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POTENT HYPOTENSIVE ACTIVITY OF 1-O-HEXADECYL-2-O-ACETYL-SN-GLYCERO-3-PHOSPHOCHOLINE IN SPONTANEOUSLY HYPERTENSIVE RAT

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Summary. Chemically synthesized 1-O-hexadecyl-2-O-acetyl-sn-glycero-3-phosphocholine possessed the most potent hypotensive activity compared with bradykinin, prostagrandin E_2 and I_2 when 5 nano moles/kg body weight of each drug were administered intravenously in spontaneously hypertensive rat. The potency and the duration of hypotensive activity of 1-O-hexadecyl-2-O-acetyl-sn-glycero-3-phosphocholine were dose dependent. Exogenous norepinephrine or angiotensin II showed pressor activity during the hypotensive action of 1-O-hexadecyl-2-O-acetyl-sn-glycero-3-phosphocholine, but did not disturb the hypotensive pattern of this ether lipid. These may suggest that 1-O-alkyl-2-O-acetyl-sn-glycero-3-phosphocholine plays an important role for the regulation of blood pressure.

INTRODUCTION

Renal medulla has been considered to have hypotensive lipids, because the lipid extract of renal medulla has potent hypotensive activity(1). The lipid granule-containing interstitial cell of renal papilla has also hypotensive activity when grafted intracutaneously in hypertensive Goldblatt rat(2). The hypotensive lipids of renal medulla hitherto known include PG E_2, antihypertensive polar renomedullary lipid (1-O-alkyl-2-O-acetyl-G-3-PC) and antihypertensive neutral renomedullary lipid(3,4), but the structure of the neutral lipid is still unknown. These lipids are thought to be synthesized mainly in renomedullary interstitial cells(3,4). Antihypertensive polar renomedullary lipid was obtained by Muirhead et al. from lipid extract of renal medulla with some chemical modification such as hydrolysis of ester bond and reesterification with acetic anhydride. Its structure turned out to be 1-O-alkyl-2-O-acetyl-G-3-PC conglomerate(4). Semi-synthetic 1-O-alkyl-2-O-acetyl-G-3-PC from bovine heart choline plasmalogen (reduction of vinyl

Abbreviation used: G-3-PC, sn-glycero-3-phosphocholine; PG, prostagrandin; MBP, mean blood pressure; SHR, spontaneously hypertensive rat.

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bond, hydrolysis of position-2 and following acetylation of position-2) also possessed very similar hypotensive activity(5).

On the other hand, interestingly, the structure of this substance is identical (or almost identical) with that of platelet activating factor produced from immunoglobulin E-sensitized basophils(6). Platelet aggregating activity of 1-O-alkyl-2-O-acetyl-G-3-PC is reported to be very potent and it occured on the concentration of approximately 10^{-10}M, in vitro(7).

In this communication, we examined the hypotensive activity of chemically synthesized 1-O-hexadecyl-2-O-acetyl-G-3-PC and compared with that of other potent hypotensive materials such as bradykinin and prostagrandins. The action of angiotensin II as a pressor hormone of norepinephrine as a chemical transmitter was also studied during the hypotensive action of 1-O-hexadecyl-2-O-acetyl-G-3-PC from the view point of the relationship between pressor substance and depressor substance.

MATERIALS AND METHOD

Chemicals. 1-O-Hexadecyl-2-O-acetyl-G-3-PC was synthesized with Mr. I. Yamatsu, Eisai Co. Ltd., Tokyo from D-mannitol according to the method of Godfroid et al. (8). This is white amorphous lipid and is mono spot on TLC plate of Kieselgel 60F254 Art5715(Merk). Rf value is 0.3 in $CHCl_3$:CH_3OH:NH_4OH = 60:25:4 (V/V). Nuclear magnetic resonance and mass spectra showed no other contamination of side product. But, after hydrolysis of 3-position of this ether lipid, the rearrangement reaction of acetyl group from position-2 to position-3 was slightly observed. Therefore, the possibility of this ether lipid to include slight contamination of 1-O-hexadecyl-3-O-acetyl-G-2-PC could not be ruled out. Bradykinin was obtained from the Peptide Institute, Protein Research Foundation, Osaka, Japan. PG E_2 and PG I_2 were kindly gift from Ono Pharmaceutical Co. Ltd., Osaka. Haemacel(3.5% of repolymerization product of decomposed gelatin, 0.85% of NaCl, 0.038% of KCl and 0.093% of $CaCl_2$) was purchased from Hoechst Japan Co. Ltd.. Other chemicals were obtained commercially.

