported by grants NSC 89-2320-B-016-011-M53 from the National Science Council and DOD-91-15 from the Ministry of National Defense as well as TAGH-039006 from the Taichung Army General Hospital, Taiwan, R.O.C.

References

1. Altura, B.M., Lefer, A.M. and Schumer, W. *Handbook of Shock and Trauma*. Vol. 1: Basic Science. NY: Raven Press, 1983.
2. Amezcua, J.L., Palmer, R.M., de Souza, B.M. and Moncada, S. Nitric oxide synthesized from L-arginine regulates vascular tone in the coronary circulation of the rabbit. *Br. J. Pharmacol*. 97: 1119-1124, 1989.
3. Arai, T., Hiromatsu, K., Kobayashi, N., Takano, M., Ishida, H., Nimura, Y. and Yoshikai, Y. IL-10 is involved in the protective effect of dibutyl cyclic adenosine monophosphate on endotoxin-induced inflammatory liver injury. *J. Immunol*. 155: 5743-5749, 1995.
4. Bearn, A.G., Billing, B. and Sherlock, S. The effect of adrenaline and noradrenaline on hepatic blood flow and splanchnic carbohydrate metabolism in man. *J. Physiol*. 115: 430-441, 1951.
5. Beck, G.C., Oberacker, R., Kapper, S., von Zabern, D., Schulte, J., van Ackern, K., van der Woude, F.J. and Yard, BA. Modulation of chemokine production in lung microvascular endothelial cells by dopamine is mediated via an oxidative mechanism. *Am. J. Respir. Cell Mol. Biol*. 25: 636-643, 2001.
6. Beckman, J.S., Beckman, T.W., Chen, J., Marshall, P.A. and Freeman, B.A. Apparent hydroxyl radical production by peroxynitrite: implications for endothelial injury from nitric oxide and superoxide. *Proc. Natl. Acad. Sci. USA* 87: 1620-1624, 1990.
7. Beutler, B. and Cerami, A. The biology of cachectin/TNFα: a primary mediator of the host response. *Ann. Rev. Immunol*. 7: 625-655, 1989.
8. Chu, A., Chambers, D.E., Lin, C.C., Kuehl, W.D., Palmer, R.M. and Moncada, S. Effects of inhibition of nitric oxide formation on basal vasomotion and endothelium-dependent responses of the coronary arteries in awake dog. *J. Clin. Invest*. 87: 1964-1968, 1991.
9. Deitsch, E.A. Multiple organ failure: pathophysiology and potential future therapy. *Ann Surg* 216: 117-134, 1992.
10. Hasko, G., Szabo, C., Nemeth, Z.H., Kvetan, V., Pastores, S.M. and Vizi, E.S. Adenosine receptor agonists differentially regulate IL-10 and TNF production in endotoxemic mice. *J. Immunol*. 157: 4634-4640, 1996.
11. Hasko, G., Szabo, C., Nemeth, Z.H., Salzman, A.L. and Vizi, E.S. Stimulation of β-adrenoceptors inhibits endotoxin-induced IL-12 production in normal and IL-10 deficient mice. *J. Neuroimmunol*. 88: 57-61, 1998.
12. Hayes, M.A., Timmins, A.C., Yau, E.H., Palazzo, M., Hinds, C.J. and Watson, D. Elevation of systemic oxygen delivery in the treatment of critically ill patients. *N. Engl. J. Med*. 330: 1717-1722,

1994.
13. Hoffman, B.B. and Lefkowitz, R.J. Catecholamines, sympathomimetic drugs, and adrenergic receptor antagonists. In Hardman, J.G., Limbird, L.E., Molinoff, P.B., Ruddon, R.W. and Gilman, A.G. (eds): *Goodman & Gilman's The Pharmacological Basis of Therapeutic*s. 9th ed. NY: The McGraw-Hill Co. Inc. 1996, pp 199-248.
