Br. J. Pharmac. (1983), **79**, 667–671

Aortic vascular and atrial responses to (±)-1-O-octadecyl-2-acetyl-glyceryl-3-phosphorylcholine

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1 The effects of (±)-1-O-octadecyl-2-acetyl-glyceryl-3-phosphorylcholine (octadecyl-AGPC) were studied in three types of aortic vascular smooth muscle preparations, namely, strips, rubbed and unrubbed rings, and an atrial preparation in normotensive rats.

2 In the resting tension state, octadecyl-AGPC did not elicit significant contractions in either rubbed or unrubbed ring preparations at concentrations lower than 1×10^{-4} M. However, at a concentration of 3×10^{-4} M, octadecyl-AGPC markedly contracted both types of ring preparations. This contractile response was partially antagonized by pretreatment with reserpine and completely blocked by phentolamine (1×10^{-6} M).

3 In preparations contracted with noradrenaline (NA), octadecyl-AGPC elicited biphasic responses in intact ring preparations; an initial relaxation followed by contraction. Octadecyl-AGPC induced only a slight contraction in strips and a slight relaxation in the rubbed ring preparation.

4 Octadecyl-AGPC did not elicit any significant effect on chronotropy or inotropy at concentrations up to 3×10^{-5} M. When the concentration was 1×10^{-4} M, octadecyl-AGPC produced significant positive chronotropic and inotropic effects on spontaneously beating right and electrically driven left atrial preparations, respectively. Both effects were blocked by propranolol (5×10^{-8} M); reserpine pretreatment antagonized only the chronotropic response.

5 In [^{3}H]-dihydroalprenolol ([^{3}H]-DHA) binding studies, octadecyl-AGPC had a K_d of 427.85 μM and thus was much less potent than isoprenaline (K_d = 465.10 nM) or propranolol (K_d = 4.4 nM) in displacing [^{3}H]-DHA in rat cardiac membrane preparations.

6 In conclusion, relaxation and contraction induced by octadecyl-AGPC in aortic preparations is an indirect rather than a direct effect. An unknown factor released from endothelial cells is responsible for aortic smooth muscle relaxation by octadecyl-AGPC while released NA appears to be responsible for aortic vascular contraction and for the positive chronotropic and inotropic effects in the atrial preparations.

Introduction

The alkyl-ether analogue of phosphatidylcholine, specifically the C_{16} alkyl-ether, has been named antihypertensive polar renomedullary lipid (APRL) (Muirhead, 1980) and/or platelet activating factor (PAF) (Demopoulos, Pinckard & Hanahan, 1979) because of its potent blood pressure lowering activity and platelet activating property, respectively. Recently we (Lai, Shepherd, Cervoni, Wissner, 1983) reported that (±)-1-O-octadecyl-2-acetyl-glyceryl-3-phosphorylcholine (octadecyl-AGPC), a totally synthetic chemical, effectively lowered arterial blood pressure in normotensive rats and decreased perfusion pressure in autoperfused hindquarter preparations. The hypotensive effect of octadecyl-AGPC has been attributed to a direct vasodilator action and not the result of cholinoceptor, histamine receptor or β-adrenoceptor interactions.

In the present paper we describe the *in vitro* rat aortic vascular responses and atrial reactivity to octadecyl-AGPC. It was noted that there were substantial differences in responsiveness to octadecyl-AGPC between *in vivo* and *in vitro* preparations and/or between smaller and larger blood vessels.

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Methods

Male Sprague-Dawley normotensive rats weighing 300–325 g were used in this study.

Preparation of aortic strips

The thoracic aortae were removed from decapitated rats and placed in Krebs solution. The blood, excess fat and connective tissues were carefully removed. The aorta was cut helically to equivalent lengths (3 cm) and widths (2.5–3 mm) by the method of Furchgott & Bhadrakom (1953).

