Polymyxin B binds to anandamide and inhibits its cytotoxic effect

Yin Wang[a], Yan Liu[a], Krishna Pada Sarker[a], Mihoko Nakashima[a], Takeshi Serizawa[b], Akio Kishida[b], Mitsuru Akashi[b], Masanori Nakata[a], Isao Kitajima[a], Ikuro Maruyama[a,*]

[a] *Department of Laboratory and Molecular Medicine, Faculty of Medicine, Kagoshima University, 8-35-1 Sakuragaoka, Kagoshima 890-8520, Japan*
[b] *Department of Applied Chemistry and Chemical Engineering, Faculty of Engineering, Kagoshima University, Kagoshima, Japan*

Received 1 February 2000; received in revised form 17 February 2000

Edited by Masayuki Miyasaka

Abstract Anandamide (ANA), an endogenous cannabinoid, can be generated by activated macrophages during endotoxin shock and is thought to be a paracrine contributor to hypotension. We discovered that ANA in saline/ethanol solution and in serum was efficiently adsorbed in a polymyxin B (PMB)-immobilized beads column and eluted with ethanol. We confirmed the direct binding of PMB to ANA by using surface plasmon resonance. The adsorption of ANA by PMB may abolish the diverse effects of ANA such as hypotension, immunosuppression, and cytotoxicity, and may suggest a new therapeutic strategy for endotoxin shock.

Key words: Cannabinoid; Anandamide; Polymyxin B; Endotoxin shock; Surface plasmon resonance

1. Introduction

Since lipopolysaccharide (LPS) shock is still a serious event with high mortality and multiple organ failure, the development of efficient treatments has been expected. Such mediators as tumor necrosis factor-α (TNF-α), interleukin-1 (IL-1), platelet activating factor, thromboxane A_2, and NO from inducible NO synthase have been proposed as candidate mediators of LPS shock. However, pharmacological antagonists, inhibitors, and monoclonal antibodies of these factors have never shown a sufficient effect on LPS shock [1,2], therefore, the existence of the other mediators has been proposed. Recently it was described that anandamide (ANA) is generated by macrophages in response to LPS during Gram-negative bacterial sepsis, and endogenous cannabinoids may have an important role in the pathomechanism of LPS shock, especially hypotension [3].

ANA as an endogenous cannabinoid was initially isolated from porcine brain, and shown to exert its diverse biological functions through cannabinoid receptors, CB1 and CB2 [4]. Thereafter it was found to exist not only in the brain but also in various peripheral tissues [5]. Functional studies have demonstrated that it may possess similar functions to cannabinoid in the brain and immune system [6–8]. It has been reported that a cannabinoid, Δ^9-tetrahydrocannabinol (THC), may regulate immune cells in a biphasic manner. Lower concentrations of THC enhanced production and release of proinflammatory cytokines including IL-1, IL-6, TNF-α, and NO, but higher concentrations suppressed the effects of the above-mentioned molecules [9,10]. ANA may also play a similar role in immune cells to THC. Recent evidence indicates that activation of vascular cannabinoid receptors contributes to hypotension caused by hemorrhagic or endotoxic shock, and that ANA and 2-arachidonoylglycerol (2-AG) probably serve as paracrine mediators of hypotension via activation of the CB1 receptor expressed in the peripheral vasculature [11–14].

Polymyxin B (PMB), which is formed from a cyclic heptapeptide moiety linked to a peptide side chain which terminates with a short fatty acid residue, blocks the biological effects of LPS through binding to LPS [15]. However, the potent toxic effect of PMB to mammalian cells limits its clinical use. To reduce this toxic effect and derive benefit from the LPS blocking property, PMB was immobilized onto insoluble fibers creating a hemoperfusion column to use as an extracorporeal endotoxin removal device for the treatment of patients with Gram-negative bacterial sepsis. Hemoperfusion using this device improves hypotension and results in an increment of survival rates in both experimental animal models and patients with LPS shock [16–19]. This led us to determine whether PMB binds to ANA resulting in the neutralization of the biological function of ANA.