14. Kavelaars, A., van de Pol, M., Zijlstra, J. and Heijnen, C.J. β_2-Adrenergic activation enhances interleukin-8 production by human monocytes. *J. Neuroimmunol*. 77: 211-216, 1997.
15. Liao, M.H. and Wu, C.C. Effects of dobutamine on circulatory failure and survival in rats with endotoxemia. *J. Med. Sci*. 20: 416-428, 2000.
16. Lipton, S.A., Choi, Y.B., Pan, Z.H., Lei, S.Z., Chen, H.S., Sucher, N.J., Loscalzo, J., Singel, D.J. and Stamler, J.S. A redox-based mechanism for the neuroprotective and neurodestructive effects of nitric oxide and related nitroso-compounds. *Nature (London)* 364: 626-632, 1993.
17. Macarthur, H., Westfall, T.C., Riley, D.P., Misko, T.P. and Salvemini, D. Inactivation of catecholamines by superoxide gives new insights on the pathogenesis of septic shock. *Proc. Natl. Acad. Sci. U.S.A.* 97: 9753-9758, 2000.
18. McLean, J.S. and Byrick, R.J. ARDS and sepsis - definitions and new therapy. *Can. J. Anesth*. 40: 585-590, 1993.
19. Metcalf, K., Jungersten, L. and Lisander, B. Effective inhibition of nitric oxide by aminoguanidine does not reverse hypotension in endotoxemic rats. *Acta Anaesthesiol. Scand*. 46: 17-23, 2002.
20. Mira, J.P., Fabre, J.E, Baigorri, F., Coste, J., Annat, G., Artigas, A., Nitenberg, G. and Dhainaut, J.F. Lack of oxygen supply dependency in patients with severe sepsis. A study of oxygen delivery increase by military antishock trouser and dobutamine. *Chest* 106: 1524-1531, 1994.
21. Moncada, S., Palmer, R.M.J., Higgs, E.A. Nitric oxide: Physiology, pathophysiology and pharmacology. *Pharmacol. Rev*. 43: 109-142, 1991.
22. Nathan, C.F. Secretory products of macrophages. *J. Clin. Invest*. 79: 319-326, 1987.
23. Nathan, C., Xie, Q.W. Regulation of biosynthesis of nitric oxide. *J. Biol. Chem*. 269: 13725-13728, 1994.
24. Panina-Bordignon, P., Mazzeo, D., Di Lucia, P., D'Ambrosio, D., Lang, R., Fabbri, L., Self, C. and Sinigaglia, F. β_2-Agonists prevent Th1 development by selective inhibition of interleukin 12. *J. Clin. Invest*. 100: 1513-1519, 1997.
25. Parker, M.M., Shelhamer, J.H., Natanson, C., Alling, D. and Parillo, J.E. Seroa hemodynamic patterns in survivors and non-survivors of septic shock in humans. *Crit. Care Med*. 15: 923-929, 1987.
26. Phang, P.T., Cunningham, K.F., Ronco, J.J., Wiggs, B.R. and Russell, J.A. Mathematical coupling explains dependence of oxygen delivery in ARDS. *Am. J. Respir. Crit. Care Med*. 150: 318-323, 1994.
27. Platzer, C., Meisel, C., Vogt, K., Platzer, M. and Volk, H.D. Up-regulation of monocytic IL-10 by tumor necrosis factor-α and cAMP elevating drugs. *Int. Immunol*. 7: 517-523, 1994.
28. Reinelt, H., Radermacher, P., Fischer, G., Geisser, W., Trunk, E., Wiedeck, H., Mezody, M., Georgieff, M. and Vogt, J. Dobutamine and dopexamine and the splanchnic metabolic response in septic shock. *Clin. Intens. Care* 8: 38-41, 1997.