Preparation of aortic rings

The thoracic aortae removed from decapitated rats were carefully cleaned of adherent fat and connective tissue. Extra caution was taken to avoid stretching and rubbbing the intima during the cleaning procedure. The aortae were cut into segments of 2 rings with a pair of fine scissors. The segments were mounted on a pair of stainless steel wire hooks. Intimal endothelial cells were removed before introduction of the rings onto the stainless steel wire hooks following the procedure developed by Furchgott & Zawadzki (1980). In brief, a small wooden stick was inserted into the lumen and rubbed gently on the intimal surface for 60 s. The effectiveness of the rubbing procedure in removing endothelial cells was ascertained by subjecting the tissue to an acetylcholine (ACh) challenge. The complete loss of the relaxant response to ACh indicated complete success in destroying the endothelial cells. Strips and rings were mounted in a 10 ml organ bath containing an oxygenated (95% O_2, 5% CO_2) Krebs solution of the following composition (mM): NaCl 126, KCl 4.6, $CaCl_2.2H_2O$ 2.3, KH_2PO_4 1.1, $MgSO_4.7H_2O$ 1.1, $NaHCO_3$ 24.9 and dextrose 11.5. An initial tension of 1 g was applied to each strip or ring preparation. The aortic preparations were allowed to equilibrate for 2 h before octadecyl-AGPC was added cumulatively to the bath. Changes in tension were recorded with a Grass FT03 force-displacement transducer coupled to a Grass polygraph. Relaxation experiments were carried out on noradrenaline (NA)-induced, moderately contracted tissues. Relaxation of the contracted tissue back to base line represented 100% relaxation.

Preparation of isolated atria

For determination of chronotropic and inotropic responses of rat isolated atria to octadecyl-AGPC, hearts were quickly removed from rats after cervical dislocation and placed in petri dishes filled with physiological saline. The combined atria were carefully dissected free of connective tissues and ventricles. The separation of left and right atria and preparation for the determination of chronotropic and inotropic responses to agonists have been described previously in detail (Cervoni, Herzlinger, Lai & Tanikella, 1981). Concentration-response curves to octadecyl-AGPC were determined by cumulative addition of the compound.

β-Adrenoceptor binding assay

Cardiac membranes for β-adrenoceptor binding were prepared essentially as described by Baker & Potter (1980). Atrial tissues were collected from a group of 10 decapitated rats and homogenized in 10 ml of cold Tris-HCl buffer (10 mM, pH 8.0) with a polytron PT-10 homogenizer set at speed 6 for 45 s. The homogenate was diluted with 30 ml of 1 M KCl, left on ice for 10 min and filtered through four layers of cheese cloth. The filtrate was centrifuged at 48,000 *g* for 10 min. Pellets were gently resuspended in 20 ml of buffer, resedimented and finally dispersed in 1.5 ml of buffer. Cardiac membranes of 300–450 μg protein were incubated in triplicate with 0.5–10 nM of (−)-[^{3}H]-dihydroalprenolol ([^{3}H]-DHA) as described previously (Cervoni *et al.*, 1981).

Calculation of K_d

The dissociation constant (K_d) for propranolol, isoprenaline and octadecyl-AGPC were calculated by the method of Cheng & Prusoff (1973) using the formula $K_d = IC_{50}/ (1 + [^3H]\text{-DHA}]/K_d$ for [^{3}H]-DHA): where the IC_{50} = concentration for drug which inhibits [^{3}H]-DHA binding by 50% (estimated from the data shown in Figure 4) and the K_d for the radioligand was obtained by the method of Scatchard (1949).

Reserpine pretreatment and noradrenaline assay

Reserpine (5 mg/kg i.p.) was given 24 h before animals were killed. Atrial and thoracic tissues were removed, blotted and rinsed, dried and kept frozen for NA determinations according to a radioenzymatic assay (Goldstein, Lai, Herzlinger & Cervoni, 1982).

