ETₐ receptor blockade attenuates the hypertension but not renal dysfunction in DOCA-salt rats

GRAHAM H. ALLCOCK, RICHARD C. VENEMA, AND DAVID M. POLLOCK

Vascular Biology Center, Medical College of Georgia, Augusta, Georgia 30912-2500

ENDOTHELIN-1 (ET-1) possesses a wide variety of biological effects, including being a potent vasoconstrictor and pressor agent (12, 43). ET-1 produces its effects by acting on specific subtypes of receptors, ETA and ETₐ, which were originally characterized by their selectivity for the endothelin isopeptides (2, 34). It has been shown that both ETA and ETₐ receptors are present on vascular smooth muscle, where they mediate vasoconstriction (7, 36). ETₐ receptors also exist on endothelial cells, where they mediate vasodilation via the release of endothelium-derived nitric oxide (7, 18, 41). ET-1 has potent actions in the kidney, where it can decrease glomerular filtration rate (GFR) (23) and induce vasoconstriction via both ETA and ETₐ receptors (8, 32, 40). Furthermore, ET-1 has both diuretic and natriuretic actions and thus may be important in the physiological regulation of sodium and water transport (for review, see Ref. 21).

The potent vasoconstrictor action of ET-1, as well as its promitogenic activity (19), has led to the suggestion of its involvement in the development and maintenance of hypertension. However, the evidence for such a role is equivocal, varying between subjects and experimental models. In the deoxycorticosterone acetate (DOCA)-salt hypertensive rat, a model of mineralocorticoid-induced volume-expanded hypertension, the evidence for a role of ET-1 is more substantial. DOCA-salt hypertensive rats exhibit increased plasma levels of ET-1 (25, 43). There have been several reports of increased ET-1 mRNA in vascular tissues (9, 36) including vessels and glomeruli within the kidney (10). In addition, ETA receptor-selective and nonselective endothelin receptor antagonists have exhibited moderate success at reducing DOCA-salt hypertension (3, 14, 27, 35). However, in DOCA-salt hypertension there is an associated reduction in GFR (38). ET-1 has a profound ability to reduce GFR, although the extent to which ET-1 may influence GFR and excretory function in DOCA-salt rats is unknown. Therefore, to further investigate the role of ET-1 in the DOCA-salt model of hypertension, we determined the effect of A-127722, an ETA receptor-selective antagonist (31, 42), on blood pressure and renal function in conscious rats.

METHODS

Animal experiments. Experiments were performed using male Sprague-Dawley rats (200–220 g; Harlan Laboratories, Indianapolis, IN) in accordance with the Guide for the Care and Use of Laboratory Animals and approved and monitored by the Medical College of Georgia Committee for Animal Use in Research and Education. Rats were housed under conditions of constant temperature and humidity and exposed to a 12:12-h light-dark cycle. A right flank incision was used to remove the right kidney under methohexital sodium (50 mg/kg Brevital; Eli Lilly, Indianapolis, IN) anesthesia. A carotid catheter was implanted and exteriorized at the back of the neck. One day later, arterial blood pressure was measured by a Synchron EL-ISE electrolyte system (Beckman, Brea, CA).

After 3 wk, animals were anesthetized with Brevital, and a carotid catheter was implanted and exteriorized at the back of the neck. One day later, arterial blood pressure was
recorded in conscious rats using a MacLab 8e data acquisition system (ADInstruments, Milford, MA). Rats were unrestrained, and arterial pressure was allowed to stabilize before being recorded over a 30-min period. Rats were then anesthe-
tized with pentobarbital sodium (65 mg/kg ip), and a blood sample was removed from the aorta by a syringe to measure plasma creatinine and ET-1. In addition, the left kidney was
removed, dissected into cortex and medulla, and frozen in liquid nitrogen. Tissues were stored at −80°C until total RNA was extracted.