Measurement of blood pressure. Male SHR(body weight 180-200g) was purchased from Keari Co. Ltd., Osaka. All chemicals except for PG I_2 were dissolved in 0.2% of Haemacel-saline solution in plastic tube to avoid the adsorption of drug. PG I_2 was dissolved in 0.1 M glycine buffer (pH 10.5) because of its instability. All reagents were freshly dissolved before use and injected through external jugular vein catheter (injection volume; 0.05-0.1 ml) and flushed completely through the intravenous cannula by saline. Blood pressure was directly monitered from femoral artery using Nihon Koden RH-5 Power Unit and Recticorder.

RESULTS AND DISCUSSION

When 5 n moles/kg bodyweight of 1-O-hexadecyl-2-O-acetyl-G-3-PC was injected to male SHR, acute drop of MBP was observed as seen in Fig, 1. Similar hypotensive action was also seen at the PG I_2 and bradykinin administration, but the slight decrease of MBP was observed when PG E_2 was administered under the same dose level.

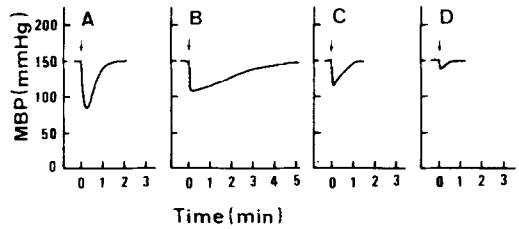

Fig. 1. Comparative hypotensive pattern of 1-O-hexadecyl-2-O-acetyl-G-3-PC, PG I_2, Bradykinin and PG E_2. 0.9 Nano mole of each drug was injected to 180 g male SHR and MBP was monitered under consciousness. A, 1-O-Hexadecyl-2-O-acetyl-G-3-PC; B, PG I_2; C, Bradykinin; D, PG E_2.

1-O-Hexadecyl-2-O-acetyl-G-3-PC showed the most potent activity for the lowering of MBP under this dose level, but the duration of hypotensive activity was the longest when administered PG I_2 (Table 1). Minimum effective dose of 1-O-hexadecyl-2-O-acetyl-G-3-PC for hypotensive activity was about 1 n mole/kg body weight (Fig. 2) The order of this effective dose is so low that its hypotensive effect may be caused through its receptor. This hypotensive effect might be derived from the direct vasodilation since its hypotensive response is so rapid. Prewitt et al. reported that the beginning of the blood pressure drop occured 3.8 seconds after administration of 1-O-alkyl-2-O-acetyl-G-3-PC conglomerate(9). Renal medulla is considered to play an endocrine-like role in the prevention of hypertension(10). These results suggest that the specific 1-O-alkyl-2-O-acetyl-G-3-PC receptor in blood vessel exists and this receptor plays an important role for blood vessel dilatation and blood pressure regulation. The potency and the duration of hypotensive activity

Table 1. Comparative hypotensive activity of 1-O-hexadecyl-2-O-acetyl-G-3-PC, PG I_2, bradykinin and PG E_2.

Drug	No of experiment	Hypotensive activity(- mmHg)	Hypotensive duration(min)
1-O-Hexadecyl-2-O-acetyl-G-3-PC	7	62 ± 2	0.45 ± 0.04
PG I_2	7	36 ± 1	1.68 ± 0.07
Bradykinin	5	34 ± 1	0.35 ± 0.03
PG E_2	5	16 ± 2	0.39 ± 0.03

(mean ± s.e.)

0.2 Nano mole of each drug was injected intravenously to 180 g male SHR. Hypotensive activity means the net change of MBP before and after administration of 1-O-hexadecyl-2-O-acetyl-G-3-PC and hypotensive duration means the half recovery time of MBP from minimum level of MBP.

