Alkyl Ether Analogs of Phosphatidylcholine Are Orally Active in Hypertensive Rabbits

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SUMMARY 1-O-alkyl ethers of phosphatidylcholine having an acetoyl in the second position were derived from fresh renal tissue. The main ether so derived had a C16:0 chain. The C16:0 alkyl ether was synthesized de novo. The renally derived and the synthetic ether exerted a similar and powerful antihypertensive action in hypertensive rabbits when given orally in divided doses. This action was prolonged, requiring more than 60 hours after the last input of the compound for recovery of the arterial pressure. As these ethers exerted their antihypertensive action, there was no evidence of adverse effects. Noteworthy was the failure of these depressor compounds to cause renin release. Diuresis-kaliuresis did not occur. A suggestion of sodium retention was noted. (Hypertension 3 (supp I): I-107–I-111, 1981)

KEY WORDS • alkyl ethers • phosphatidylcholine • antihypertensive action • vasodilator • rabbit • renal medulla • platelet factor

The polar antihypertensive lipid derived from fresh renal medulla of the rabbit and designated as the antihypertensive polar renal medullary lipid (APRL) was identified as a conglomerate of 1-O-alkyl ethers of phosphatidylcholine, having an acetoyl in the second position (fig. 1). The chain lengths and saturation status of the ethers were determined by high resolution mass spectrometry as C16:0 (67%), C16:1 (16%), C18:1 (11%), C18:0 (4%), and C15:0 (2%). Since these observations were recorded, we have obtained the C16:0 ether, the main compound of the conglomerate derived from renal tissue, as a pure compound derived by de novo synthesis.

At this time, we wish to compare the antihypertensive action of the conglomerate of alkyl ethers derived from renal tissue with that of the pure synthetic C16:0 alkyl ether when both agents were given by mouth to hypertensive rabbits. Emphasis will be placed on the effect of these agents on the arterial pressure and on renal excretory function and plasma renin concentration.

Material and Methods

Production of Hypertension in Rabbits

The one-kidney, one wrap Page model was used. The white rabbits (obtained from the B and D Commercial Rabbitry, Malvern, Arkansas) were 14- to 16-week-old males weighing approximately 3.0 kg. Under pentobarbital anesthesia (30 mg/kg i.v.) the left kidney was isolated, its capsule teased away with a pair of tweezers, and a previously prepared silk sac
placed over the kidney and secured in place by a purse-string tie about the hilum. The tie was loose so as not to compromise the renal vessels and ureter. The right kidney was removed 1 week later under similar anesthesia. Four weeks later, when the hypertensive state had leveled, the animals were ready for the experiments.

Cannulation of Abdominal Aorta and Arterial Pressure Measurement

A specially devised indwelling catheter was inserted into the abdominal aorta well below the renal arteries and was brought out at the back of the neck, as previously described. The mean aortic pressure (MAP) was determined via a Statham transducer (P23GB) and a Model 7 Grass polygraph. The animals were conscious and quiet in a box while the recording was obtained over 30 minutes. Blood samples were obtained via the aortic catheter.

Sodium, Potassium, Water Excretion

Sodium, potassium, and water excretion via the urine was measured for 5 days before and 5 days during and after the medications. The rabbits were in metabolic cages, and the procedures used were described earlier.

Platelet Count, Leucocyte Count, and Hematocrit Reading

After emptying the blood in the catheter dead space, a 0.3 ml sample of blood was obtained and placed in a Microtainer (Becton, Dickinson and Company) containing 0.39 mg EDTA as anticoagulant. The blood and anticoagulant were mixed. The platelet and leucocyte counts were obtained in a Coulter S automated portable counter under quality control status. The hematocrit reading was obtained by the micropipette method.