Creatinine assay. Plasma and urine creatinine concentra-
tions were measured by the picric acid colorimetric method. A picric acid solution was created by a tenfold dilution of saturated picric acid with 1% NaOH. Next, 50 µl of plasma or 50 µl of a 1:10–25 dilution of urine was added to 200 µl of the picric acid-NaOH solution in a microtiter plate. The samples were allowed to incubate for 15 min before being read at 490 nm in a microplate reader set to correct for absorbance at 620 nm. A standard curve was prepared on the same assay plate to derive the creatinine concentration.

Plasma ET-1 assay. ET-1 concentrations in plasma were determined by ELISA (R & D Systems, Minneapolis, MN). Briefly, 1 ml of plasma was added to 1.5 ml of extraction solvent (a mixture of acetone, 1 M HCl, and distilled water in ratios of 40:1.5, respectively). The sample was centrifuged at 3,000 rpm at 4°C for 20 min. The supernatant was decanted and dried down in a centrifugal evaporator for 4 h at 37°C. The pellet was then reconstituted in 0.25 ml sample diluent. Anti-ET-1 horseradish peroxidase was added to each well of the 96-well plate provided with the kit. Standards, controls, or reconstituted plasma was added to separate wells and allowed to incubate at room temperature for 1 h. Each well was then aspirated and washed with buffer six times. After the final aspiration, substrate was added to each well fol-
lowed by a 30-min incubation period at room temperature. Stop solution was then added to each well, and the plate was
read at dual wavelengths of 450 and 620 nm within 30 min. The manufacturer reports the cross-reactivity of the ELISA to be <1% for big ET-1, 45% for ET-2, and 14% for ET-3; the
inter- and intra-assay variation are reported to be <5% and <6.5%, respectively; and the limit of assay sensitivity is reported to be 0.25 pg/ml.

Northern blot analysis. Renal cortex or medulla was pulver-
ized while still frozen. Total RNA was extracted from the pulverized tissue by a guanidine isothiocyanate-phenol- 
chloroform method (4) using TRizol reagent. Total RNA samples (20 µg) were made up to equal volumes in nuclease-
free diethyl pyrocarbonate (DEPC) water and then incubated at 65°C for 5 min with 10× MOPS, 37% formaldehyde, and
formamide. Ten microliters of 5× glycerol loading dye and 1 µl of ethidium bromide (10 mg/ml) were then added, and the samples were mixed before they were loaded on a 1% agarose gel containing 72 ml DEPC water, 10 ml 10× MOPS, and 18 ml formaldehyde. The samples were run on the gel sur-
rounded by 1× MOPS at 120 V for 1.5–2 h. The samples were then transferred from the gel to a positively charged nylon membrane (TotalBlot +; Amresco, Solon, OH) by capillary action with 3 M NaCl and 0.3 M sodium citrate (20× standard sodium citrate (SSC)) overnight. Membranes were washed with 2× SSC for 10 min and then allowed to dry before locations of the 18S and 28S rRNA species were revealed by
observing the ethidium bromide staining under ultraviolet light. Membranes were prehybridized at 42°C for 3 h in 2× SDS, 50%formamide, and a prehybridization solution containing 12× SSC, 10× Denhardt’s solution, and 200 µg/ml sheared, denatured salmon sperm. The membranes were
hybridized for 18–20 h at 42°C in the above-mentioned
prehybridization solution with 10% dextran sulfate and 25 ng of the 32P-labeled probe, [α-32P] deoxyctosine triphosphate (dCTP). The probe p3B-3 is a 511-bp fragment of rat ET-1 that had been cut out of a cloned vector pGEM-3z (Promega) using BamHI. The membranes were then washed two times for 30 min in 2× SSC, 1% SDS at 55°C, and finally for 30 min in 0.2× SSC and 0.1% SDS at 55°C. Membranes were dried and then exposed to autoradiographic film (NEN, Boston, MA) for 1–4 days. Autoradiograms and the ethidium bromide staining of the 18S and 28S rRNA bands were analyzed by densitometry (IS-1000 digital imaging system, Alpha Inno-
tech, San Leandro, CA).

