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Evidence for the lack of interaction between (±)- 1-O-octadecyl-2-acetylglyceryl-3-phosphorylcholine and α-adrenoceptors *in vivo* and *in vitro*

P. Cervoni, B.M. Goldstein, H. Herzlinger, F.M. Lai & G.J. Quirk

Cardiovascular Biological Research, Medical Research Division, American Cyanamid Company, Pearl River, New York 10965 U.S.A.

1 The interactions of (±)-1-O-octadecyl-2-acetylglyceryl-3-phosphorylcholine (octadecyl-AGPC) with α-adrenoceptors were studied in rat mesenteric artery, cat nictitating membrane and on the blood pressure of the cat and spontaneously hypertensive (SH) rat.

2 Using a direct radioligand α-adrenoceptor binding assay in particulate fractions of rat mesenteric arteries, octadecyl-AGPC was found to be 5×10^7 and 75 times less potent than prazosin and noradrenaline (NA), respectively, in displacing (2,6-dimethoxyphenoxyethyl)-aminomethyl-1,4-benzodioxane ([^{3}H]-WB 4101 — a selective probe for the identification of α-adrenoceptors).

3 In the cat, intravenous infusions of octadecyl-AGPC, which produce a hypotensive response, did not attenuate nictitating membrane contractions *in vivo* in response to intravenous injections of NA, adrenaline (Ad) or to electrical stimulation of the postganglionic fibres of the superior cervical ganglion. In these experiments, the pressor responses to NA or Ad were not affected by octadecyl-AGPC. Phentolamine, on the other hand, attenuated nictitating membrane contractions and blood pressure responses to Ad or NA.

4 In the SH rat, octadecyl-AGPC decreased mean arterial blood pressure (MABP). After an intravenous dose of phentolamine which lowered MABP, the depressor response to octadecyl-AGPC was reduced. When MABP in the phentolamine-treated SH rat was restored to its initial level with an infusion of angiotensin II (AII), the depressor response to octadecyl-AGPC was restored to its original magnitude. The effectiveness of α-adrenoceptor blockade under these experimental conditions was monitored with intravenous NA and Ad.

5 Thus, based on radioligand binding studies and pharmacological studies, it is concluded that octadecyl-AGPC lacks the ability to interact with α-adrenoceptors.

Introduction

A series of phospholipids isolated from renomedullary tissues as well as interstitial cells has been identified and shown to possess potent anti-hypertensive and vasodilator activity (Muirhead, 1980). The structure of the lipid extract has been elucidated and found to consist of 1-O-alkyl ether analogues of phosphatidylcholine and has been named as the antihypertensive polar renomedullary lipid (APRL). The main constituents of this extract are C16-C18 alkyl ethers. APRL has also been shown to be structurally identical to platelet activating factor (Paf-acether) (Demopoulos *et al.*, 1979). APRL is a highly potent hypotensive substance which lowers blood pressure in animal models of experimental hypertension by a mechanism of direct vasodilatation (Muirhead, 1980). However, several reports in the literature have proposed that APRL possesses some α-adrenoceptor blocking properties *in vivo* and *in vitro* (Smith *et al.*, 1981; Gaillard *et al.*, 1981). Recently, we (Lai *et al.*, 1983) have described the hypotensive and vasodilator activity of a totally synthetic chemical, namely, (±)-1-O-alkyl-2-acetylglyceryl-3-phosphorylcholine (octadecyl-AGPC) in the normotensive rat. It is the purpose of this paper to relate the interaction of octadecyl-AGPC with α-adrenoceptors utilizing a variety of experimental procedures including a direct α-adrenoceptor radioligand binding technique.

