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INHIBITION OF RENIN SECRETION BY PLATELET ACTIVATING FACTOR (ACETYLGLYCERYL ETHER PHOSPHORYLCHOLINE) IN CULTURED RAT RENAL JUXTAGLOMERULAR CELLS

Josef Pfeilschifter, Armin Kurtz and Christian Bauer

Physiologisches Institut der Universität Zürich,
Winterthurerstrasse 190, CH-8057 Zürich, Switzerland

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:etylglyceryl ether phosphorylcholine (AGEPC), commonly known as .atelet activating factor, was found to strongly inhibit renin :cretion in cultures rich in juxtaglomerular cells. This inhibitory :tion of AGEPC was accompanied by an enhanced calcium permeability of ıe cell membrane as evaluated from measurements of the uptake of ·Ca. Simultaneous addition of the calcium channel blocker vera-- ımil abolished the effects of AGEPC on both renin secretion and ılcium permeability. Furthermore, addition of AGEPC to the cell ultures led to a decrease of ^{32}P-labeled phosphatidylinositol ,5-bisphosphate and to an increase in ^{3}H-labeled diacylglycerol, ndicating an activation of phospholipase C by AGEPC.

atelet activating factor (PAF) was first described as an inductor of atelet aggregation during IgE-mediated anaphylaxis in the rabbit ,2). PAF has recently been identified as 1-O-alkyl-2-acetyl-sn-glyce- -3-phosphorylcholine (AGEPC) (3). This phospholipid causes marked va- ıconstriction and increased permeability of blood vessels (4) as well ; contraction of ileal smooth muscle (5). AGEPC is produced from baso- ıils on immunological challenges and from platelets, neutrophils and ıcrophages in response to specific stimuli (6-11) and has also been ɜmonstrated in the venous effluent of isolated perfused rat kidney 12) and also in isolated glomeruli (28). Authors in (13) have shown ıat AGEPC possesses marked antihypertensive activity when given to Goldblatt" one-kidney, one-clip hypertensive rats. Hypertension in this ondition is known to be due to increased levels of plasma renin with ubsequent production of angiotensin II. In view of the fact that AGEPC as vasoconstrictive properties and vasoconstrictive agents such as

bbreviations used: AGEPC, acetylglyceryl ether phosphorylcholine; AF, platelet activating factor; AI, angiotensin I; PIP_2, phospha- idylnositol 4,5 bisphosphate; DAG, 1,2-diacylglycerol; IP_3, nositoltrisphosphate.

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angiotensin II and arginine-vasopressin markedly inhibit renin secretion (14), it is tempting to speculate that the antihypertensive properties of AGEPC in Goldblatt hypertension might be due to a direct inhibition of renin secretion in the kidney.

Recently we have established a cell culture system from rat kidneys containing about 60% of juxtaglomerular cells which allows study of renin secretion in some detail. Aim of the present study was to investigate the influence of AGEPC on renin secretion as well as on some pertinent cellular processes like calcium permeability of the cell membrane (15) and phosphoinositide metabolism (16-19).

Materials and Methods

Stock solutions of AGEPC (Sigma), (10^{-3}M) in ethanol were prepared and complexed with 0.3 mg/ml of bovine serum albumin (BSA) in MEM to give a concentration of 10^{-6}M. The BSA bound AGEPC was added to the cells to give a final concentration of 10^{-8}M or 10^{-10}M. Carrier free $^{32}P_i$, ^{45}Ca and (^{3}H)-glycerol were from Amersham International. T.L.C. plates (precoated silicagel 60 with concentration zone, 0,25 mm thick) came from Merck, Darmstadt, F.R.G. Lipid standards and BSA (essentially fatty acid-free) were obtained from Sigma. Cell culture dishes and cell culture media were from Greiner, Nürtingen, F.R.G. and Boehringer-Mannheim, respectively.

Cell Cultures rich in juxtaglomerular cells were prepared as described previously (15). In brief, rat kidneys were perfused in situ with Hank's balanced salt solution supplemented with 1 g glucose, 12.11 g sucrose, 1,2g $NaHCO_3$, 2.6 mM glutamine, 0,84 g Na-citrate and 10 mg BSA per liter. Cortical pieces (1 mm^3) were incubated with this buffer, containing 0,25% trypsin and 0,1% collagenase instead of Na-citrate. The suspension was gassed with 5% CO_2 in air and stirred with a magnetic bar at 37°C. After 120 min. the solution was poured over a 0,22 mm sieve. Cells passing the sieve were washed two times with culture medium (RPMI 1640, 25mM Hepes, 0,66 U/ml insulin, 100 µg/ml streptomycin, 100 U/ml penicillin and 10% fetal bovine serum) and seeded in tissue culture dishes at a density of 10^5 cells/cm^2. Cultures were kept at 37 °C in a humidified atmosphere at 5% CO_2 in air. After two days of culture, 60% of the adherent cells displayed a strong immunoreactivity for a specific antibody against renin. The renin content of the cultured cells was about 1µg AI/h x 10^6 cells at that time.