Creatinine and saturated picric acid were obtained from Sigma (St. Louis, MO). Pentobarbital sodium, A-127722, and the ET-1 cDNA probe were obtained from Abbott Laboratories (Abbott Park, IL). Brevial was obtained from Eli Lilly (Indianapolis, IN). DOCA and placebo pellets were obtained from Innovative Research of America (Sarasota, FL). The ET-1 ELISA kit came from R & D Systems. Ten times MOPS, 20× SSC, formamide, formaldehyde, TotalBlot + nylon mem-
branes, and agarose were all obtained as part of the TotalBlot Northern kit (Amresco, Solon, OH). SDS, 5× glycerol gel loading buffer, and DEPC-treated nuclease-free water were also obtained from Amresco. Ethidium bromide was obtained from Bio-Rad (Hercules, CA). TRizol reagent and 2× prehy-
bridization-hybridization solution came from Gibco: LifeTech-
ologies (Grand Island, NY). [α-32P]dCTP was obtained from Amersham (Arlington Heights, IL). Two-way analysis of variance with Fisher’s protected least-significant differences post hoc test was used for statistical evaluation of data. Values are reported as means ± SE. P < 0.05 was considered significant.

RESULTS

Three weeks after the uninephrectomy, the mean arterial blood pressure (MAP) of placebo-treated rats was 133 ± 3 mmHg (Fig. 1A). Rats receiving DOCA-salt for 3 wk had a significantly elevated MAP (197 ± 6 mmHg). Chronic treatment with A-127722 significantly attenuated the hypertension (156 ± 8 mmHg) in DOCA-
salt rats but had no effect on MAP in placebo rats (134 ± 3 mmHg). As expected, creatinine clearance (Fig. 1B) and urinary creatinine excretion rate (Fig. 2) were significantly reduced in DOCA-salt animals compared with the placebo controls. Treatment with A-127722 did not significantly change the creatinine
clearance or excretion of either the placebo or DOCA-
salt rats. Plasma creatinine levels were not signifi-
cantly elevated and were not different between these groups after 3 wk (data not shown). Concentrations of
ET-1 in the plasma were greater in DOCA-salt rats compared with placebo rats (Fig. 1C). Rats receiving A-127722 had significantly higher plasma ET-1 levels than their untreated counterparts.

PreproET mRNA was expressed in greater amounts in the medulla than in the cortex in all groups of rats (Fig. 3). Treatment with DOCA-salt and/or A-127722 had no significant effect on the renal expression of
preproET mRNA.

Water intake remained unchanged for both placebo rats and A-127722-treated placebo rats throughout the 3-wk treatment period (Fig. 4A). DOCA-salt treatment induced an increase in water intake. A-127722 attenuated the increase in water intake for the first, but not
the subsequent, weeks of DOCA-salt treatment. Food intake of placebo-treated rats was not significantly affected by treatment with A-127722 (Fig. 4B). After treatment with DOCA-salt, rats consumed significantly less food than placebo-treated rats. A-127722 significantly attenuated the reduction in food intake in DOCA-salt-treated rats in the third week after treatment but not in the two preceding weeks.

Before treatment with DOCA-salt or placebo, urine volume and sodium excretion were not different between groups of animals. Urine volume and sodium excretion changed little throughout the course of the study for placebo-treated rats receiving vehicle or A-127722 (Figs. 5, A and B). For DOCA-salt-treated rats, both urine volume and sodium excretion were greater than in placebo-treated animals for the 3 wk after pellet implantation. Treatment of DOCA-salt rats with A-127722 attenuated this increase in urine volume and sodium excretion for the first, but not the subsequent, weeks after pellet implantation. Water balance (water intake – urine output) of DOCA-salt-treated rats was elevated over controls in the first week after uninephrectomy but did not significantly differ in the following weeks (Table 1). A-127722 attenuated the initial DOCA-salt-induced water retention. DOCA-salt-treated rats retained more sodium than placebo rats after uninephrectomy. A-127722 treatment had no effect on the sodium balance of placebo rats but did attenuate the sodium retention of DOCA-salt rats in the initial, but not subsequent, weeks after uninephrectomy.

