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A NEW CLASS OF ANTIHYPERTENSIVE NEUTRAL LIPID: 1-ALKYL-2-ACETYL-sn-GLYCEROLS, A PRECURSOR OF PLATELET ACTIVATING FACTOR

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SUMMARY— A new type of neutral lipid is described that possesses hypotensive activity in genetic hypertensive (SHR) and normotensive (WKY) rats. 1-Alkyl-2-acetyl-sn-glycerols and 1-alkyl-2-propionyl-sn-glycerols are both equally effective in eliciting the hypotensive response. Requirement for the 1-alkyl and 2-acetyl or 2-propionyl structure of the active isomer was documented by the negative responses obtained with closely related neutral lipid analogs (1-alkyl-2-acyl-, 1-alkyl-3-acetyl-, 1-acyl-2-acetyl-, 1-alkyl-2,3-diacetyl-, and 1-alkyl-glycerols). Although less potent than PAF (1-alkyl-2-acetyl-sn-glycero-3-phosphocholine), the 1-alkyl-2-acetyl-sn-glycerols produce a response of significantly longer duration and may have fewer immediate side effects than PAF. The mechanism for the biological activity is unknown; however, we have demonstrated previously that the enzymatic synthesis of 1-alkyl-2-acetyl-sn-glycerols to PAF occurs via a specific cholinephosphotransferase and therefore the observed blood pressure response might be due to the conversion of the neutral lipid precursor to PAF in vivo.

The diverse biological activities and biochemical properties of acetylated alkyl ether glycerophosphocholines, also known as platelet activating factor or PAF,[1] have been reviewed (1-6). PAF possesses a potent hypotensive activity that has been observed in the Goldblatt (7,8) and SHR genetic hypertensive rats (8-13), as well as normotensive animals (8,12-15). Other phospholipids (16,17) and an uncharacterized neutral lipid fraction, termed ANRL, isolated from rabbit kidneys (18) have also been reported to possess antihypertensive activities.

Earlier work from our laboratory (19) demonstrated that a number of rat tissues contain high activities of a specific cholinephosphotransferase that transfers the phosphocholine moiety from CDP-choline to 1-alkyl-2-acetyl-sn-glycerol to produce the bioactive PAF product according the following reaction:

1-alkyl-2-acetyl-sn-glycerol + CDP-choline ⟶
1-alkyl-2-acetyl-sn-glycero-3-phosphocholine (PAF) + CMP

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[1] Abbreviations:
PAF, platelet activating factor, 1-alkyl-2-acetyl-sn-glycerol-3-phosphocholine; CDP, cytidine diphosphocholine; TLC, thin-layer chromatography; MAP, mean arterial pressure.

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The specific cholinephosphotransferase that forms PAF differs from the one that utilizes diradylglycerols [i.e., diacyl (20,21) or alkylacyl (22) species] to produce phospholipids with long chain aliphatic moieties at both the sn-1 and sn-2 positions of the glycerolipids. Those reactions that form the long chain phospholipids from alkylacyl- and diacyl-glycerols appear to be catalyzed by the same cholinephosphotransferase (23). The unique selectivity of the cholinephosphotransferase in PAF synthesis is based on the fact that dithiothreitol (5 mM) inhibits the long chain diradylglycerol cholinephosphotransferase but not the alkylacetylglycerol cholinephosphotransferase (19).

In view of the relatively high activities of 1-alkyl-2-acetyl-sn-glycerol: CDP cholinephosphotransferase in rat tissues, we pursued the possibility that 1-alkyl-2-acetyl-sn-glycerols (a neutral lipid precursor of PAF) may elicit a hypotensive response via its conversion to PAF. Indeed this neutral lipid precursor lowers the blood pressure of both SHR hypertensive and normotensive rats albeit at higher levels than found for PAF. Furthermore, the hypotensive effect obtained with 1-alkyl-2-acetyl-sn-glycerols is of considerably longer duration than with PAF.

MATERIALS AND METHODS

1-Hexadecyl-sn-glycerol (>98% purity, Sigma Chemical Co., St. Louis, MO) was acetylated by heating 200 mg in a solution of 2 ml of acetic anhydride and 0.5 ml of pyridine for 1 hr in a sealed tube at 100°C. After addition of 2 ml of water, the 1-hexadecyl-2,3-diacetyl-sn-glycerol was extracted from the reaction mixture with hexane:diethyl ether (1:1, v/v); the extract was dried over Na_2SO_4 and the solvents evaporated with a stream of N_2. Only a single component possessing the same R_f as commercial preparations of diacetates of batyl and selachyl alcohols (Western Chemical Industries, Vancouver, Canada) was found after thin-layer chromatography (TLC) on 250-μm layers of Silica Gel G developed in chloroform:methanol (98:1.5, v/v) (19).