Plasma Renin Concentration

The plasma renin concentration was determined according to the method of Carvalho et al. The rabbit was conscious and quiet in the rabbit box with the aortic catheter protruding out through an opening. The fluid in the dead space of the catheter was removed into a tuberculin type syringe along with a small volume of aortic blood, and retained. The blood sample (0.3 ml) was withdrawn into a second syringe and injected into a capillary whole blood collector (Microtainer of Becton, Dickinson and Company), the same as that used to collect for platelet, WBC counts, and hematocrit reading. The EDTA and blood were mixed; the plasma was recovered by centrifugation and frozen at -20°C until analyzed. The mixture of blood and saline in the retained syringe was injected back into the animal, and the dead space was filled with saline.

The antibody and antigen used were obtained from the Clinical Assays, Inc. kit (Cambridge, Massachusetts). The renin substrate (plasma from rabbits 24 hours after nephrectomy) was derived in our laboratory. The control plasma samples were those of the kit. Our results using these controls were proper (kit values 7.32 and 3.66, our recovery 7.0 and 3.2, respectively).

**FIGURE 2.** Conglomerate of alkyl ether analogs of phosphatidylcholine was given by mouth (at arrows) to hypertensive rabbits. The statistical analyses are between the control values (zero day) and the values between Days 1 and 2.
Alkyl Ether of Phosphatidylcholine with Acetoyl in the Second Position

The alkyl ether conglomerate was derived from fresh renomedullary tissue, as previously described. The synthetic alkyl ether (1-O-hexadecyl-glyceryl-3-phosphorylcholine) was prepared by Curt Roos of Loradan Lipids AB of Malmo, Sweden. The second position was acetylated in our laboratory. The structure of this compound was verified by high resolution mass spectrometry. The acetylated compound caused an acute depressor effect in low doses (< 500 ng).

Administration of the Alkyl Ether to Conscious Hypertensive Rabbits

Group 1

There were two groups. Group 1 (n = 6) was given two doses of the conglomerate (APRL), each about 25 μg, 6 hours apart, on each of 2 consecutive days. The ether was dissolved in 0.5 ml saline by 20 seconds of sonification in a Bronson Sanogen Model D-50 sonicator, followed by 20 seconds of sonification in a Heat Systems Ultrasonics cell disruptor Model W185F instrument. It was then taken up in a 1 ml plastic syringe, the needle removed, and the syringe placed far back in the mouth while the ether solution trickled slowly as the animal swallowed. The body weight and MAP were determined before each dose. The platelet and leucocyte counts and hematocrit reading were obtained 1 hour after the first three doses and 20 hours after the fourth dose.

Group 2

Group 2 (n = 6) was treated precisely as Group 1 but received 25 μg of the pure synthetic C16:0 alkyl ether. In addition, this group had the food intake and the water, sodium, and potassium excretion determined for 5 days before the treatment and for 5 days after the treatment was started.

Plasma renin concentration of three animals in each group was determined before treatment and periodically thereafter until the MAP had recovered.

Statistical Analysis

The analysis was by the paired Student’s t test.

Results

Group 1

After the first oral dose (~25 μg) of the alkyl ether conglomerate in hypertensive rabbits, the MAP dropped from 106 to 90 mm Hg; after the second dose, to 89 mm Hg; and after the third dose, to 82 mm Hg (−24 mm Hg) (fig. 2). It required more than 60 hours after the last input of the alkyl ethers for the MAP to return to prior hypertensive levels. There was no change in the body weight as the pressure changes occurred.

As the MAP declined under the influence of the oral alkyl ether, there was no change in the platelet and leucocyte counts and no change in the hematocrit reading.

Figure 3. This group of hypertensive rabbits was given the synthetic C16:0 alkyl ether by mouth (at arrows). The statistical analyses are between the values of zero day and those of the other days.
Group 2

The hypertensive MAP of this group was lower than that of Group 1, averaging 95 mm Hg. The authentic (pure) C16:0 ether (~25 µg 6 hours apart in 2 days) given by mouth dropped the MAP to 79 mm Hg. Recovery again was slow. As the MAP receded (fig. 3), there was no change in body weight (average, 3.85 kg before, 3.86 kg after), no change in blood platelets and leucocytes, and no indication of diuresis, natriuresis, or kaliuresis (fig. 4). The plasma renin concentration (fig. 5) remained steady throughout (near 3 to 4 ng Al/ml/hr). Food intake was the same before and after the compound(s).