Before administration of the DOCA or placebo pellets, rats weighed between 200 and 220 g (212 ± 6 g, placebo; 209 ± 7 g, placebo + A-127722; 210 ± 5 g, DOCA-salt; 201 ± 9 g, DOCA-salt + A-127722). After 3 wk, the placebo-treated rats gained significantly more weight than DOCA-salt rats (Fig. 6). A-127722 treatment had no effect on the increase in body weight

Fig. 1. Effects of A-127722 and/or deoxycorticosterone acetate (DOCA)-salt treatment on mean arterial pressure (A), creatinine clearance (B), and plasma endothelin (ET)-1 concentration (C). Rats were treated with placebo (n = 8), placebo + A-127722 (n = 7), DOCA-salt (n = 8), or DOCA-salt + A-127722 (n = 5). Each value represents mean ± SE. *P < 0.05 compared with placebo rats, †P < 0.05 compared with DOCA-salt-treated rats.

Fig. 2. Urinary creatinine excretion of rats treated with placebo, placebo + A-127722, DOCA-salt, or DOCA-salt + A-127722. Each point represents mean ± SE. *P < 0.05 compared with placebo rats.
observed in placebo-treated rats. Three weeks after treatment, DOCA-salt rats treated with A-127722 gained more weight than the DOCA-salt rats without ETA receptor antagonist, although there was no difference during the initial 2 wk.

**DISCUSSION**

In addition to confirming that chronic ETA receptor antagonism can attenuate the hypertension in DOCA-salt rats, results of the present study indicate several novel findings. These include the inability of ETA receptor antagonism to restore the diminished renal function observed after 3 wk of treatment with DOCA-salt. However, there were some indications that ETA receptor blockade may have a beneficial effect during the initial week of DOCA-salt treatment as suggested by the improved salt and water balance during that period. In addition, we show that the ETA receptor antagonist A-127722 can increase plasma endothelin levels.

A-127722 is a recently developed, orally bioavailable, nonpeptidic ETA receptor-selective antagonist that has been previously characterized in vitro and in vivo (31, 33, 35, 42). The dose of A-127722 used in this study was chosen because of its efficacy and selectivity demonstrated in previous studies. Administration of 10 or 30 mg/kg of A-127722 by gavage produces a maximum plasma concentration of the antagonist after 1 h. At that time point, the ETA receptor-mediated pressor response to an exogenous dose of ET-1, but not the ETB receptor-mediated depressor response, was significantly blocked (31). In addition, administration of A-127722 in the drinking water at doses comparable to those used in the present study severely attenuated the pressor response to exogenous ET-1 (35) or big ET-1 (33). These findings indicate that A-127722 given in the drinking water at doses used in the current

---

Fig. 3. Representative Northern blot for preproET-1 mRNA isolated from cortical and medullary renal tissue (A). Bar graph (B) indicates mean ± SE of each blot as assessed by densitometry and standardized to 28S ribosomal RNA band to account for slight variations in loading. Rats were treated with placebo (P), placebo + A-127722 (P + A), DOCA-salt (D), or DOCA-salt plus A-127722 (D + A); n = 5 for each group.

Fig. 4. Water intake (A) and food intake (B) of rats treated with placebo, placebo + A-127722, DOCA-salt, or DOCA-salt + A-127722. Each point represents mean ± SE. *P < 0.05 compared with placebo rats, †P < 0.05 compared with DOCA-salt-treated rats.
study inhibits \( \text{ET}_A \), but not \( \text{ET}_B \), receptor-mediated responses.