1-Hexadecyl-2,3-dipropionyl-sn-glycerol was prepared in the same manner as the 1-hexadecyl-2,3-diacetyl-sn-glycerol except that propionic anhydride was used instead of acetic anhydride. 1-Hexadecyl-2,3-diacetyl-sn-glycerol, the diacetates of selachyl (18:1) and batyl (18:0) alcohols (100 mg of each), and 1-hexadacyl-2,3-dipropionyl-sn-glycerol were treated with 20-25 mg of porcine pancreatic lipase (Nutritional Biochemicals Co., Cleveland, OH) in 2.75 ml of 0.73 M Tris-HCl buffer (pH = 8.0) that contained 45 mg $CaCl_2$ and 2.5 mg of sodium deoxycholate. After the mixture was shaken vigorously for 2 hr at room temperature, the hydrolytic products were separated on TLC layers prepared with a 4% boric acid solution and developed in chloroform:methanol (98:1.5, v/v) (19). Developed TLC plates were sprayed with a solution of 0.025% rhodamine 6G in ethanol and the lipid bands were visualized under ultraviolet light. Both isomeric forms of the hydrolytic products produced from the 1-hexadecyl-2,3-diacetyl-sn-glycerol (1-hexadecyl-2-acetyl-sn-glycerol and 1-hexadecyl-3-acetyl-sn-glycerol) were isolated from the silica gel by extraction with diethyl ether. However, with the other preparations only the 1-alkyl-2-acetyl-sn-glycerols (derived from selachyl and batyl diacetates) or the 1-alkyl-2-propionyl-sn-glycerol fraction were isolated from the silica gel. After addition of one volume of hexane to the diethyl ether extracts, the organic layers were washed three times with water, dried over Na_2SO_4, and the solvent evaporated. The lipid preparations were stored in chloroform at -20°C. The purity of all these compounds was estimated at >98% by TLC analysis (19).

A number of other lipid analogs were prepared as described below for testing, including PAF which was used as a reference for comparison purposes.

The PAF preparation, 1-hexadecyl-2-acetyl-sn-glycero-3-phosphocholine, was synthesized as previously described (9). The ester analog of PAF, 1-hexadecanoyl-2-acetyl-sn-glycero-3-phosphocholine, was made by acetylation of 1-hexadecanoyl-2-lyso-sn-glycero-3-phosphocholine (16 mg, Sigma Chemical Co., St. Louis, MO) in a reaction with 0.5 ml of acetic anhydride and 20 mg of 4-dimethylaminopyridine in 1.25 ml of toluene; the sealed tube was heated at 100°C for 2 hr and, after evaporation of the solvents with a stream of N_2, the products were extracted by the method of Bligh and Dyer (24). The product, 1-hexadecanoyl-2-acetyl-sn-glycero-3-phosphocholine, was then treated with phospholipase C (25) to produce 1-hexadecanoyl-2-acetyl-sn-glycerol, which was estimated on the basis of TLC to have a purity >95%. The neutral ether analog of a diradylglycerol with a long chain fatty acid at the sn-2 position, 1-hexadecyl-2-octadecenoyl-sn-glycerol, was isolated by preparative TLC from the reaction products formed after treatment of rac-hexadecyl-2-octadecenoyl-glycerophosphocholine (R. Berchtold, Biochemisches Labor, Bern, Switzerland) with phospholipase C.

Male (10-month-old) spontaneous hypertensive (SHR) or (1-year-old) normotensive (WKY) rats (both from Charles River Breeding Laboratories, Inc., Wilmington, MA) were used for measurements of mean arterial pressure (MAP) before and after administration of the test substances. The animals were anesthetized with sodium pentobarbital and connected to a pressure transducer inserted into the decending aorta and the blood pressure tracings were obtained with a physiograph as previously described in detail (9). All neutral lipid preparations tested for their effect on blood pressure were dissolved at a concentration of 100 nmol/0.1 ml of 1% Tween-20 in saline and then injected into the vena cava over a period of a few seconds.