Results

Aortic vascular responses to octadecyl-AGPC

Responses of aortic vascular preparations to octadecyl-AGPC were studied at resting tension and on tissues which had developed a tension with NA. Under the resting tension, there were no noticeable

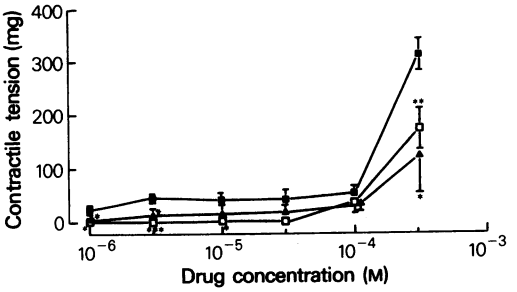

Figure 1 Cumulative concentration-response curves for octadecyl-AGPC in aortic preparations which were at resting tension. Rubbed rings (□) and unrubbed rings (■) were preparations in which the endothelium was removed or was left intact, respectively. Reserpinized rings (△) were prepared from rats which were pretreated with reserpine (5 mg/kg i.p.) 24 h before they were killed. Each point represents the mean of preparations from 5–6 animals; s.e.mean shown by vertical lines. Statistically significant differences from unrubbed rings are indicated by $*P<0.05$; $**P<0.01$; $***P<0.001$.

effects of octadecyl-AGPC on either rubbed or unrubbed ring preparations up to concentrations of 1×10^{-4}M. However, when the concentrations were increased to 3×10^{-4}M, octadecyl-AGPC significantly increased tension in both types of ring tissue. Intact ring preparations generated more tension than endothelial damaged rings. The data are summarized in Figure 1.

Phentolamine (1×10^{-6}M) completely blocked, while reserpine pretreatment partially antagonized, contractile responses to octadecyl-AGPC in the unrubbed aortic ring preparations.

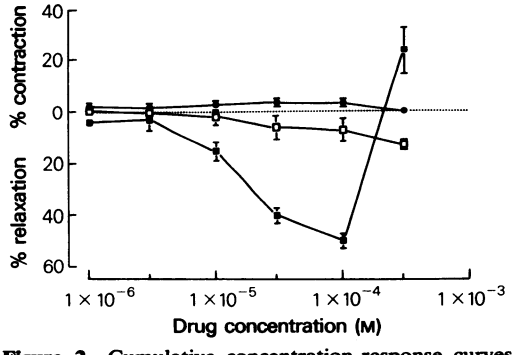

Figure 2 Cumulative concentration-response curves for octadecyl-AGPC in aortic preparations which were contracted with noradrenaline (1×10^{-8}M) to induce a moderate tension. Rubbed rings (□) and unrubbed rings (■) were preparations in which the endothelium was removed or was left intact, respectively. Strips (●) refer to helically cut preparations. Each point represents the mean of preparations from 5–6 animals; s.e.mean shown by vertical lines.

When aortic tissues were previously contracted with NA, octadecyl-AGPC elicited a slight contraction in aortic strip preparations and a slight relaxation in rubbed aortic ring preparations. However, in the endothelial intact unrubbed ring preparations, octadecyl-AGPC elicited biphasic responses; an initial relaxation followed by contraction which was concentration-dependent. Relaxation was concentration-dependent within the concentration range of 1×10^{-5}–1×10^{-4}M. When concentrations were increased to 3×10^{-4}M, octadecyl-AGPC did not induce further relaxation but instead contracted the relaxed tissue to reach a tension greater than the tension in response to the initial NA exposure (Figure 2).