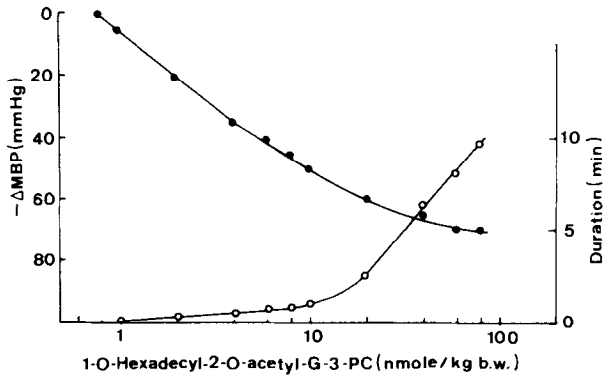

Fig. 2. Dose dependency of hypotensive activity of 1-O-hexadecyl-2-O-acetyl-G-3-PC in SHR under consciousness. Closed circle is the net change of MBP before and after administration of various amounts of 1-O-hexadecyl-2-O-acetyl-G-3-PC and open circle is the half recovery time of MBP from minimum level.

were dose-dependently increased (Fig. 2). When dosage amount of 1-O-hexadecyl-2-O-acetyl-G-3-PC was increased, the MBP was decreased and reached minimum level. On the other hand, the duration of activity increased dose-dependently even after when MBP reached minimum level.

Exogenous norepinephrine (15 n moles/kg body weight) or angiotensin II (0.3 n mole/kg body weight) showed pressor activity before and after 1-O-hexadecyl-2-O-acetyl-G-3-PC administration (12 n moles/kg body weight) (Fig. 3). Smith et al. (11) reported that the antihypertensive polar renomedullary lipid containing 10% of the active alkyl ether conglomerate blocked the pressor activity of exogenous

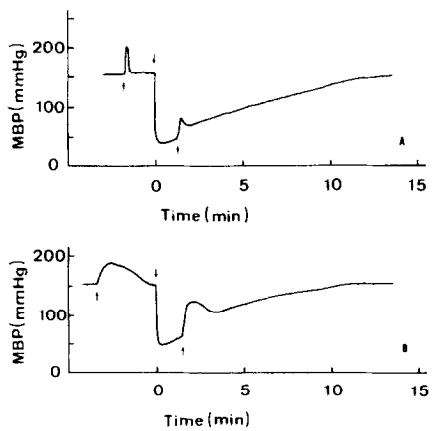

Fig. 3. Effect of norepinephrine or angiotensin II on MBP during hypotensive action of 1-O-hexadecyl-2-O-acetyl-G-3-PC in SHR. Norepinephrine (15 n moles/kg) or angiotensin II (0.3 n mole/kg) was injected before and after administration of 1-O-hexadecyl-2-O-acetyl-G-3-PC (12 n moles/kg) to SHR under pentobarbital anesthesia. ↑, Norepinephrine (A) or angiotensin II (B) administration; ↓, 1-O-Hexadecyl-2-O-acetyl-G-3-PC administration.

norepinephrine, but the pressor action of angiotensin II was not blocked by the lapine 1-O-alkyl-2-O-acetyl-G-3-PC conglomerate. This discrepancy is not fully explained, by the ether lipid we used is chemically synthesized 1-O-hexadecyl one and the antihypertensive polar renomedullary lipid from lapine is a conglomerate consists of $C_{16:0}$(67%), $C_{16:1}$(16%), $C_{18:1}$(11%), $C_{18:0}$(4%) and $C_{15:0}$(2%). In platelet aggregation system, 1-O-alkyl conglomerate has been reported to be slightly more active than 1-O-hexadecyl analogue(12). On the other hand, both pressor substances did not disturb the duration pattern of hypotensive action of 1-O-hexadecyl-2-O-acetyl-G-3-PC. These may imply that the 1-O-hexadecyl-2-O-acetyl-G-3-PC receptor site is different from the receptor site of angiotensin II or norepinephrine, and these substances do not compete with each other. 1-O-Hexadecyl-2-O-acetyl-G-3-PC plays a potent hypotensive action by its specific mechanism.

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