In the present study, we show that PMB directly binds to ANA and neutralizes its biological activity. These observations may present a new strategy for the treatment of endotoxin-induced hypotension.

2. Materials and methods

2.1. Materials

ANA, LPS (*Escherichia coli* strain 055:B5), phosphatidylcholine (PC) and arachidonic acid (AA) were obtained from Sigma Chemical Co. (St. Louis, MO, USA). 2-AG was obtained from Calbiochem-Novabiochem Co. (La Jolla, CA, USA). PMB sulfate was obtained from Wako Pure Chemical Co. (Osaka, Japan), and Affi-Prep polymyxin matrix from Bio-Rad Laboratories (Hercules, CA, USA). Anhydrate benzene, acetonitrile and water were of HPLC grade. All other reagents used were of analytical grade. Stock solution of anandamide was prepared in ethanol (EtOH) and stored in aliquots at −80°C.

2.2. Cell culture and cell viability test

Rat pheochromocytoma (PC-12) cells were grown as described previously [20] in RPMI 1640 medium, supplemented with 10% heat-inactivated (at 56°C for 30 min) horse serum, and 5% fetal bovine serum, 100 μg/ml penicillin, and 100 μg/ml streptomycin under 5% CO_2 and 100% humidity at 37°C. Cell viability (cytotoxicity) was assayed by fluorescence-activated cell sorting (FACS) analyzer (EPICS, Coulter, USA) using propidium iodide (PI), as described previously [21]. Briefly, PC-12 cells were plated at a density of 2.5×10^5 cells per 35 mm dish. Following 18 h culture, monolayers were washed with serum-free RPMI medium once, and pretreated with the indicated concentrations of PMB, and the monolayers were incubated with or without 10 μM ANA for 24 h in RPMI medium without serum. Then the harvested cells were washed with PBS, and centri-

*Corresponding author. Fax: (81)-99-275 2629.
E-mail: rinken@khosp2.kufm.kagoshima-u.ac.jp

PII: S0014-5793(00)01313-2

fuged (200×*g* for 5 min). The pellet was resuspended in 70% ethanol (2 ml), and incubated at −20°C for 20 min. Following an additional 15 min of incubation with PI (5 μg/ml of PBS solution) in the dark in the presence of RNase (5 μg/ml), cell viability was tested (2×10^4 cells each time) using a FACS analyzer.

2.3. PMB-immobilized beads affinity chromatography

A chromatography column (0.5×3 cm), which was packed with 500 μl of 50% Affi-Prep PMB suspension, was washed with 1 ml of saline and centrifuged to remove the saline. Then the various amounts of ANA dissolved in 100 μl of 50% saline/ethanol were loaded on the columns and the effluents were collected. The columns were washed with 500 μl of saline and the washing solution was collected. The ANA adsorbed on the beads was eluted by 200 μl of 95% ethanol. ANA in all solutions was assayed using reverse phase high performance liquid chromatography (RP-HPLC). In the experiment of verifying the capacity of PMB-immobilized beads to adsorb ANA in the presence of human serum, various amounts of ANA dissolved in 1 ml solutions with increasing concentrations of human serum were loaded onto the column packed with PMB-immobilized beads. After intensive washing with saline, ANA adsorbed on the beads was eluted with 200 μl of 95% ethanol and the total contents in the collected eluates were determined by RP-HPLC.

2.4. HPLC analysis

HPLC was performed as described by Lang et al. [22]. Briefly, a Gilson System (Gilson Inc., USA) equipped with a 305 pump and a 118 UV-Vis detector was used and separation was carried out on a TSK gel ODS 80TM (50×4.6 mm, i.d. 5 μm) column (Tosoh, Tokyo, Japan) with acetonitrile and 8.5% aqueous phosphoric acid (9:1, v/v) at a flow rate of 1 ml/min. Quantitation was based on the integration of peak areas at 204 nm. The amount of product formed was calculated from calibration curves of appropriate standards.