Determination of Renin Secretion. Renin activity was determined by its ability to generate angiotensin I (AI) from the plasma of bilaterally nephrectomized rats exactly as described in (20). AI was determined by radioimmunoassay (IDW, Dreieich, F.R.G.) Renin secretion was investigated at the second day of culture. The culture medium was substituted by a prewarmed buffer consisting of 132 mM NaCl, 5mM KCl, 0,8mM $MgSO_4$, 2mM $CaCl_2$, 10mM Na-acetate, 2mM NaH_2PO_4, 10mM glucose, 20mM hepes (pH = 7,3) and the culture dishes were placed on a heating block at a temperature of 37°C. The renin secretion rate was calculated from the linear increase of the renin activity of the cell-conditioned buffer.

Measurement of ^{45}Calcium-uptake. The culture medium was substituted by a prewarmed Hepes buffered saline (HBS) consisting of 20mM He-

pes, 148mM NaCl, 5mM KCl, 2mM $CaCl_2$, 1mM $MgCl_2$, 6mM Glucose, pH 7,3 supplemented with ^{45}Ca (4μ Ci/ml). Simultaneously, AGEPC or verapamil or a combination of both was added. At different times, buffer was withdrawn and the cells quickly washed with 10 x 1 ml ice-cold HBS containing 10mM $CaCl_2$. The cells were lysed by the addition of 1 ml of 0,5N NaOH and the radioactivity was counted in 10 ml Aquasol 2 (New England Nuclear) in a β-scintillation counter.

Prelabeling of cells with $^{32}P_i$ and (^{3}H)-glycerol. After two days of culture, medium was removed and the cells were incubated for 24 hours in phosphate-poor MEM (50μM P_i) containing 100μCi/ml $^{32}P_i$ or with MEM containing 5μCi/ml (^{3}H)-glycerol. After this prelabeling period the medium was replaced by fresh medium either without (control) or with AGEPC (10^{-8}M).

Extraction, separation and quantitation of Phosphoinositides and Diacylglycerol. Incubations were terminated by addition of ice-cold methanol-chloroform, and lipid extraction was done as described (21). The extracted ^{32}P-labeled phospholipids were separated on thin-layer plates impregnated with potassium oxalate and EDTA with a solvent system consisting of chloroform/methanol/4N NH_4OH (45/35/10, by vol.) Neutral lipids were separated using n-heptane/ diethyl ether/acetic acid (75/25/4, by vol.) as described (21). Phospholipids and neutral lipids were localized by comigration of standards followed by iodine staining and ^{32}P-labelled lipids additionally by autoradiography. The spots were scraped from the chromatographs and counted by liquid scintillation in water (Cerenkov counting) for ^{32}P labelled lipids. (^{3}H)-labeled lipids were analysed with a TLC-linear analyzer LB 2821 from Berthold, München, F.R.G. The detection efficiency for (^{3}H) was about 1%. Protein determination was done according to the method of Lowry (22) with BSA as standard.

Results

Fig. 1 shows that AGEPC strongly inhibited the spontaneous renin secretion in a concentration dependent manner. Whilst in control experiments, the rate of renin secretion was about 390 pg AI/h per min per dish, AGEPC at concentrations of (10^{-10}M) and (10^{-8}M) reduced the secretion rate to 175 pg AI/h and to 72.5 pg AI/h per min. and dish respectively. Note, that the term AI/h is formally equivalent with one unit of renin activity. This inhibition could only be observed during the first 15 minutes and reached control levels after 60 minutes. Simultaneous addition of verapamil (10^{-5}M) completely abolished the inhibitory effect of AGEPC, whilst the addition of verapamil alone greatly enhanced renin secretion (Fig. 1).

Fig. 2 shows that calcium uptake by the cultured cells was increased in the presence of AGEPC (10^{-8}M). Verapamil (10^{-5}M) strongly reduced the calcium uptake and the simultaneous addition of AGEPC and verapamil also reduced the calcium uptake, but to a slighter degree than did verapamil alone.