RESULTS

A typical blood pressure response curve after an intravenous injection of 100 nmol 1-hexadecyl-2-acetyl-sn-glycerol is shown in the upper panel of Fig. 1. The MAP began to decrease approximately 5 sec after injection of 1-hexadecyl-2-acetyl-sn-glycerol and reached a maximum depression 1 to 2 min

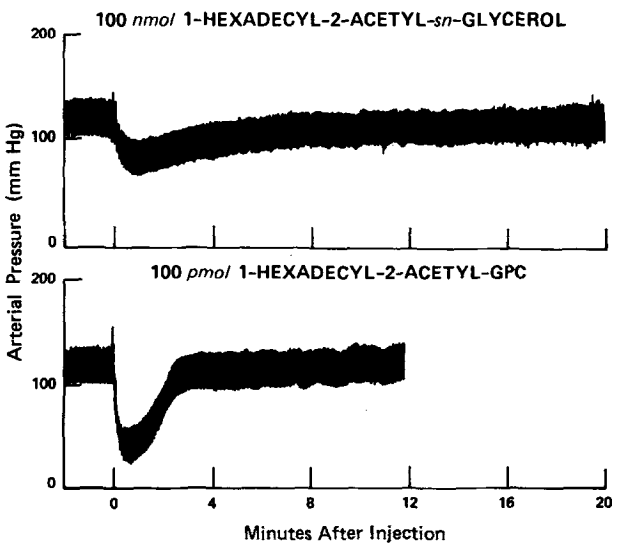

Fig. 1. Physiograph tracings of arterial pressure in anesthesized SHR rats after bolus intravenous injections of 100 nmol 1-hexadecyl-2-acetyl-sn-glycerol (upper panel) or 100 pmol 1-hexadecyl-2-acetyl-sn-glycero-3-phosphocholine (lower panel).

TABLE 1

EFFECT OF 1-HEXADECYL-2-ACETYL-sn-GLYCEROL ON THE MEAN ARTERIAL PRESSURE IN SHR RATS

1-Hexadecyl-2-acetyl-sn-glycerol injected	Decrease in MAP	Time to recover MAP
(nmol)	(%)	(min)
50 (n=3)	12.3 ± 1.3	10.5 ± 0.5
100 (n=6)	25.5 ± 2.8	13.2 ± 1.6
200 (n=8)	39.5 ± 3.2	15.4 ± 2.0

Values represent the mean ± S.E.M.

later; the arterial pressure returned to pre-injection levels within about 15 min. Repeated injections of the 1% Tween-20 solution alone (i.e., without lipid) did not affect the MAP. We were unable to detect any changes in heart rate during the antihypertensive response elicited by 1-hexadecyl-2-acetyl-sn-glycerol (309 ± 31 beats/min before injection and 294 ± 21 beats/min after injection, n=4). For comparsion a tracing of the MAP response obtained after an intravenous injection of 100 pmol of PAF, 1-hexadecyl-2-acetyl-sn-glycero-3-phosphocholine (in a 0.25% bovine serum albumin solution), is shown in the lower panel of Fig. 1. PAF lowers the MAP more rapidly and to a greater extent than does 1-hexadecyl-2-acetyl-sn-glycerol, but the duration of the response is considerably longer for the active neutral lipid than with PAF.

The main effect of increasing the amounts of the 1-hexadecyl-2-acetyl-sn-glycerol injected is the extent that the MAP is lowered; however, the duration of the antihypertensive response (Table 1) was also lengthened to some extent by higher doses of 1-hexadecyl-2-acetyl-sn-glycerol. 1-Octadec-9-enyl-2-acetyl-sn-glycerol prepared from the diacetate of selachyl alcohol had about the same effect in lowering the MAP (40.2 ± 0.9% by 200 nmol, n=3) as the same dose of 1-hexadecyl-2-acetyl-sn-glycerol, whereas the 1-octadecyl-2-acetyl-sn-glycerol prepared from batyl alcohol was much less effective (20.4 ± 2.3% by 200 nmol, n=4). Injections of 200 nmol of 1-hexadecyl-sn-glycerol, diacetates of batyl or selachyl alcohol, 1-hexadecanoyl-2-acetyl-sn-glycerol, 1-hexadecyl-2-octadecenoyl-sn-glycerol, or 1-hexadecyl-3-acetyl-sn-glycerol failed to exhibit any significant effect on the MAP of hypertensive SHR (<1% of the overall MAP response (9) compared with an equivalent amount of 1-hexadecyl-2-acetyl-sn-glycerol).