Atrial responses to octadecyl-AGPC

Effects of octadecyl-AGPC on chronotropy and inotropy were studied in spontaneously beating right and electrically driven left atrial preparations, respectively. Octadecyl-AGPC did not elicit any significant positive or negative effects on atrial rate or contractile force up to concentrations of 3×10^{-5}M. When the bath concentration was increased to 1×10^{-4}M,

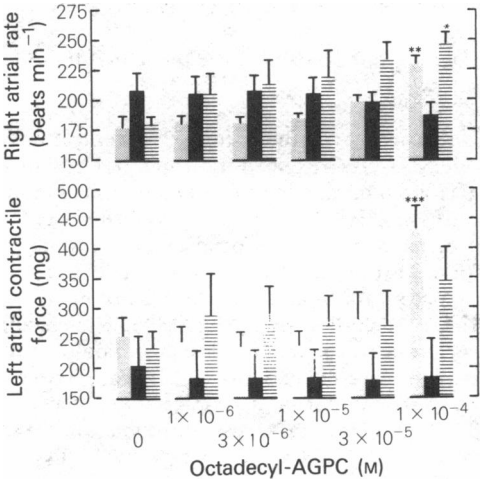

Figure 3 Effects of octadecyl-AGPC on the inotropic responses in isolated electrically driven left atria and chronotropic responses in spontaneously beating right atria from control and reserpine-treated normotensive rats. Reserpinized atrial preparations were taken from rats which were pretreated with reserpine (5 mg/kg i.p.) 24 h before they were killed. Each column and bar represent the mean and s.e. of preparations from 5 animals: stippled columns, control; solid column, propranolol-treated; hatched columns, reserpine-treated. Statisically significant differences from basal conditions are indicated by $*P<0.05$; $**P<0.01$; $***P<0.001$.

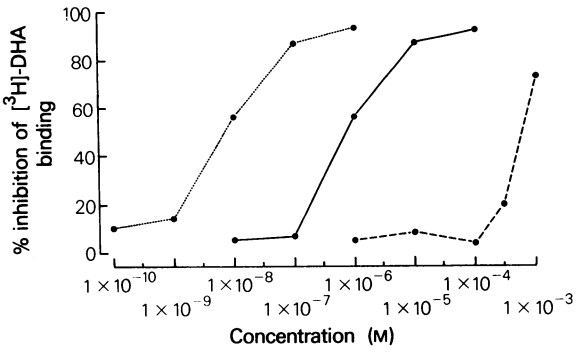

Figure 4 Concentration-response curves for the inhibition of [^{3}H]-dihydroalprenolol ([^{3}H]-DHA)-binding by propranolol (●·····●), isoprenaline (●——●) and octadecyl-AGPC (●– –●) in atrial membrane preparations. Each point is the mean of triplicate determinations in three separate experiments.

octadecyl-AGPC elicited significantly positive inotropic and chronotropic effects. Propranolol (5×10^{-8} M) attenuated the positive chronotropic and inotropic responses. Reserpine pretreatment 24 h previously, attenuated chronotropic but not the inotropic response to octadecyl-AGPC (Figure 3).

Inhibition of [^{3}H]-dihydroalprenolol binding by octadecyl-AGPC

To discover whether high concentrations of octadecyl-AGPC exerted inotropic or chronotropic effects via β-adrenoceptor interaction, its inhibitory activity on [^{3}H]-DHA-binding was compared with that of isoprenaline and propranolol in atrial preparations. Propranolol and isoprenaline were much more potent than octadecyl-AGPC in competing for [^{3}H]-DHA binding sites (Figure 4). The calculated dissociation constants (K_d) for propranolol and isoprenaline were 4.4 nM and 465.10 nM, respectively. Thus, propranolol and isoprenaline were about 100,000 and 900 times, respectively, more potent in displacing [^{3}H]-DHA than octadecyl-AGPC whose K_d is 427.85 μM.

Effect of reserpine treatment on tissue noradrenaline content

Reserpine pretreatment effectively depleted NA from aorta and atrial tissue. The NA (ng g^{-1} tissue) content of normal aorta and atria was 41.93 ± 14.2(3) and 590.48 ± 105.13(3), respectively. The NA content of the same tissues from reserpine-treated rats was 1.35 ± 0.42(3) and 31.21 ± 9.48, respectively which were significantly different from those of control rats.