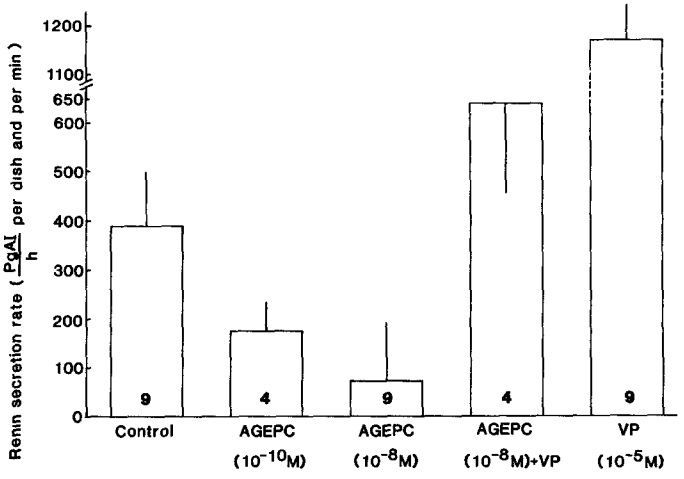

Fig. 1 Renin secretion rate of cultured juxtaglomerular cells in the presence of acetylglyceryl ether phosphorylcholine (AGEPC) (10^{-8}M) and (10^{-10}M), verapamil (VP) (10^{-5}M) and the combination of AGEPC (10^{-8}M) and VP (10^{-5}M). Data are mean +/- S.E.M. Figures at the bottom of the columns indicate the number of independent experiments.

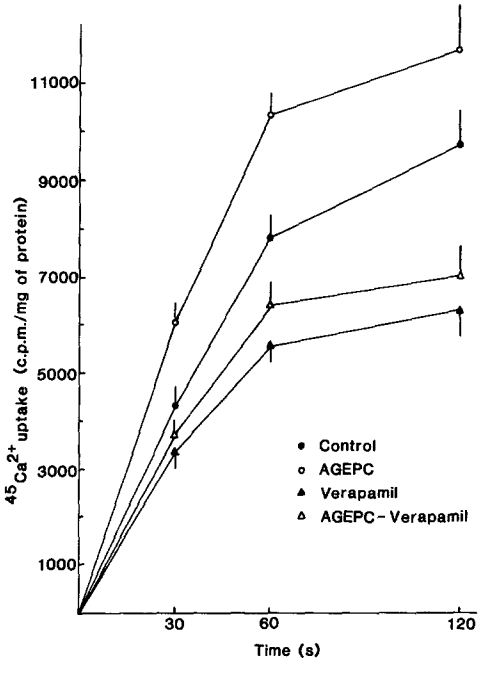

Fig. 2 ^{45}Calcium uptake in the presence of AGEPC (10^{-8}M), verapamil (10^{-5}M) and after simultaneous addition of AGEPC (10^{-8}M) and verapamil (10^{-5}M). Results are presented as mean +/- S.E.M. of eight experiments.

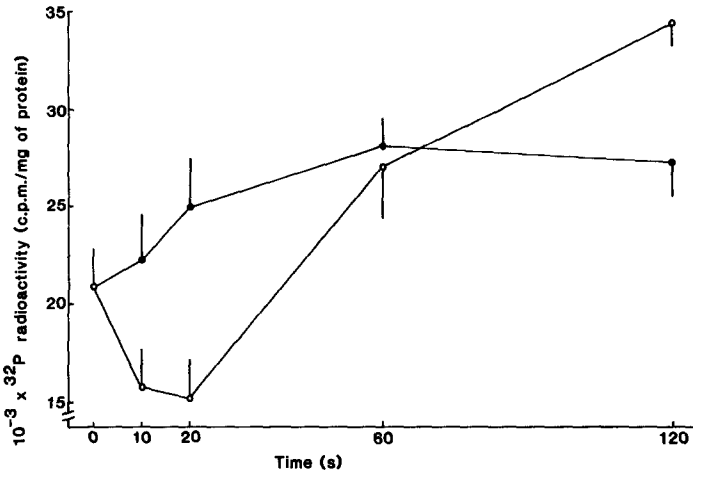

Fig. 3 Time course of ^{32}P radioactivity in phosphatidylinositol 4,5-bisphosphate. Open symbols represent stimulation with AGEPC (10^{-8}M); filled symbols represent control values. Values are mean +/- S.E.M., n = 5.