29. Repine, J.E. Scientific perspectives on adult respiratory distress syndrome. *Lancet* 339: 466-469, 1992.
30. Severn, A., Rapson, N.T., Hunter, C.A. and Liew, F.Y. Regulation of tumor necrosis factor production by adrenaline and beta-adrenergic agonists. *J. Immunol*. 148: 3441-3445, 1992.
31. Shoemaker, W.C., Appel, P.L. and Kram, H.B. Hemodynamic and oxygen transport effects of dobutamine in critically ill general surgical patients. *Crit. Care Med.* 14: 1032-1037, 1986.
32. Silverman, H.J., Penaranda, R., Orens, J.B. and Lee, N.H. Impaired beta-adrenergic receptor stimulation of cyclic adenosine monophosphate in human septic shock: association with myocardial hyporesponsiveness to catecholamines. *Crit. Care Med.* 21: 31-39, 1993.
33. Stoclet, J.C., Fleming, I., Gray, G., Julou-Schaeffer, G., Schneider, F., Schott, C., Schott, C. and Parratt, J.R. Nitric oxide and endotoxemia. *Circulation* 87(Suppl V): V77-V80, 1993.
34. Strassmann, G., Patil-Koota, V., Finkelman, F., Fong, M., Kambayashi, T. Evidence for the involvement of interleukin 10 in the differential deactivation of murine peritoneal macrophages by prostaglandin E_2. *J. Exp. Med*. 180: 2365-2370, 1994.
35. Sugino, K., Dohi, K., Yamada, K. and Kawasaki, T. Changes in the levels of endogenous antioxidants in the liver of mice with experimental endotoxemia and the protective effects of the antioxidants. *Surgery* 105: 200-206, 1989.
36. Szabo, C. Alterations in nitric oxide production in various forms of circulatory shock. *New Horizons* 3: 2-32, 1995.
37. Szabo, C., Hasko, G., Zingarelli, B., Nemeth, Z.H., Salzman, A.L., Kvetan, V., Pastores, S.M. and Vizi, E.S. Isoproterenol regulates tumor necrosis factor, interleukin-10, interleukin-6 and nitric oxide production and protects against the development of vascular hyporeactivity in endotoxemia. *Immunology* 90: 95-100, 1997.
38. Taffet, S.M., Singhel, K.J., Overholtzer, J.F. and Shurtleff, S.A. Regulation of tumor necrosis factor expression in a macrophage-like cell line by lipopolysaccharide and cyclic AMP. *Cell Immunol*. 120: 291-300, 1989.
39. Thiemermann, C. The role of the L-arginine: nitric oxide pathway in circulatory shock. *Adv. Pharmacol*. 28: 45-79, 1994.
40. Thiemermann, C., Wu, C.C., Szabo, C., Perretti, M. and Vane, J.R. Role of tumor necrosis factor in the induction of nitric oxide synthase in a rat model of endotoxin shock. *Br. J. Pharmacol*. 110: 177-182, 1993.
41. Tracey, K.J. and Cerami, A. Tumor necrosis factor, other cytokines and disease. *Annu. Rev. Cell Biol*. 9: 317-343, 1993.
42. Vallet, B., Curtis, S.E. and Chopin, C. Prognostic value of the dobutamine test in patients with sepsis syndrome: a prospective multicenter study. *Crit. Care Med*. 23: 415-416, 1995.
43. Wu, C.C., Chiao, C.W., Hsiao, G., Chen, A and Yen, M.H. Melatonin prevents endotoxin-induced circulatory failure in rats. *J. Pineal Res*. 30: 147-156, 2001.
44. Wu, C.C., Liao, M.H., Chen, S.J., Chou, T.C., Chen, A. and Yen, M. H. Terbutaline prevents circulatory failure and mitigates mortality in rodents with endotoxemia. *Shock* 14: 60-67, 2000.
45. Wu, C.C., Liao, M.H., Chen, S.J. and Yen, M.H. Pentoxifylline improves circulatory failure and survival in murine models of endotoxemia. *Eur. J. Pharmacol.* 373: 41-49, 1999.