Methods

α-Adrenoceptor radioligand binding in rat mesenteric vasculature

A particulate fraction of the mesenteric artery containing predominantly smooth muscle was prepared using the method described by Colucci *et al.* (1981). Male Sprague-Dawley rats (Charles River, Wilmington, MA), 300–325 g, were killed by a blow on the head and cervical dislocation. A midline incision was made in the abdomen and polyethylene (PE) 50 tubing was inserted into the mesenteric artery at its origin from the aorta. The entire mesenteric artery was perfused with 0.15 M phosphate buffered saline, pH 7.4, at a constant rate of 5 ml min^{-1} until the residual blood was completely removed. The mesenteric vasculature was freed from the intestine and transferred to a petri dish filled with phosphate buffered saline. The mesenteric vein, fat and lymph nodes were carefully removed. Arteries from 2 animals were placed in 4 ml of cold sucrose solution (0.25 M) in a glass homogenizer. Mild homogenization of the tissue was performed with a hand-held Teflon pestle, which was loosely fitted to the tube, to remove fatty adventitial tissues. Arteries from rats, cleaned in this fashion, were pooled, minced and homogenized with a Brinkman Polytron (2 × 10 s at a setting of 8) in 75 ml sucrose solution. The homogenate was centrifuged at 3500 r.p.m. for 10 min at 4 °C. The supernatant was filtered through nylon mesh and cheesecloth and recentrifuged at 35,000 r.p.m. for 30 min at 4 °C. The resultant pellet was resuspended in 1.5 ml of assay buffer (contains: Tris-HCl 50 mM and $MgCl_2$, 5 mM, pH 7.55). The mesenteric artery pellets from 10 rats were combined for each experiment to yield a final protein content of approximately 550 μg per assay tube (150 μl total volume). Tissue protein was determined according to the method of Lowry *et al.* (1951).

The α-adrenoceptor binding assay described by Colucci *et al.* (1981) was used. Briefly, arterial membrane preparations of approximately 550 μg protein were incubated in triplicate with 0.5 to 1.0 nM of tritium labelled (2,6-dimethoxyphenoxyethyl)-aminomethyl-1,4-benzodioxane ([^{3}H]-WB 4101), with and without antagonist, in a final volume of 150 μl for 20 min at 22 °C. At the end of the incubation period, the reaction was stopped by immersing the assay tubes in ice-cold water. Ice-cold assay buffer (4.5 ml) was added to each tube and the fluid was filtered through a Whatman GF/C glass fibre filter. Each tube and filter was rinsed with two additional 4.5 ml aliquots of assay buffer. The filter was placed in a scintillation counting vial, 5 ml of Ready-Solv HP added and the samples counted. Specific binding for [^{3}H]-WB 4101 was defined as the percentage of total bound counts minus the non-specific counts. The dissociation constants and maximum number of receptors were determined by the method of Scatchard (1949).

Cat nictitating membrane preparation

Mongrel cats were conditioned. Conditioning consisted of a minimum of 21 days of quarantine during which time the cats were immunized for rabies, feline distemper, rhinotracheitis, calici virus and treated for other diseases and parasitic infestations as determined by caprology, haematological examination and clinical chemistry. All findings, immunizations and treatments along with body weight record and vendor's records become part of the individual animal's medical record. Cats of either sex, weighing 2.5–4.0 kg, were anaesthetized with sodium pentobarbitone (35 mg kg^{-1} i.p.). Each cat was placed in the supine position on a canvas sling within a Flexaframe rack. The head was immobilized by placing the frame rod between the cat's jaw and tying the jaws together. A tracheal cannula was inserted via a tracheotomy and the cats respired spontaneously. A PE 90 cannula was placed in the right femoral artery for recording blood pressure. The arterial cannula was connected to a Statham P23Db pressure transducer and a Gould recorder. The right femoral vein was cannulated for infusion of octadecyl-AGPC. The left femoral vein was cannulated for administration of supplemental anaesthesia and various agonists. Body temperature was maintained at 38 °C with a Hamilton Aquamatic K Module heating pad. Nictitating membrane (NM) contractions were elicited by stimulation of postganglionic fibres of the right superior cervical ganglion at various frequencies as described by Mirkin & Cervoni (1962). Rectangular pulses of 0.5 ms duration and supramaximal voltage were applied for 10 s at all frequencies used. The response at each frequency was determined at 2 min intervals. Statistical analysis was carried out using the two-tailed Student's *t* test for paired data.