DOCA-salt hypertensive rats had a drastically reduced GFR compared with placebo-treated rats as determined by creatinine clearance in conscious rats. \( \text{ET}_A \) receptor blockade with A-127722 failed to produce any improvement in GFR as assessed by creatinine clearance. Because plasma creatinine was not increased, urinary creatinine excretion suggests that GFR is reduced early in the development of DOCA-salt hypertension and is not improved even during the initial weeks of treatment with A-127722. These findings are in keeping with acute \( \text{ET}_A \) receptor blockade in DOCA-salt hypertensive rats.

**Table 1. Effect of 3 wk treatment with \( \text{ET}_A \) receptor antagonist A-127722 on water and sodium balance in DOCA-salt and placebo rats**

<table>
<thead>
<tr>
<th>Week</th>
<th>Water balance, ml/day</th>
<th>Sodium balance, mmol/day</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>14.0±0.5</td>
<td>15.5±0.5</td>
</tr>
<tr>
<td>1</td>
<td>15.9±1.3</td>
<td>16.3±0.9</td>
</tr>
<tr>
<td>2</td>
<td>15.5±0.7</td>
<td>17.2±0.7</td>
</tr>
<tr>
<td>3</td>
<td>17.1±1.3</td>
<td>16.0±0.7</td>
</tr>
</tbody>
</table>

*Data are means ± SE. Water and sodium balance = intake − excretion. ET, endothelin. *P < 0.05 compared with placebo rats. †P < 0.05 compared with deoxycorticosterone acetate (DOCA)-salt rats.

Rats treated with DOCA-salt for 4 wk where GFR has been reported to be unchanged (29) or even reduced (13) after antagonist infusion. When considered in conjunction with the present studies, it is now apparent that \( \text{ET}_A \) receptors are not responsible for the reduced GFR.

**Fig. 5. Urine output (A) and sodium excretion (B) in rats treated with placebo, placebo + A-127722, DOCA-salt, or DOCA-salt + A-127722. Each point represents mean ± SE. *P < 0.05 compared with placebo rats, †P < 0.05 compared with DOCA-salt-treated rats.**

**Fig. 6. Body weight of rats treated with placebo, placebo + A-127722, DOCA-salt, or DOCA-salt + A-127722. Each point represents mean ± SE. *P < 0.05 compared with placebo rats, †P < 0.05 compared with DOCA-salt-treated rats.**
in this model. This does not preclude, however, a role for ET-1 in hemodynamic and other aspects of renal function via ET<sub>B</sub> receptors. Further examination of ET<sub>B</sub> receptor actions in the kidneys of DOCA-salt rats awaits the availability of receptor-selective antagonists with oral bioavailability and prolonged duration of action.

Although A-127722 was without effect on GFR after 3 wk, it was noted that in the first week, the ETA receptor blocker attenuated the increase in urine output, water intake, and sodium excretion rate. Thus ETA receptors could be involved in the development of renal dysfunction associated with DOCA-salt hypertension. It is possible that the role of renal ETA receptors diminishes during DOCA-salt hypertension. Fujita and colleagues (15) observed in renal clearance experiments on rats treated with DOCA-salt for 4 wk that acute administration of an ETA receptor antagonist, FR-139317, exhibited little effect on renal function. This is consistent with our findings that long-term inhibition of ETA receptors has no effect on renal function after the first week of DOCA-salt treatment.