2.5. Surface plasmon resonance (SPR) analysis

SPR analysis was performed using a BIAcore 2000 biosensor system (Pharmacia Biosensor AB, Uppsala, Sweden). All experiments were performed at 20°C with a constant flow rate of 10 μl/min. PMB was covalently immobilized directly on a carboxymethylated dextran matrix (CM5 sensor chips, Pharmacia Biosensor) at a concentration of 40 mg/ml in 10 mM sodium acetate, pH 4.8. PMB was coupled through its amino group according to the amine coupling kit supplied by the manufacturer. Nearly 3000 resonance units (RU) of PMB were immobilized under these conditions, where 1 RU corresponds to an immobilized PMB concentration of ∼1 pg/mm^2. The unreacted moieties on the surface were blocked with 35 μl of 1 mM ethanolamine pH 8.5. The immobilization of PMB was carried out in 10 mM HEPES, pH 7.4, 150 mM NaCl and 3.4 mM EDTA. Interaction of ANA and PMB was carried out as follows: the sensor chip (CM5) with immobilized PMB was brought into contact with saline containing 4% ethanol buffer. The flow rate of the buffer solution was kept at a high level (10 μl/min) in order to prevent mass transport limitations at the sensor surface/solution interface. After a stable baseline was obtained, 30 μl ANA or saline containing ethanol was injected through the PMB surface. Next, 40 μl saline containing 4% ethanol buffer was injected to observe the dissociation.

3. Results

3.1. PMB neutralizes the cytotoxic effect of ANA

ANA at a dose of 10 μM induced the death of PC-12 cells cultured in serum-free medium (Fig. 1). Treatment of the cells with PMB prevented cell death induced by ANA in a dose-dependent manner, as compared to the vehicle (0.01% ethanol), because ANA was dissolved in ethanol. This result suggests that PMB adsorbs ANA and neutralizes or inhibits the biological function of ANA.

3.2. PMB directly binds to ANA

To elucidate the mechanism of the inactivation of the cytotoxic effect of ANA on PC-12 cells in culture, we investigated the interaction of PMB and ANA in vitro using PMB-immobilized beads. ANA was dissolved in saline containing 50% ethanol and loaded onto a column packed with PMB-immobilized beads. The contents of the feed (before column), effluent (after column), and eluates of saline and 95% ethanol, respectively, were determined by RP-HPLC. The chromatograms of ANA in each case mentioned above are shown in Fig. 2. In our analytic system, the retention time for standard ANA is 4.77 min. Before passing through the PMB-immobilized beads, ANA appeared as a high and sharp peak at 4.77 min, but after flowing through the column, the 4.77 min peak disappeared in both the effluent and eluate of saline. A large amount of saline failed to elute ANA from the column, whereas 95% ethanol eluted almost all ANA from the column (Fig. 2, eluate of 95% ethanol). Recovery of ANA in the eluate of 95% ethanol was as high as 95%. These results demonstrated that PMB-immobilized beads strongly adsorbed ANA.

3.3. Capacity of PMB-immobilized beads to adsorb ANA

To verify the specificity and capacity of ANA binding to PMB-immobilized beads, we investigated the binding study using increasing amounts of ANA as shown in Table 1. Different amounts of ANA in 100 μl of a 1:1 ethanol/saline solution were loaded on a column packed with 500 μl of a 50% suspension of Affi-Prep polymyxin support. The content of ANA in the effluent of each concentration was determined

Table 1
Capacity of PMB-immobilized beads to adsorb ANA, AA and 2-AG

Loaded substance (μg)	Recovery (%)				
	effluent	saline	50% ethanol	70% ethanol	95% ethanol
ANA					
5	0	0	0	47.1 ± 5.1	91.5 ± 3.2
25	0	0	0.77 ± 0.23	53.3 ± 4.3	93.4 ± 2.7
50	0	0	4.30 ± 0.68	61.2 ± 3.8	94.2 ± 2.1
100	0.89	0.2	10.9 ± 1.5	70.4 ± 3.5	94.6 ± 2.3
300	67	–	–	–	–
AA					
100	4.3	1.5	26.5 ± 4.7	82.1 ± 4.7	95.4 ± 3.2
2-AG					
100	0.21	0	8.2 ± 1.1	65.3 ± 1.8	93.8 ± 2.6

Various amounts of ANA, 100 μg of AA or 100 μg of 2-AG in 100 μl of 1:1 ethanol/saline were loaded on a column packed with 500 μl of suspension of PMB-immobilized beads. The ANA amount in the effluent, eluate of 1.5 ml saline and eluate of 200 μl ethanol at indicated concentrations was determined by RP-HPLC. The recovery of ANA in effluent and each eluate is represented as a percentage relative to the amount of ANA prior to loading.