The experimental protocol was as follows: control NM responses to adrenaline (Ad) and noradrenaline (NA) and a frequency-response curve to postganglionic nerve stimulation were obtained. Octadecyl-AGPC was then infused until a significant stable decrease in mean arterial blood pressure (MABP) was observed. With the octadecyl-AGPC infusion continuing, NM responses to Ad and NA and the frequency-response curve were again determined. The infusion was stopped and the cat's MABP allowed to return to the control level. After the MABP had stabilized at or near the control level, NM responses to Ad, NA and nerve stimulation were once again determined. One hour later, the control responses to Ad, NA and nerve stimulation were re-

peated. A 2 mg kg^{-1} i.v. bolus of phentolamine was administered and when the hypotensive response to phentolamine was at a steady state, the frequency-response curve and the Ad and NA challenges were repeated.

Blood pressure studies in conscious spontaneously hypertensive (SH) rats

Under ether anaesthesia, Weeks type cannulae (Peterson Technics, Monmouth Junction, NJ) were surgically implanted in the abdominal aorta and vena cava of SH rats (Taconic Farms, Germantown, NY), passed subcutaneously and exteriorized at the back of the neck. The cannulae were filled with saline, plugged, and the rats returned to single cages and allowed food and water *ad libitum*. Following a 4–7 day period, a PE cannula was implanted in the caudal vein under local anaesthesia. Approximately 24 h later, the rats were weighed and placed in Broome-style restraining cages. The plug was removed from the aortic cannula and connected to a Statham P23ID pressure transducer using PE 100 tubing and a stepdown connector fabricated from stainless steel hypodermic tubing. Arterial pulse pressure was monitored on a Grass Model 7 recorder. MABP was obtained by electrical damping of the pulse pressure channel. The plug was removed from the vena cava catheter and a PE 20 tubing extension was added using a piece of stainless steel hypodermic tubing. The other end of the extension was terminated with a 27G needle and a 1 ml syringe. The caudal vein cannula was similarly attached to a PE 10 tubing extension and used for drug infusions.

To serve as controls, two responses were obtained to the following agonists in the doses indicated: angiotensin II (AII; 0.3 μg kg^{-1}), NA (1 μg kg^{-1}), Ad (1 μg kg^{-1}) and octadecyl-AGPC (1 μg kg^{-1}). Phentolamine (3 mg kg^{-1}) was administered intravenously and after waiting 10 min, single responses to each agonist were again obtained. AII (0.1 μg kg^{-1} min^{-1}) was infused and after approximately 20 min (when MABP had stabilized at a new level), the agonists were re-administered. Finally, a second dose of phentolamine (3 mg kg^{-1}) was given while continuing the AII infusion and after 10 min, the agonists were injected again.

Drugs

Drugs used in this study were: angiotensin II amide, phentolamine methanesulphonate (Ciba-Geigy, Summit, NJ); adrenaline chloride solution (Parke-Davis Co., Ann Arbor, MI), (−)-noradrenaline hydrochloride (Aldrich Chemical Co., Milwaukee, WI), octadecyl-AGPC (Lederle Laboratories, Pearl River, NY) and prazosin hydrochloride (Pfizer, New York, NY). All drugs were dissolved in 0.9% w/v NaCl solution (physiological saline) and doses expressed in terms of base.

Results

α-Adrenoceptor radioligand binding in rat mesenteric vasculature

The inhibitory effects of prazosin, NA and octadecyl-AGPC on the binding of [^{3}H]-WB 4101 to α-adrenoceptors in rat mesenteric vasculature are depicted in Figure 1. The selective α-adrenoceptor antagonist, prazosin, and the α- and β-adrenoceptor

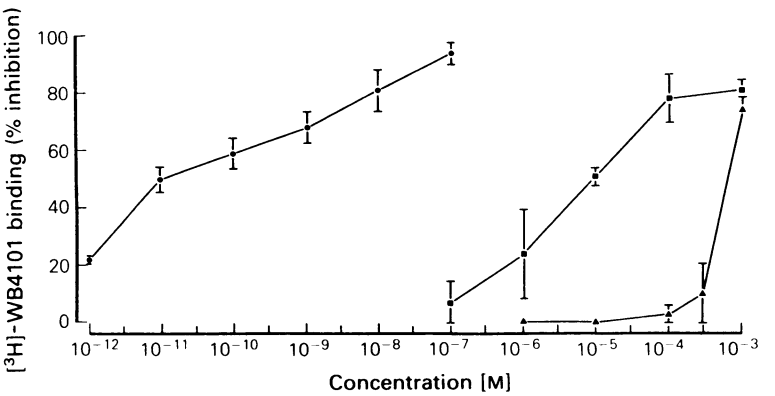

Figure 1 Concentration–response curves for the inhibition of [^{3}H]-WB 4101 binding by noradrenaline (■), octadecyl-AGPC (▲) and prazosin (●) in rat mesenteric artery membrane preparations. Each point is the mean of triplicate determinations of three separate experiments.