1-Alkyl-2-acetyl-sn-glycerols also exhibit a hypotensive effect in normotensive rats; the blood pressure tracings obtained after an intravenous administration of 200 nmoles of the 1-hexadecyl-2-acetyl-sn-glycerol to one-year-old WKY rats gave essentially the same response pattern as seen in

Fig. 1 for the SHR animals, except the recovery of the MAP in the normotensive rats appeared to be somewhat longer than the SHR animals. Intravenous injections of 1-hexadecyl-2-propionyl-sn-glycerol (200 nmol) was also found to elicit an identical hypotensive response (41.4% lower MAP, based on maximum decrease) in the normotensive rats as found with 1-hexadecyl-2-acetyl-sn-glycerol (41.3% lower MAP, based on maximum decrease).

DISCUSSION

Our studies report the discovery of a new class of neutral glycerolipids, 1-alkyl-2-acetyl-sn-glycerols, that possess hypotensive properties closely related to those previously described by us (7,9) for PAF (1-alkyl-2-acetyl-sn-glycero-3-phosphocholine). However, it should be noted that PAF is a much more potent antihypertensive agent than the 1-alkyl-2-acetyl-sn-glycerols. A dose of only 25 pmol of 1-alkyl-2-acetyl-sn-glycero-3-phosphocholine lowers the MAP 65% with a recovery time of about 3 min in the hypertensive rat (SHR) (9), whereas nanomole levels of the antihypertensive neutral lipid are required to evoke a similar hypotensive response. The rate of decrease in MAP after injection of PAF (7,9) is much more rapid (maximum hypotension is reached within 10 sec after injection) than with the 1-alkyl-2-acetyl-sn-glycerols (1 to 2 min). However, the MAP is decreased for a longer time after injection of 50 to 200 nmol of 1-alkyl-2-acetyl-sn-glycerols (Fig. 1 and Table 1) than with 25-100 pmol of PAF.

The nature of the antihypertensive response after injection of 1-alkyl-2-acetyl-sn-glycerols and the amounts needed to produce this response appear to be similar to those reported by Muirhead et al. (18) for an antihypertensive neutral lipid (termed ANRL) isolated from the rabbit renal medulla. However, the chemical structure of ANRL has never been characterized, and therefore it is difficult to say at this time whether the active component of ANRL is 1-alkyl-2-acetyl-sn-glycerol, but this possibility appears likely.

Since 1-hexadecanoyl-2-acetyl-sn-glycerol, 1-hexadecyl-sn-glycerol, 1-hexadecyl-3-acetyl-sn-glycerol, 1-alkyl-2,3-diacetyl-sn-glycerols and 1-hexadecyl-2-octadecenoyl-sn-glycerol exhibited no hypotensive activities, it appears that an alkyl ether group at the sn-1 position, an acetate group (or other short chain acyl group such as propionate) at the sn-2 position, and a free hydroxyl group at the sn-3 position of glycerol are the essential requirements for expression of the biological activity of this neutral glycerolipid. However, since 1-alkyl-2-acetyl-sn-glycerol can be converted to 1-alkyl-2-acetyl-sn-glycero-3-phosphocholine by a CDP-cholinephosphotransferase enzyme (19); the actual compound responsible for the decrease in MAP after injection of 1-alkyl-2-acetyl-sn-glycerol to rats may be its metabolic product, PAF (1-alkyl-2-acetyl-sn-glycero-3-phosphocholine). Although the structural requirements at the sn-1 and sn-2 positions for biological activity of the

antihypertensive neutral lipid and PAF are identical (9), the actual biochemical mechanism that accounts for the hypotensive action of the neutral lipid is unknown.

Previous studies by Renooij and Snyder (19) demonstrated that 1-alkyl-2-acetyl-sn-glycerols could be converted to the bioactive PAF molecule by 1-alkyl-2-acetyl-sn-glycerol:CDP-choline cholinephosphotransferase (EC 2.7.8.16). This enzyme transfers the phosphocholine moiety to the sn-3 position of 1-alkyl-2-acetyl-sn-glycerol. The exact location for such an enzymatic conversion when 1-alkyl-2-acetyl-sn-glycerol is administered in vivo remains to be determined, although our original studies (19) demonstrated that rat tissues (kidney, spleen, lung, liver, heart) contain sufficient amounts of the cholinephosphotransferase activity to synthesize the biologically active lipid (PAF) from the 1-alkyl-2-acetyl-sn-glycerol precursor. The larger doses of the antihypertensive neutral lipid required to obtain a lowering of blood pressure and the longer duration of the response compared with PAF is consistent with this hypothesis.

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