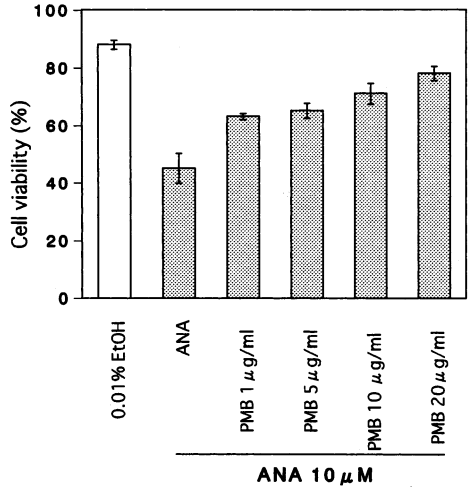

Fig. 1. PMB prevents ANA-induced PC-12 cell death. PC-12 cells were plated onto a 6 well dish at a density of 2×10^5 cells/well. Following 18 h culture, cells were pretreated with PMB for 1 h, then incubated with 10 μM of ANA or 0.01% ethanol (EtOH, as a vehicle) for an additional 24 h in serum-free medium. The cell viability was determined as described in Section 2. The results are means ± S.D. of three independent experiments.

by RP-HPLC. Only 0.89% of total ANA was detected in the effluent of 100 μg/ml ANA, while 67% of total ANA corresponding to 300 μg of ANA passed through the column of PMB-immobilized beads, indicating that about 100 μg of ANA was adsorbed by the PMB-immobilized beads (Table 1). Thus, the capacity of PMB-immobilized beads to adsorb ANA is calculated to be about 0.4 mg/ml of PMB beads suspension. As shown in Table 1, although a high concentration of ANA was loaded, saline failed to elute ANA from the column. 50% ethanol was unable to elute ANA from the column until the amount of loaded ANA exceeded 25 μg, and even when 100 μg of ANA was loaded, only 10.9% of ANA adsorbed in the column was eluted by 50% ethanol. In contrast, 75% ethanol and 95% ethanol recovered ANA at a

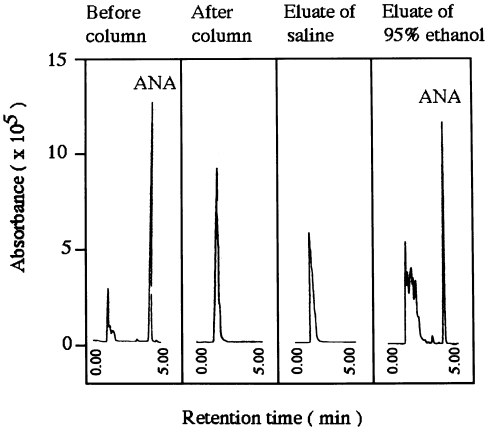

Fig. 2. RP-HPLC profiles showing the adsorption of ANA by PMB-immobilized beads. 10 μg of standard ANA was dissolved in 100 μl of 1:1 ethanol/saline and loaded on a column packed with 500 μl of PMB bead suspension. The content of ANA in the loading solution (before column), effluent (after column), eluate of 1.5 ml saline, and eluate of 200 μl of 95% ethanol was analyzed by RP-HPLC. The results were from five separate analyses. The recovery of ANA in the eluate of 95% ethanol is more than 95% with respect to the amount prior to loading.

high efficiency, especially 95% ethanol, which eluted more than 91% of ANA from the PMB beads at all concentrations of ANA loaded. Generally, with an increase in the concentration of ethanol, the capacity of ethanol to elute ANA from the column was increased, and only a high concentration of ethanol was capable of eluting ANA completely. PMB also adsorbed 2-AG and AA. Using the same concentrations as ANA, the adsorptive capacity of PMB for 2-AG seemed to be greater, but that for AA lower than for ANA.