Table 1 The effects of octadecyl-AGPC and phentolamine on contractions of the cat nictitating membrane in response to exogenously administered adrenaline (Ad) and noradrenaline (NA) and postganglionic nerve stimulation

	Control (g)	*During octadecyl-AGPC infusion* (g)	*Control* (g)	*After phentolamine* (g)
Adrenaline ($2\,\mu g\,kg^{-1}$ i.v.)	2.9±0.5	3.1±0.6	3.0±0.7	0*
Noradrenaline ($5\,\mu g\,kg^{-1}$ i.v.)	1.5±0.3	1.3±0.4	1.8±0.5	0*
Postganglionic nerve stimulation				
1 Hz	8.3±1.3	7.3±1.6	6.3±0.3	0.8±0.4*
3 Hz	12.0±1.4	12.3±1.9	9.8±0.5	2.5±0.3*
10 Hz	16.0±2.2	15.8±2.5	13.0±0.9	5.5±0.3*
30 Hz	20.3±2.7	20.0±2.7	16.5±1.3	9.0±1.1*

The postganglionic nerve fibres of the superior cervical ganglion were stimulated with bipolar silver electrodes with square wave stimuli of 5 ms duration and supramaximal voltage. Octadecyl-AGPC was infused at $1-2\,\mu g\,kg^{-1}\,min^{-1}$ causing a decrease in mean arterial blood pressure (MABP). Phentolamine was given as an i.v. $2\,mg\,kg^{-1}$ injection causing a decrease in MABP. Ad and NA were given as i.v. bolus injections. Values are means±s.e. ($n=4$). *$P<0.05$ compared to control.

agonist, NA, have estimated IC_{50}s of 0.014 nM and 9500 nM, respectively, which are significantly less than that of octadecyl-AGPC (700,000 nM).

The effect of octadecyl-AGPC on responses of the cat nictitating membrane to agonists and nerve stimulation

Octadecyl-AGPC infused at 1 or $2\,\mu g\,kg^{-1}\,min^{-1}$ reduced MABP in 4 cats from 110.0±8.7 to 55.5±7.2 mmHg. Under these conditions, the NM responses to Ad, NA or nerve stimulation were unchanged (Table 1). A bolus injection of phentolamine, $2\,mg\,kg^{-1}$ i.v., decreased MABP from 87.5±3.9 to 54.0±4.3 mmHg. The NM contractions to Ad or NA were completely inhibited (100%) after phentolamine. In these experiments, the octadecyl-AGPC infusions had no effect on the pressor responses to Ad or NA (Table 2) while phentolamine significantly decreased the pressor responses to Ad and NA.

Table 2 The effects of octadecyl-AGPC and phentolamine on the pressor responses to adrenaline (Ad) and noradrenaline (NA) in cats

		Control MABP (mmHg)	*MABP during octadecyl-AGPC infusion* (mmHg)	*Control MABP* (mmHg)	*MABP after phentolamine* (mmHg)
Adrenaline ($2\,\mu g\,kg^{-1}$ i.v.)	Before Ad	110.0± 8.7	55.5±7.2	87.5±3.9	54.0±4.3
	After Ad	152.3± 9.2	92.5±4.1	132.5±7.2	60.0±4.6
	Change	42.3± 3.8	37.0±6.2	45.0±7.5	6.0±1.0*
Noradrenaline ($5\,\mu g\,kg^{-1}$ i.v.)	Before NA	106.8± 8.4	56.0±6.4	87.3±2.8	58.5± 4.1
	After NA	192.3±14.4	130.8±7.6	158.8±4.4	84.8±11.1
	Change	85.5± 7.2	74.8±3.4	71.5±3.7	26.3±8.1*

Octadecyl-AGPC was infused at $1-2\,\mu g\,kg^{-1}\,min^{-1}$ while phentolamine was given as an i.v. bolus injection ($2\,mg\,kg^{-1}$).
Ad and NA were given as bolus injections. Mean arterial blood pressure (MABP) values shown are means ± s.e. ($n=4$). *$P<0.05$ compared to control.

