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SPECIFIC ANTAGONISTS OF PLATELET ACTIVATING FACTOR-MEDIATED VASOCONSTRICTION AND GLYCOGENOLYSIS IN THE PERFUSED RAT LIVER*

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Abstract—Stimulation of hepatic glycogenolysis and vasoconstriction of the hepatic vasculature in response to acetyl glyceryl ether phosphocholine (AGEPC; platelet activating factor) was inhibited by two structural analogues of AGEPC, U66985 (1-*O*-octadecyl-2-*O*-acetyl-*sn*-glycero-3-phosphoric acid-6′-trimethyl ammonium hexyl ester) and CV3988 [*rac*-3-(*N*-*n*-octadecylcarbamoyloxy)-2-methoxypropyl-2-thiazolioethyl phosphate]. Infusion of CV3988, 10^{-7} M, increased the AGEPC dose needed for half-maximal hemodynamic response by approximately 5-fold, while U66985 at 10^{-7} M increased by twenty times the dose of AGEPC required to give the half-maximal response. Glucose output responses were similarly inhibited. U66985, 10^{-6} M, completely abolished both hemodynamic and glycogenolytic responses to AGEPC, 2×10^{-10} M, while in the presence of CV3988, 10^{-6} M, approximately 15% of the uninhibited responses remained. Perfusion of livers for 20 min after termination of inhibitor infusion, in the absence or presence of bovine serum albumin, resulted in only a slightly smaller extent of inhibition than simultaneous infusion of agonist and antagonist. Specificity of the inhibitors was demonstrated by only a minimal inhibition of glycogenolytic response to the α-adrenergic agonist phenylephrine at a sub-maximal dose.

Platelet activating factor (acetyl glyceryl ether phosphocholine; AGEPC) is a biologically active phospholipid, which has been implicated as a primary mediator of acute inflammatory and allergic reactions [1–4]. The structure of this potent agonist has been identified as 1-*O*-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine [5]. In addition to the role of AGEPC in modulating platelet and polymorphonuclear leukocyte function, several non-inflammatory tissues have been shown to respond to AGEPC. Negative inotropic effects of AGEPC on isolated, perfused guinea pig hearts have been shown [6, 7], and Camussi *et al.* [8] have demonstrated biphasic ionotropic effects of AGEPC on guinea pig papillary muscle. Contraction of rat intestinal strips [9] and isolated guinea pig ileum [10, 11] has been shown in response to AGEPC, while this phospholipid also causes vasoconstriction of guinea pig pouch microvasculature [12]. In contrast, Kamitani *et al.* [13] have reported hypotensive effects of AGEPC in intact rats, and vasodilation of precontracted aortae, while Muirhead *et al.* [14] using antihypertensive polar renomedullary lipid (APRL), a kidney derived fraction reported to contain a mixture of 1-*O*-alkylphosphatidylcholine ethers, have shown dilation of hindquarter resistance vessels in perfused rat vascular beds, and reduced mean arterial pressure in intact rats.

Recent work in our laboratories has demonstrated a potent glycogenolytic action of AGEPC in the perfused rat liver [15, 16] and has indicated that AGEPC causes a dramatic transient vasoconstriction of the hepatic vasculature.‡ This vasoactive response of AGEPC in the hepatic circulation is very much in keeping with the transient increase in portal vein pressure observed in the dog during acute circulatory collapse in response to injection of AGEPC [17].

Production of platelet activating factor activity by the liver in response to infusion of immune aggregates also has been demonstrated [18].

Terashita *et al.* [19] have reported that CV3988 [*rac*-3-(*N*-*n*-octadecylcarbamoyloxy)-2-methoxypropyl-2-thiazolioethyl phosphate], an analogue of AGEPC, inhibits specifically AGEPC-induced rabbit platelet aggregation, and also the dose-dependent hypotension observed in anesthetized rats in response to i.v. administration of AGEPC. In the present study, the effects of CV3988 on hepatic glycogenolytic and hemodynamic responses to AGEPC were investigated and compared with the effects of another AGEPC analogue, U66985 (1-*O*-octadecyl-2-*O*-acetyl-*sn*-glycero-3-phosphoric acid-6′-trimethyl ammonium hexyl ester).