Appearance of Rabbits

At no time did we observe any outward indication of a reaction to the orally administered alkyl ether conglomerate in either group. The rabbits remained placid, breathed normally and, when placed on the floor, jumped about in the usual manner.

Discussion

The alkyl ether analogs of phosphatidylcholine represent a new class of biologically active lipids. The extent of their biologic activity is not known at present. Several activities have been described. Some of these compounds are potent platelet activators. They apparently mediate part of the inflammatory reaction, perhaps being involved in the delayed phase of the vascular response. In the guinea pig and the rabbit, certain parenteral doses cause bronchoconstriction. It has been suggested that this group of compounds may be involved in the anaphylactic response.

In separate presentations we have shown that these compounds are potent vasodilators as indicated by hemodynamic measurements of the whole animal and by direct effect on the microcirculation. Part of their action appears due to α-adrenergic antagonism.

Previously, we recorded a prolonged depressor effect in the hypertensive rat when these compounds, mainly the C16:0 alkyl ether, were introduced either by vein or mouth. At lower doses, the effect appeared to be due mainly to arterial-arteriolar dilation, as there was minimal effect on the cardiac output. There was also little change in heart rate. At higher doses, the cardiac output became depressed, a change interpreted as due to venous dilatation, as cardiac contractility did not appear to be altered.

It is therefore of interest that the conglomerate of ethers derived from renal tissue (mainly the C16:0 ether) and the synthetic and pure C16:0 ether gave rise to the same type of antihypertensive action in the hypertensive rabbit when given by mouth. The action did not stimulate renin release and did not alter the excretion of potassium and water. There was a suggestion of some sodium retention. Thrombocytopenia and leukopenia were not induced, and there was no effect on the hematocrit reading. Moreover, there was no indication of an anaphylactoid response when 25 Hg was given orally. We are left with the likelihood that these alkyl ether analogs of phosphatidylcholine are effective oral antihypertensive agents, acting as vasodilators.

The prolonged antihypertensive action of these alkyl ethers is one of the most intriguing aspects of their biologic behavior. It is characterized by the arterial pressure remaining depressed, without a change in cardiac output, for 30 to 72 or more hours after the last input of the compound given by mouth or parenterally. Since these compounds are rapidly inactivated in the circulation, the prolonged antihypertensive effect may be due to a prolonged action at the level of the resistance vessels, as has been described for the vasodilator, minoxidil, and its active metabolite, or an amplification of another system.

Minoxidil causes renin release, apparently by increasing adrenergic activity, while the alkyl ethers do not modify the state of the plasma renin concentration. In this respect, the alkyl ethers are unique as vasodilators, as most dilators stimulate renin release. The antihypertensive action is accompanied by a tendency to retain sodium, a feature that mimics the
action of other dilators. It remains to be demonstrated whether long-range antihypertensive activity can be attained with these compounds and whether the sodium retention, in time, will necessitate the use of diuretics for continued blood pressure control.

The pertinent features of the presently reported observations include a potent and prolonged antihypertensive activity of the 1-O-alkyl ether analogs of phosphatidylcholine with a short ester in the second position when given orally and no indication of other biologic activities attributed to these compounds. Other activities of these compounds have been described either by in vitro technique or by intravenous bolus injections. These include some adverse effects. It remains to be determined whether the oral administration of these compounds alters their structure, thus modifying their biologic activity, or whether they are absorbed intact and the rate of introduction into the body is such as to prevent activities other than the antihypertensive ones.

References
Alkyl ether analogs of phosphatidylcholine are orally active in hypertensive rabbits.
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