Table 3 The effects of phentolamine, alone and in combination with angiotensin II (AII), on the changes in mean arterial blood pressure (MABP) in response to various agonists in conscious spontaneously hypertensive rats

	AII (0.3 μg kg^{-1} i.v.)	*NA* (1 μg kg^{-1} i.v.)	*Ad* (1 μg kg^{-1} i.v.)	*Octadecyl-AGPC* (1 μg kg^{-1} i.v.)
Control	63.5 ± 3.2	39.6 ± 1.7	15.5 ± 1.3	−43.3 ± 4.9
After phentolamine (3 mg kg^{-1} i.v.)	84.8 ± 5.0*	11.0 ± 2.6*	−16.8 ± 4.5*	−23.3 ± 4.8*
After phentolamine during AII infusion (0.1 μg kg^{-1} min^{-1} i.v.)	27.3 ± 5.0*	7.8 ± 1.0*	−44.0 ± 5.0*	−61.3 ± 6.4
During AII infusion and additional phentolamine	26.5 ± 5.3*	3.3 ± 2.9*	−49.5 ± 2.1*	−47.3 ± 5.6

AII, noradrenaline (NA), adrenaline (Ad) and octadecyl-AGPC were given as bolus injections. Values are mean changes in MABP in mmHg ± s.e. ($n = 4$). *$P < 0.05$ compared to control.

Blood pressure studies in conscious spontaneously hypertensive rats

The changes in MABP produced by the 4 agonists and the effect of the various treatments on these responses are shown in Table 3. Phentolamine significantly enhanced the MABP response to AII while attenuating responses to NA, Ad and octadecyl-AGPC. After establishing an infusion of AII, the response to an injection of AII was significantly decreased and responses to NA and Ad remained depressed. However, the response to octadecyl-AGPC was restored and was even larger than the control response. Additional administration of phentolamine during the AII infusion did not cause any further alteration of the responses to AII, NA or Ad. However, the magnitude of the octadecyl-AGPC response was reduced to the original control level.

The initial MABP before administration of each of the 4 agonists and the effects of the various treatments on this parameter are shown in Table 4. α-Adrenoceptor blockade with phentolamine significantly decreased MABP before injection of each agonist. Initial MABP was restored to the pre-phentolamine level by the infusion of AII. Additional administration of phentolamine resulted in a small decrease in MABP but these values were not significantly different from the control MABP measured at the start of the experiment.

Discussion

Among current studies described in the literature, Smith *et al.*, (1981) using APRL and Gaillard *et al.* (1981) using Paf-acether, have proposed that a component of the vasodilatation produced by these compounds is due to α-adrenoceptor blockade. The evidence for this stems from the attenuation of the pressor response to NA administration during the

Table 4 Blood pressure measurements (MABP) of conscious spontaneously hypertensive rats after phentolamine administration, alone and in combination with angiotensin II (AII), before intravenous injection of various agonists

	AII	*NA*	*Ad*	*Octadecyl-AGPC*
Control	152.9 ± 4.3	160.9 ± 3.0	161.1 ± 5.0	156.4 ± 3.0
After phentolamine	97.5 ± 3.4*	87.8 ± 5.4*	91.3 ± 5.7*	91.5 ± 6.4*
After phentolamine during AII infusion	163.5 ± 6.4	163.0 ± 7.2	159.8 ± 4.5	159.3 ± 4.3
During AII infusion and additional phentolamine	156.0 ± 6.7	146.0 ± 6.0	145.3 ± 5.6	144.3 ± 7.1

Values are mean MABP in mmHg ± s.e. ($n = 4$). *$P < 0.05$ compared to control.