MATERIALS AND METHODS

1-*O*-Hexadecyl-2-*O*-acetyl-*sn*-glycero-3-phosphocholine (AGEPC) was obtained from Bachem, Bubendorf, Switzerland. CV3988, synthesized as described by Terashita *et al.* [19] was a

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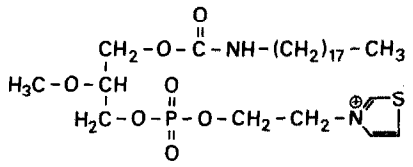
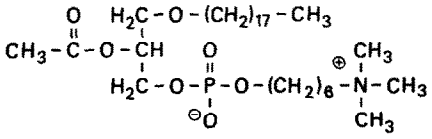


A) CV3988



B) U-66985

Fig. 1. Structures of (a) CV3988 [*rac*-3-(*N*-*n*-octadecylcarbamoyloxy)-2-methoxypropyl 2-thiazolioethyl phosphate] and (b) U66985 (1-*O*-octadecyl-2-*O*-acetyl-*sn*-glycero-3-phosphoric acid-6′-trimethyl ammonium hexyl ester).

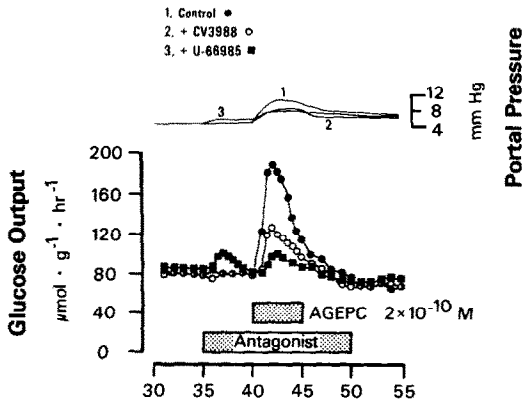


Fig. 2. Inhibition of hepatic glucose output and portal pressure increase responses to AGEPC by antagonists. Key: (●—●) control, no antagonist, (○—○) CV3988, 10^{-7} M, coinfused, and (■—■) U66985, 10^{-7} M, coinfused. AGEPC and antagonists were infused as shown by the horizontal bars. The experiments shown are representative of at least four experiments for each condition.

gift of Takeda Chemical Ind., Ltd., Osaka, Japan. U69985 was provided by D. E. Ayer, Lipids Research, The Upjohn Co., Kalamazoo, MI. Figure 1 shows the structures of CV3988 and U66985. AGEPC, CV3988 and U66985 were dissolved in chloroform/methanol (1:1, v/v) and stored at −20°. Before infusion into perfused rat livers, the samples were dried with nitrogen gas and dissolved in 0.15 M NaCl containing bovine serum albumin, 2.5 mg/ml.

Livers from male Sprague–Dawley rats, 160–200 g body weight, fed *ad lib.*, were perfused *in situ* using a non-recirculating hemoglobin-free perfusion system [20]. The perfusion medium was Krebs–Henseleit [21] bicarbonate buffer, calcium concentration, 1.25 mM, pH 7.4, saturated with a mixture of 95% oxygen and 5% carbon dioxide, and maintained at 37°. Hepatic oxygen consumption was monitored using a Clark-type oxygen electrode placed in the perfusion circuit immediately after the liver. Portal vein pressure, an index of intrahepatic pressure [22], was monitored using a Statham P23 ID pressure transducer in conjunction with a Grass model 7 Polygraph, connected to the portal cannula line. Basal portal vein pressure was 4.9 ± 0.3 mm Hg. Effluent perfusate was collected for 30-sec intervals for measurement of glucose. Livers were perfused for 30 min prior to commencement of antagonist or agonist infusion, to ensure removal of endogenous hormones and to stabilize glucose output. Glucose was measured by the method of Bergmeyer *et al.* [23].

Glucose production rates are expressed as μmoles glucose per gram wet weight. Results are presented as means ± standard errors.