lition of AGEPC (10^{-8}M) to the cells provoked a rapid decrease of P) radioactivity found in phosphatidylinositol 4,5-bisphosphate [P_2) (Fig. 3). This effect could be detected as early as 10 seids after addition of AGEPC and was maximal (60% of control) within 20 One minute after addition of AGEPC, the level of ^{32}P in PIP_2 creased and approached control levels, followed by an increase above itial levels at 2 minutes. This early decrease of ^{32}P-label in P_2 was followed by a subsequent increase of (^3H)-glycel-label in diacylglycerol (DAG) which had a maximum 30 s after addion of AGEPC (Fig. 4).

scussion

e strong inhibition of renin secretion by AGEPC (Fig. 1) is comparable that of other vasoconstrictive agents like angiotensin II and argini--vasopressin (14). In analogy to what we have recently shown for giotensin II, AGEPC inhibits renin secretion probably by enhancing the -permeability of the cell membrane, thereby increasing the intracellur calcium concentrations (15). The complete suppression by verapamil the action of AGEPC on renin secretion suggests that AGEPC acts by ening calcium channels. The fact that an inhibition of renin secretion uld be observed only during the first 15 min. after addition of AGEPC

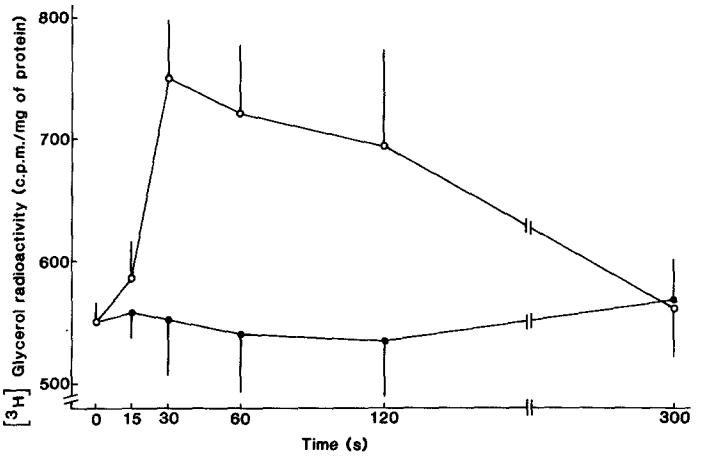

Fig. 4 Time course of (^{3}H)glycerol radioactivity in 1,2-diacylglycerol in juxtaglomerular cells stimulated with AGEPC (10^{-8}M); open symbols represent stimulation with AGEPC (10^{-8}); filled symbols represent control values.

s very likely to be due to the fast degradation of the agent (23).
t now appears to be generally accepted that hormones and neurotransmit-
ers that use calcium as second messenger specifically hydrolyse membra-
e phosphoinositides. Michell suggested in 1975 that calcium mobiliza-
ion is linked to phosphatidylinositol breakdown (24). However, more
ecent experiments have demonstrated that the primary event in receptor
timulated inositol phospholipid metabolism is by an action of
hospholipase C (PLC) upon phosphatidylinositol 4,5-bisphosphate
PIP_2) (for review see 25-26). Since AGEPC is known to cause
IP_2 degradation in platelets (17-19) and hepatocytes (16), we have
xamined the effect of AGEPC on PIP_2 metabolism in our cell culture
ystem. The results presented in Figs. 3 and 4 clearly show that AGEPC
eads to a loss of radioactivity from PIP_2 and an increase of
H)-glycerol label in diacylglycerol (DAG). Therefore, AGEPC
parently stimulates PLC in our cell culture system. We have observed
ry similar effects of angiotensin II and arginine-vasopressin on the
nositol phospholipid metabolism in these cells (manuscript submitted).
th of the cleavage products of PIP_2 metabolism, i.e. DAG and
ositol 1,4,5-trisphosphate (IP_3) are important second messengers
or review see (27)). IP_3 is thought to be the intracellular sig-
l to promote hormone induced calcium mobilization and DAG activates
otein kinase C which in turn phosphorylates various cellular proteins.
have recently found that the phorbol ester, 12-O-tetradecanoyl-

bol-13-acetate, which is widely used as an activator of protein ki-
C, enhances the calcium permeability in cultured glomerular mesan-
cells (unpublished results). In view of this, it can be speculated
an activation of protein kinase C by DAG is causally related to the
ease in calcium permeability observed in the present experiments
. 2).

his report we have presented evidence, that AGEPC strongly influen-
renin secretion and phosphoinositide metabolism in cultures rich in
al juxtaglomerular cells. The low concentration at which AGEPC inhi-
s renin secretion (10^{-10}M) and also the fact that AGEPC is pro-
ed by the kidney itself (12, 28) supports the notion that AGEPC is
olved in the regulation of kidney renin secretion and may be part
an intrarenal feedback mechanism controlling renin secretion.

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