Discussion

Recently, we demonstrated that octadecyl-AGPC (Lai *et al.*, 1983) is a potent vasodilator which in the microgram range effectively decreased perfusion pressure in the rat autoperfused hindquarter preparation. It was also suggested that the hypotension was the result of direct vasodilatation and not the result of an interaction with cholinoceptors, histamine receptors or β-adrenoceptors. In the present study, octadecyl-AGPC exerted a relaxant effect upon aortic rings with intact endothelial cells. However, the degree of relaxation was limited and the threshold concentration (1×10^{-5} M) was relatively higher than that of the *in vivo* studies (0.01 μg) (Lai et al., 1983). The reason for the discrepancy between the threshold doses *in vivo* and *in vitro* is not clear, although the size of the blood vessels in these two studies were different; the blood vessels in the rat hindquarter preparation, in general, are much smaller in diameter than the aorta. These results imply that different size blood vessels might respond differently to octadecyl-AGPC.

The reason that octadecyl-AGPC exerts relaxant effects upon aortic ring preparations with intact endothelial cells but not aortic preparations with damaged endothelial cell is not known, although an unknown factor(s) released from endothelial cells is an attractive speculation. Endothelial cell contribution to the vasodilator action of other drugs has been reported (Furchgott & Zawadzki, 1980; Folco, Rossoni, McGiff & Spokas, 1982). Regardless of the exact mechanism of the relaxation, these results clearly demonstrate that endothelial cells are required for the induction of aortic vascular smooth muscle relaxant responses to octadecyl-AGPC. In view of such findings, it is tempting to speculate that the potent hypotensive effect of octadecyl-AGPC in animals is the combined effect of direct vasodilatation and vascular relaxant responses mediated by unknown factor/factors released from vascular endothelial cells.

Octadecyl-AGPC contracted all three types of aortic vascular preparations at high concentrations. The fact that phentolamine completely antagonized and reserpine pretreatment partially blocked the octadecyl-AGPC-induced contraction suggests that high concentrations of octadecyl-AGPC are capable of releasing NA from adrenergic nerves in aortic vascular tissue and that the released NA mediates the contraction. In the unrubbed ring preparation, vascular relaxation was converted into a contractile response when concentrations of octadecyl-AGPC were increased to 1×10^{-4} M. This could be the end result of the NA-mediated contraction overcoming the relaxation mediated by factors released from endothelial cells.

In atrial preparations, octadecyl-AGPC-induced positive chronotropic and inotropic effects were attenuated by propranolol. However, reserpine pretreatment attenuated only the chronotropic responses to octadecyl-AGPC. The reason for the failure of reserpine pretreatment to attenuate both inotropic and chronotropic responses to octadecyl-AGPC is somewhat puzzling. Nonetheless, these results suggest that octadecyl-AGPC, at higher concentrations, is capable of releasing NA from cardiac tissue.

The question arises as to the possibility of a direct interaction between octadecyl-AGPC and adrenoceptors in mediating the vascular contraction and positive chronotropic and inotropic responses. The receptor binding study provides some answer to this question. Propranolol and isoprenaline displaced [^{3}H]-DHA from cardiac membrane preparations with a K_d of 4.4 nM and 465.10 nM, respectively. These agents are much more potent in displacing [^{3}H]-DHA than octadecyl-AGPC. The K_d for octadecyl-AGPC was 427.85 μM which indicates that it has a weak affinity for the cardiac β-adrenoceptor. The displacement of [^{3}H]-DHA binding by octadecyl-AGPC at 1 mM is probably the result of a nonspecific interaction with the cardiac membrane and therefore suggests that octadecyl-AGPC does not produce its effect by direct interaction with cardiac adrenoceptors.

In conclusion, the relaxation and contraction induced by octadecyl-AGPC in aortic vascular smooth muscle and the positive chronotropic and inotropic effects in atrial tissues are the result of an indirect rather than a direct effect by octadecyl-AGPC. An unknown factor(s) released from endothelial cells is responsible for aortic relaxation while released NA is responsible for aortic contraction and the positive chronotropic effects in atrial preparations.

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(Received October 29, 1982. Revised January 18, 1983.)