RESULTS

The effects of CV3988 and U66985, 10^{-7} M, on glucose output and portal vein pressure in the perfused rat liver are shown in Fig. 2. CV3988 alone, at 10^{-7} M, had no effect on basal glucose output or portal vein pressure, but inhibited the hepatic response to subsequent coinfusion of AGEPC, 2×10^{-10} M. U66985 alone, 10^{-7} M, caused a small, transient increase in hepatic glucose output and increased portal pressure slightly. However, when AGEPC at 2×10^{-10} M was co-infused with either one of the two antagonists, a greater inhibition of the hepatic response to AGEPC was observed with U66985 than with CV3988. In Fig. 3, A and B, dose–

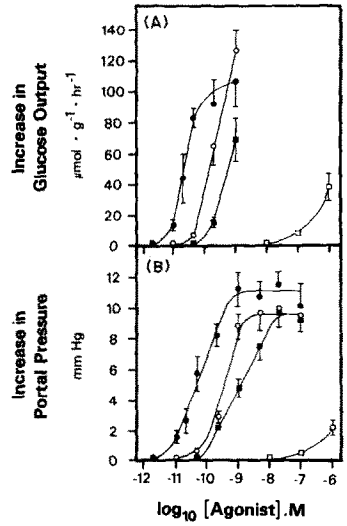


Fig. 3. Effects of antagonists on the AGEPC dose–response curves for (A) maximal hepatic glucose output increase and (B) maximal portal vein pressure increase. Key: (●—●) AGEPC, no antagonist, (○—○) AGEPC + CV3988, 10^{-7} M, (■—■) AGEPC + U66985, 10^{-7} M, and (□—□) agonist effects of U66985 in absence of AGEPC. Experimental protocol was shown in Fig. 2. Each point represents means ± standard errors for four to eight livers.

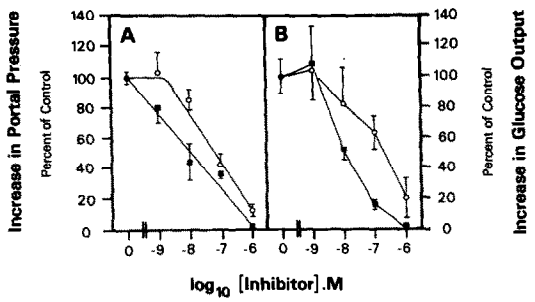


Fig. 4. Effect of antagonist concentration on hepatic responses to AGEPC, 2×10^{-10} M. Key: (■—■) + U66985 and (○—○) + CV3988. Experimental protocol was as shown in Fig. 2. Each point represents results from four to six livers. One hundred percent values (no antagonist) in this series of experiments were: maximal increase in glucose output, 100 ± 11 μmoles·hr^{-1}·g^{-1}; maximal increase in portal pressure, 6.1 ± 0.3 mm Hg.

response curves are shown for hepatic glycogenolytic and hemodynamic responses to AGEPC alone and to AGEPC in the presence of CV3988 at 10^{-7} M, or U66985 at 10^{-7} M. For livers perfused with AGEPC alone, half-maximal increase in portal pressure occurred at 5×10^{-11} M AGEPC (Fig. 3B). In the presence of CV3988 at 10^{-7} M, the AGEPC concentration required to give half-maximal portal pressure increase was increased to 2.5×10^{-10} M. Since the glucose output response to AGEPC becomes biphasic at high concentrations of AGEPC [16], it is not possible to determine the AGEPC dose giving half-maximal increase in glucose output, but it can be seen from Fig. 3A that the inhibitors caused shifts in the AGEPC-induced glucose output response similar to those seen in the portal pressure response.

Figure 3 also illustrates responses to U66985. Both glucose output and portal pressure were increased in a dose-dependent fashion by U66985, the minimum effective concentration tested being 10^{-7} M. The inhibitor is thus 4–5 orders of magnitude less active as an agonist than AGEPC. CV3988 showed no agonist activity at concentrations as high at 10^{-6} M.