maximum decrease in MABP caused by APRL or Paf-acether. In the present study, using synthetically prepared octadecyl-AGPC, one of the active alkyl ether analogues of phosphatidylcholine in APRL or Paf-acether, we have obtained direct proof of a lack of interaction of octadecyl-AGPC with α-adrenoceptors in the rat mesenteric artery particulate fraction using the radioligand [^{3}H]-WB 4101, a selective probe for identification of α_1-adrenoceptors (Kapur & Mottram, 1978; U'Prichard & Snyder, 1978; Raisman *et al.*, 1979). Previously, we showed that the threshold hypotensive dose for octadecyl-AGPC in the anaesthetized, normotensive rat was 0.03 $\mu g\,kg^{-1}$ (Lai *et al.*, 1983). This dose could be equated to approximately 0.6 μM based on the utilization of a 300 g rat with a total blood volume of 25 ml. In the present study, octadecyl-AGPC did not inhibit the binding of [^{3}H]-WB 4101 to α-adrenoceptors at concentrations up to 100 μM (about 160 times greater than could be achieved in the whole animal *in vivo* using the assumption outlined above). Even at 300 μM, the displacement of binding of [^{3}H]-WB 4101 was still less than 20%.

Pharmacological evidence for a lack of interaction of octadecyl-AGPC with α-adrenoceptors was also obtained in the cat. Contractions of the NM in response to Ad or NA or postganglionic nerve stimulation, responses mediated by activation of α-adrenoceptors, were not diminished by infusions of octadecyl-AGPC that produced about a 50 mmHg decrease in MABP. Similarly, the pressor responses to Ad or NA were unchanged during the octadecyl-AGPC infusion. Phentolamine, the α-adrenoceptor blocking agent, completely inhibited nerve-stimulated responses of the NM and attenuated the NM contractile response and pressor response to Ad and NA.

To clarify further some of the earlier observations in the described cardiovascular studies on APRL or Paf-acether and α-adrenoceptors, we studied the effects of octadecyl-AGPC and phentolamine, in SH rats under various conditions. It was hypothesized that, if a component of the Paf-acether vasodilator response is indeed due to α-adrenoceptor blockade, then prior inhibition of the α-adrenoceptor should result in a reduction in the Paf-acether response approximately equivalent to the α-adrenoceptor blocking component.

α-Adrenoceptor inhibition following phentolamine administration was confirmed by the decrease of the NA pressor response and Ad reversal. The response to AII following phentolamine was augmented as has been found previously (Day & Owen, 1970). The response to octadecyl-AGPC after phentolamine administration was significantly reduced, from which it can be inferred that a portion of the octadecyl-AGPC vasodilator response in untreated animals may be due to an action on the α-adrenoceptor.

However, it must be kept in mind that in addition to producing changes in response to the various agonists observed, phentolamine also caused a significant reduction in the initial MABP of the animals. Initial MABP was restored to control (pre-phentolamine) levels by continuously infusing AII. Subsequent administration of NA confirmed the effectiveness of the α-adrenoceptor blockade as did an even greater reversal of the Ad response. The AII response was now significantly reduced probably owing to the development of tachyphylaxis to AII. The response to octadecyl-AGPC was fully restored to control levels when the MABP was raised to its initial control value (pre-α-adrenoceptor blockade). Subsequent administration of additional phentolamine still failed to reduce the octadecyl-AGPC vasodilator response below control values.

The effects of a number of agents on blood pressure have been found to be sensitive to the blood pressure level at the time of administration. Among these are Ad, tyramine and acetylcholine (Korol & Brown, 1967). For Ad and acetylcholine the slope of the regression line of response versus initial blood pressure is positive while for tyramine it is negative. Since the response to octadecyl-AGPC was reduced when initial blood pressure was lowered and restored as the initial blood pressure was normalized, the slope of the regression line for octadecyl-AGPC should also be positive. That is, the vasodilator response produced by octadecyl-AGPC will be reduced by interventions which result in a reduction of initial MABP. This, it is felt, adequately explains the initial observations of this study which could be interpreted as an α-adrenoceptor blocking component contributing to the octadecyl-AGPC vasodilatation. However, more rigorous investigation of the changes which occurred and their correction make it clear that a normal response (control level) to octadecyl-AGPC may be obtained in an animal that for all practical purposes has all its α-adrenoceptors blocked. Therefore, α-adrenoceptor blockade does not appear to contribute to the vasodilator action of octadecyl-AGPC. Based on pharmacological and radioligand binding studies, it is concluded that octadecyl-AGPC lacks the ability to interact with α-adrenoceptors.

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