3.4. PMB adsorbs ANA in the presence of serum

It is known that the binding of LPS to PMB is markedly reduced because of the presence of LPS binding protein in the serum. It has also been reported that specific transporter of ANA is present on the surface of human astrocytoma cell lines [23]. Thus, to elucidate whether the molecule that binds to ANA is present in the serum, we examined the capacity of PMB-immobilized beads in the column to adsorb ANA in the presence of the increasing amounts of human serum (0, 20, 40, 80, and 100% of serum). Addition of the serum to the solution containing different concentrations of ANA (50, 100, 200, 500, and 1000 ng/ml) gave rise to a decreased adsorption of ANA by PMB beads. However, the effect of serum was not saturable, even at the maximum concentration (100%) of

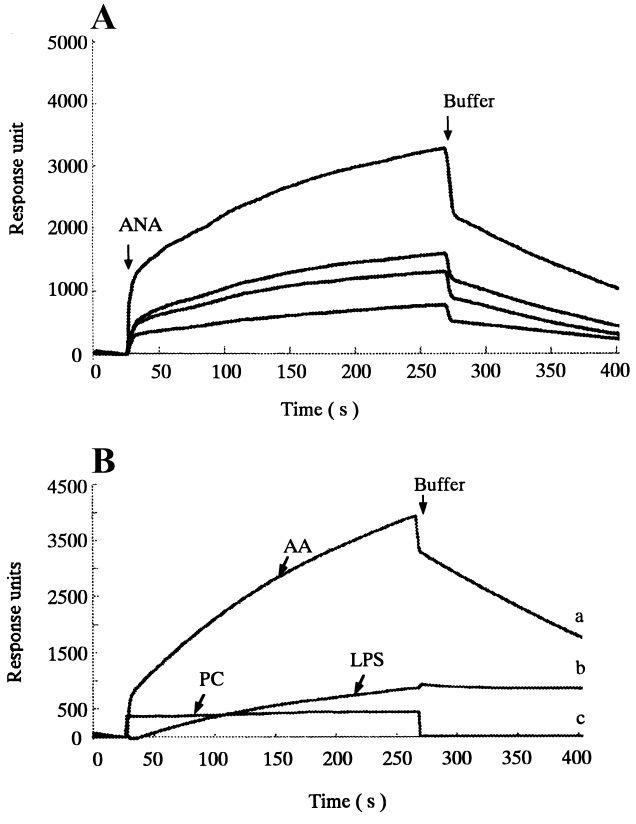

Fig. 3. Overlay plot of sensorgrams depicting the interaction of ANA with immobilized PMB. A: Interaction of PMB with ANA. ANA in a concentration range of 5.76–57.6 μM was injected for 250 s at a flow rate of 10 μl/min. The concentration of ANA from the bottom to the top is 5.76, 11.5, 29.8 and 57.6 μM. Following 250 s injection, the buffer was passed over the chip at the same flow rate as above. B: Interaction of PMB with AA, LPS and PC. AA, LPS and PC at the same concentration of 29.8 μM were injected for 250 s at a flow rate of 10 μl/min. The sensorgrams show their binding to PMB.

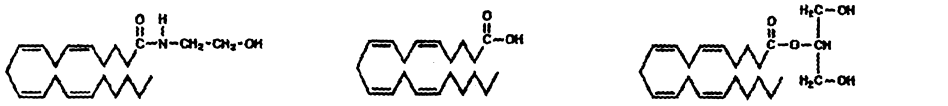

Fig. 4. Chemical structures of ANA, AA and 2-AG.

serum containing each mentioned concentration of ANA. Moreover, the decrement rate of ANA adsorption by PMB beads was linear and serum concentration-dependent. Nevertheless, about 75% of 1000 ng/ml of ANA was recovered at the maximum concentration (100%) of serum (data not shown). Thus, we supposed that there are no molecules that can specifically bind to ANA. However, more investigations are required to draw a final conclusion at this point. The specific and strong binding capacity of PMB to ANA in the presence of serum may introduce a new method to determine ANA in the serum or other fluids.