In Fig. 4, the effects of inhibitor concentration on the hepatic responses to AGEPC, 2×10^{-10} M, are demonstrated. Infusion of U66985 at concentrations as low as 10^{-9} M resulted in a significant inhibition of the AGEPC-induced portal pressure increase (Fig. 4A). Increasing the U66985 concentration produced a progressive increase in the extent of inhibition, until at 10^{-6} M U66985 the portal pressure response to AGEPC was prevented completely. Glucose out-

Table 1. Limited reversal of antagonist action of CV3988 and U66985 after removal of inhibitors

Condition	Maximal increase in glucose output (μmoles·g^{-1}·hr^{-1})	Maximal increase in portal vein pressure (mm Hg)
CV3988, 10^{-6} M, 5 min; then no addition, 20 min; then plus AGEPC, 2×10^{-10} M, 5 min	33 ± 10	2.8 ± 0.9
CV3988, 10^{-6} M, 5 min; then plus bovine serum albumin, 25 μg/ml, 20 min; then plus AGEPC, 2×10^{-10} M, 5 min	40 ± 17	2.6 ± 0.7
CV3988, 10^{-6} M, 5 min; then CV3988, 10^{-6} M, plus AGEPC, 2×10^{-10} M, 5 min	20 ± 13	0.7 ± 0.2
U66985, 10^{-6} M, 5 min; then no addition, 20 min; then plus AGEPC, 2×10^{-10} M, 5 min	14 ± 4*	0.9 ± 0.3*
U66985, 10^{-6} M, 5 min; then plus bovine serum albumin, 25 μg/ml, 20 min; then plus AGEPC, 2×10^{-10} M, 5 min	18 ± 5*	2.0 ± 0.4†
U66985, 10^{-6} M, 5 min; then U66985, 10^{-6} M, plus AGEPC 2×10^{-10} M	0	0

Livers per perfused as described in Materials and Methods. CV3988, 10^{-6} M, or U66985, 10^{-6} M, was infused for 5 min. The livers were then perfused for a further 20 min with no addition or with bovine serum albumin, 25 μg/ml. AGEPC, 2×10^{-10} M, was then infused for 5 min, and the maximal increases in glucose output and portal pressure were measured. In another set of livers, AGEPC, 2×10^{-10} M, was coinfused with the antagonist, AGEPC infusion commencing 5 min after initiation of antagonist infusion. AGEPC infused alone without antagonist gave increases of 100 ± 11 μmoles·g^{-1}·hr^{-1} and 6.1 ± 0.3 mm Hg respectively, for maximal glucose output and maximal portal vein pressure. Values represent means ± standard errors for three to four livers. Symbols (*,†) indicate significant differences.

* $P < 0.02$ vs simultaneous infusion of antagonist.

† $P < 0.005$ vs simultaneous infusion of antagonist; $P < 0.02$ vs perfusion without bovine serum albumin.

Table 2. Effects of CV3988 and U66985 on the glycogenolytic response to phenylephrine

Condition	Maximal increase in glucose output (μmoles·g^{-1}·hr^{-1})
No addition, 5 min; then phenylephrine, 5×10^{-7} M, 5 min	105 ± 3
CV3988, 10^{-7} M, 5 min; then CV3988, 10^{-7} M, plus phenylephrine, 5×10^{-7} M, 5 min	127 ± 16
U66985, 10^{-7} M, 5 min; then U66985, 10^{-7} M, plus phenylephrine, 5×10^{-7} M, 5 min	87 ± 5*

Livers were perfused as described in Materials and Methods.
Values represent means ± standard errors for four livers.
* $P < 0.01$ vs no antagonist addition.

put responses were inhibited similarly, although no inhibition of glucose output was seen below 10^{-8} M U66985 (Fig. 4B). CV3988 was approximately 10-fold less effective as an antagonist of AGEPC than U66985, with 10^{-8} M the minimum effective dose for inhibiting portal pressure increases, and with 10^{-7} M the minimum dose for significant inhibition of glucose output. Significant AGEPC activity was still observed in the presence of 10^{-6} M CV3988.