3.5. Confirmation of ANA binding to PMB by SPR

Further confirmation of binding of PMB to ANA and its molecular interaction was sought by SPR. PMB was covalently immobilized onto carboxymethylated dextran matrix covered on the sensor chip. During a 60 min interval when the sensor was in contact with the PMB solution, an increase in RU was observed, which indicated binding of PMB to the surface. Nearly 3000 RU of PMB was immobilized under these conditions. A typical sensorgram for the binding of the varying concentrations of ANA with PMB immobilized on the CM5 sensor chip is shown in Fig. 3A. The rapid increase of RU by binding of ANA to PMB with time reflects the associated changes in mass during the reaction, demonstrating the direct interaction between PMB and ANA. This interaction can also be observed between PMB and AA as shown in Fig. 3B. An immediate increase in mass was observed when a solution of AA was passed over the sensor chips to which PMB was bound (Fig. 3B, curve a). As a positive control, direct binding of LPS to the PMB sensor chip surface was also observed (Fig. 3B, curve b). However, this selective binding was not observed when a solution of PC was passed over the sensor chip containing PMB (Fig. 3B, curve c).

4. Discussion

PMB is formed from a cyclic heptapeptide moiety and a peptide side chain that terminates with a short fatty acid residue. It is known that PMB can inhibit biological effects of LPS through its high-affinity binding to lipid A, the biologically active moiety of LPS [15]. However, the molecular mechanism and manner of binding are still under debate [24–26].

ANA is composed of a highly hydrophobic *cis*-tetraene C20 chain, a polar carboxamide group and a hydroxyethyl head group [1,27]. Unlike LPS, ANA has only a long *N*,*O*-acyl chain and a hydroxy group, and does not contain any ionic group within the molecule. Hence, the interaction between ANA and PMB is probably driven primarily by hydrophobic force. Based on this deduction, the PMB-immobilized beads adsorbed ANA from 1:1 ethanol/saline or full serum probably by hydrophobic force. The hydrophobic force between PMB and ANA is so strong that saline and low concentrations of ethanol did not elute ANA from PMB-immobilized beads; only high concentrations of ethanol did. SPR, a rapid method for evaluating the elementary interaction between a molecule and its complementary ligand [28–30], reinforced the finding that PMB adsorbed ANA (Fig. 3A). In the sensorgram of ANA, a much higher RU can be seen than that in LPS, indicating that more ANA was adsorbed on the PMB-immobilized sensor chip. Some of the ANA adsorbed on the PMB-immobilized chip was dissociated by 4% ethanol/saline solution but LPS adsorbed on the same chip was not (Fig. 3A,B). However, neither 4% ethanol/saline solution nor 50% ethanol/saline was able to dissociate all ANA adsorbed on the PMB-immobilized chip, so the sensorgram curve of ANA cannot return to baseline following washing with the ethanol/saline solution above. In contrast, 50% ethanol/saline failed to elute ANA from PMB-immobilized beads. The high recovery of ANA after washing the column with a large amount of 50% ethanol/saline indicated that a strong adsorption occurred between ANA and PMB. The difference may be due to the possibility that a large amount of PMB was covalently linked to beads, and the stereoscopic conformation of PMB formed by each carrier facilitated PMB adsorption of ANA both in larger amounts and at a stronger force than PMB on the SPR sensor chip.

To elucidate which moiety in the ANA molecule plays the main role in the adsorption process of ANA by PMB, we investigated whether other lipids such as AA, 2-AG and PC are adsorbed to PMB. PMB-immobilized beads adsorbed AA and 2-AG as measured by RP-HPLC (Table 1), but not PC, which was detected by thin layer chromatography (data not shown). Since the same structure of the hydrophobic *cis*-tetraene C20 chain is shared by ANA, AA and 2-AG, but not by PC (Fig. 4), the conformation of the *cis*-tetraene C20 chain may probably match the conformation of PMB and lead the interaction of ANA with PMB by hydrophobic force. The fact that there was no significant difference in the adsorptive capacity of these lipids by PMB indicated that the electrostatic interaction is probably a minor contributor to the interaction with PMB (Table 1). Like the sensorgram of ANA, the high RU in the AA sensorgram declined after washing with the 4% ethanol/saline buffer. However, washing with 4% ethanol/saline or even with 50% ethanol/saline did not elute all AA absorbed on the PMB-immobilized sensor chip, since the sensorgram curve of AA did not decline to the baseline by washing with these ethanol/saline buffers (Fig. 3B). PC does not contain the *cis*-tetraene C20 chain in its molecule, so it shows no interaction with PMB, as previously reported [31,32]. One thing must be explained. Since ANA, AA, and PC were dissolved in a solution that is different from the running buffer,