Since desensitization of the response to AGEPC by prior exposure to the agonist has been demonstrated in a number of systems, including smooth muscle contraction [10], platelet secretion and aggregation [24], enzyme secretion from neutrophils [25], and hepatic glucose output [16] and portal pressure increase,* it was of interest to investigate the recovery of the AGEPC response after removal of antagonist. Table 1 shows that perfusion for 20 min after cessation of antagonist infusion led to only a minor reversal of the inhibition. Infusion of bovine serum albumin during the 20-min period did not alter appreciably the inhibition of the subsequent AGEPC response, suggesting that the continued residual inhibition was not due merely to inefficient removal of the hydrophobic inhibitors in the absence of bovine serum albumin as a binding agent.

To ensure that the antagonists were inhibiting specifically the response to AGEPC, the effects of these inhibitors on the hepatic glycogenolytic and oxygen consumption responses to phenylephrine, an α-adrenergic agonist, were tested at a sub-maximal dose of phenylephrine (5×10^{-7} M). CV3988, 10^{-7} M, did not inhibit the increased glucose output observed in response to the catecholamine, while U66985, 10^{-7} M, caused only a small (e.g. 17%) inhibition of the glycogenolytic effect of phenylephrine (Table 2).

DISCUSSION

Structural analogues of acetyl glyceryl ether phosphocholine, CV3988 and U66985, were effective inhibitors of the hepatic responses to AGEPC. CV3988 at a concentration of 10^{-7} M increased the AGEPC concentration required for half-maximal portal pressure response approximately 5-fold, while U66985, 10^{-7} M, caused a much greater inhibition, increasing the half-maximal AGEPC dose nearly 20-fold. AGEPC is believed to act through a specific receptor; specific high-affinity binding sites have been demonstrated on human platelets and polymorphonuclear leukocytes [25–29], and on guinea pig smooth muscle and rabbit platelets [30]. There is evidence to suggest that desensitization by prior exposure to AGEPC may involve loss of receptors, perhaps through internalization or structural alteration of the membrane receptor [25–28]. The finding that antagonism of the AGEPC response by U66985 and CV3988 was not readily reversible raises the possibility that binding of the inhibitors may lead to receptor loss. Alternatively, the inhibitor binding may be very tight, dissociation taking place only very slowly.

CV3988 showed no agonist activity in liver at concentrations up to 10^{-6} M, consistent with the results of Terashita *et al.* [19] who found this compound to be without aggregating activity toward rabbit platelets at concentrations as high as 10^{-3} M. U66985 showed some stimulation of hepatic glucose output and portal pressure at high concentrations ($\geq 10^{-7}$ M), but was 4–5 orders of magnitude less potent than AGEPC. Interestingly, U66985 showed very weak agonist activity towards washed rabbit platelets and then only on occasion even at concentrations higher than 10^{-6} M. On the other hand, both CV3988 and U66985 consistently exhibited comparable patterns of inhibition of AGEPC-induced aggregation, secretion and inositol phospholipid turnover in washed rabbit platelets to those observed for the hepatic responses to AGEPC [31].

While CV3988, 10^{-7} M, caused no inhibition of the glycogenolytic response to the α-adrenergic agonist phenylephrine, 5×10^{-7} M U66985 gave a modest inhibition (17%). By comparison, at an AGEPC concentration (2×10^{-10} M) giving a similar glycogenolytic response to the catecholamine, U66985 inhibited the glucose output response to AGEPC by 85%. It is noteworthy that α-adrenergic antagonists have been demonstrated to inhibit, at high concentrations, binding of AGEPC to platelets, leading to the suggestion that the catecholamine and AGEPC receptors may share some structural similarity [32].

* Buxton *et al.*, *J. biol. Chem.*, in press.

The results of the present study support the potential value of U66985 and CV3988 for investigating the role of AGEPC in the regulation of metabolic and hemodynamic homeostasis in situations such as systemic anaphylaxis. Also, the present study suggests possible therapeutic uses of these and related compounds for blocking potentially damaging effects of AGEPC in systemic anaphylaxis [33] and acute serum disease [34].

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