there were a sharp increase and an equally sharp decrease in RU at the time when the loading buffer and running buffer were switched, respectively.

Several studies have demonstrated that ANA and 2-AG could elicit CB1 receptor-mediated hypotension in rats [11–14]. The endogenous ANA was identified in LPS-activated macrophages in vitro, and was thought to be a paracrine mediator of LPS-induced hypotension via the activation of the vascular CB1 receptor, because hypotension is prevented by pretreatment of LPS-recipient rats with the CB1 receptor antagonist SR141716A. When endotoxic shock patients are treated with a PMB-immobilized fiber hemoperfusion column, which is generally applied for removing endotoxin, the hypotension is improved significantly, however, the mechanism of this anti-hypotensive effect remains unknown [18,19]. Supposing that endogenously generated ANA by the LPS stimulation could be adsorbed with a PMB-immobilized hemoperfusion column, we examined the columns which were used for patients with LPS shock and could detect that a large amount of ANA was adsorbed onto the column (data not shown). Therefore, the removal of endogenous ANA from the circulation of shock patients by the adsorption of a PMB-immobilized hemoperfusion column may contribute to the improvement of endotoxin-induced hypotension.

It has been reported that THC induces neuronal cell death under serum-free culture conditions [33]. We found that ANA was able to induce PC-12 cell death under the same culture conditions as THC. However, the cell death induced by ANA was prevented by pretreatment with PMB. This result indicates that PMB adsorbs ANA and neutralizes the cytotoxic function of ANA.

In conclusion, we first found that PMB adsorbed ANA, which is significant in elucidation of the mechanism of septic shock-induced hypotension, and extends the application of PMB-immobilized beads or membranes in clinical treatment of endotoxin-induced shock and analysis of ANA.

Acknowledgements: We thank Prof. P.W. Majerus at Washington University in St. Louis, MO, USA for the criticism and comments to our manuscript. We also thank Toray Chemical Co. for supplying the polymyxin B-immobilized membrane column.

References

[1] Stone, R. (1994) Science 264, 365–367.
[2] Natanson, C., Hoffman, W.D., Suffredini, A.F., Eichacker, P.Q. and Danner, R.L. (1994) Ann. Intern. Med. 120, 771–783.
[3] Wagner, J.A., Varga, K., Ellis, E.F., Rzigalinski, B.A., Martin, B.R. and Kunos, G. (1997) Nature 390, 518–521.
[4] Devane, W.A., Hanus, L., Breuer, A., Pertwee, R.G., Stevenson, L.A., Griffin, G., Gibson, D., Mandelbaum, A., Etinger, A. and Mechoulam, R. (1992) Science 258, 1946–1949.
[5] Felder, C.C., Nielsen, A., Briley, E.M., Palkovits, M., Priller, J., Axelrod, J., Nguyen, D.N., Richardson, J.M., Riggin, R.M., Koppel, G.A., Paul, S.M. and Becker, G.W. (1996) FEBS Lett. 393, 231–235.
[6] Di Marzo, V., Fontana, A., Cadas, H., Schinelli, S., Cimino, G., Schwartz, J.C. and Piomelli, D. (1994) Nature 372, 686–691.
[7] Facci, L., Dal Toso, R., Romanello, S., Buriani, A., Skaper, S.D. and Leon, A. (1995) Proc. Natl. Acad. Sci. USA 92, 3376–3380.
[8] Calignano, A., La Rana, G., Giuffrida, A. and Piomelli, D. (1998) Nature 394, 277–281.
[9] Lou, Y.D., Patel, M.K., Wiederhold, M.D. and Ou, D.W. (1992) Int. J. Immunopharmacol. 14, 49–56.
[10] Klein, T.W., Newton, C. and Friedman, H. (1998) Immunol. Today 19, 373–381.
[11] Stein, E.A., Fuller, S.A., Edgemond, W.S. and Campbell, W.B. (1996) Br. J. Pharmacol. 119, 107–114.
[12] Lake, K.D., Compton, D.R., Varga, K., Martin, B.R. and Kunos, G. (1997) J. Pharmacol. Exp. Ther. 281, 1030–1037.
[13] Calignano, A., La Rana, G., Beltramo, M., Makryannis, A. and Piomelli, D. (1997) Eur. J. Pharmacol. 337, R1–R2.
[14] Varga, K., Wagner, J.A., Bridgen, D.T. and Kunos, G. (1998) FASEB J. 12, 1035–1044.
[15] Palmer, J.D. and Rifkind, D. (1974) Surg. Gynecol. Obstet. 138, 755–759.
[16] Hanasawa, K., Tani, T. and Kodama, M. (1989) Surg. Gynecol. Obstet. 168, 323–331.
[17] Jaber, B.L., Barrett, T.W., Neto, M.C., Sundaram, S., King, A.J. and Pereira, B.J.G. (1998) ASAIO J. 44, 54–61.
[18] Kodama, M., Tani, T., Hanasawa, K., Hirata, K., Hirasawa, H., Oda, S., Otsuka, T., Yamamoto, Y., Kanesaka, S., Takahashi, Y., Maekawa, K., Wakabayashi, Y., Tamakuma, S. and Sugimoto, T. (1997) J. Endotoxin Res. 4, 293–300.
[19] Aoki, H., Kodama, M., Tani, T. and Hanasawa, K. (1994) Am. J. Surg. 167, 412–417.
[20] Greene, L.A. and Tischler, A.S. (1976) Proc. Natl. Acad. Sci. USA 73, 2424–2428.
[21] Sarker, K.P., Abeyama, K., Nishi, J., Nakata, M., Tokioka, T., Nakajima, T., Kitajima, I. and Maruyama, I. (1999) Thromb. Haemost. 82, 1071–1079.
[22] Lang, W.S., Qin, C., Hill, W.A.G., Lin, S.Y., Khanolkar, A.D. and Makriyannis, A. (1996) Anal. Biochem. 238, 40–45.
[23] Beltramo, M., Stella, N., Calignano, A., Lin, S.Y., Makriyannis, A. and Piomelli, D. (1997) Science 277, 1094–1097.
[24] Rustici, A., Velucchi, M., Faggioni, R., Sironi, M., Ghezzi, P., Quataert, S., Green, B. and Porro, M. (1993) Science 259, 361–365.
[25] Thomas, C.J., Gangadhar, B.P., Surolia, N. and Surolia, A. (1998) J. Am. Chem. Soc. 120, 12428–12434.
[26] Thomas, C.J. and Surolia, A. (1999) FEBS Lett. 445, 420–424.
[27] Piomelli, D., Beltramo, M., Glasnapp, S., Lin, S.Y., Goutopoulos, A., Xie, X.Q. and Makriyannis, A. (1999) Proc. Natl. Acad. Sci. USA 96, 5802–5807.
[28] Vadgama, P. and Crump, P.W. (1992) Analyst 117, 1657–1670.
[29] Brigham-Burke, M., Edwards, J.R. and O'Shannesy, D.J. (1992) Anal. Biochem. 205, 125–131.
[30] O'Shannesy, D.J., Brigham-Burke, M. and Peck, K. (1992) Anal. Biochem. 205, 132–136.
[31] Teuber, M. and Miller, I.R. (1977) Biochim. Biophys. Acta 467, 280–289.
[32] El Mashak, E.M. and Tocanne, J.F. (1980) Biochim. Biophys. Acta 596, 165–179.
[33] Chan, G.C., Hinds, T.R. and Impey, S. (1998) J. Neurosci. 18, 5322–5332.