Previous studies have shown differing effects of DOCA-salt on the circulating levels on ET-1. Some groups have found plasma ET-1 levels to be raised in DOCA-salt hypertension (14, 42), whereas others have found no change (11, 27, 30). We observed that DOCA-salt rats had significantly higher plasma ET-1 concentrations than placebo animals, which would account for at least part of the elevated MAP in DOCA-salt hypertension, because ETA receptor blockade reduced arterial pressure in this model. Indeed, others have observed a direct correlation between the level of hypertension and the amount of plasma ET-1 in this model (44). The elevated circulating levels of ET-1 are consistent with studies reporting an increase in ET-1 mRNA in vascular tissues (24, 26). A-127722 increased circulating ET-1 levels in both placebo and DOCA-salt rats. It was previously shown that ETA receptor-selective and nonselective endothelin antagonists increase plasma ET-1 (16, 28). This increase in plasma ET-1 induced by ETA receptor antagonist may be due to displacement of ET-1 from receptors into the circulation (28) and/or an antagonism of an ET-1 clearance pathway (16). Although previous reports have shown that ETA receptor antagonists rarely increase plasma ET-1 (28), it has been observed that ETA receptors can internalize ET-1 similar to ET<sub>B</sub> receptors (5). This could be a mechanism by which A-127722 blocks the removal of ET-1 from the circulation. An alternative explanation for the A-127722-induced increase in plasma ET-1 is the existence of a receptor-regulated negative feedback mechanism similar to that commonly observed with the many classical hormone systems; chronic receptor antagonism leads to increased hormone release. However, there is no experimental evidence to currently support that such a mechanism exists for ET-1.

To investigate whether production of ET-1 was specifically altered within the kidneys of DOCA-salt rats, we also measured preproET-1 mRNA expression in the cortex and the medulla. PreproET-1 mRNA expression was greater in the medulla than in the cortex, which was expected because the inner medullary collecting duct is a major source of ET-1 in the kidney (37). Neither DOCA-salt treatment nor A-127722 had any effect on renal expression of preproET-1 mRNA. In contrast, in situ hybridization studies have shown that ET-1 mRNA is increased in vessels and glomeruli of the kidney of DOCA-salt rats (9, 10). Because vessels and glomeruli make up a small proportion of the kidney, it may be that small changes were not detected in our whole cortical or medullary mRNA preparations. Nevertheless, the apparent lack of change in locally produced ET-1 is in accord with our finding that ETA receptor antagonism failed to restore renal function in DOCA-salt rats. However, because circulating levels of ET-1 were elevated, a role for renal ET<sub>B</sub> receptors in regulating renal function in DOCA-salt cannot be discounted.

In the present study, chronic A-127722 treatment attenuated the hypertension of DOCA-salt rats by ~40 mmHg. This is comparable to previous studies with ETA receptor antagonists in which FR-139317 decreased the blood pressure of DOCA-salt rats by ~35 mmHg over 1 h (10), and 3 days of treatment with BMS-182874 reduced blood pressure by ~30 mmHg in DOCA-salt rats (3). Bosentan, a nonselective antagonist, was reported to lower the hypertension of DOCA-salt rats by ~20 mmHg (25, 27). Thus ETA receptor antagonists appear to reduce the blood pressure of DOCA-salt rats to a slightly greater extent than nonselective antagonists. The apparent quantitative difference between ETA-selective and nonselective antagonists to lower pressure may be due to simple variations between laboratories. Indeed, one laboratory that showed that bosentan reduces the DOCA-salt hypertension by ~20 mmHg (25, 27) also showed that A-127722 can reduce MAP by ~27 mmHg (35). In addition, it has previously been observed that ETA receptor antagonists are more effective than nonselective antagonists at reducing an ET-1-induced pressor response (1). These authors suggested that ETA receptor antagonists limit an ET-1-induced elevation in MAP. This effect may be especially true of DOCA-salt rats in which it has been shown that short-term administration of the ETA receptor antagonist Ro-46–8443 actually increases blood pressure (6). Therefore, it appears that ETA receptor activation plays a role in elevating the blood pressure of DOCA-salt rats, whereas ET<sub>B</sub> receptors may serve to limit this increase in pressure.

In our study, DOCA-salt rats gained less weight and consumed less food than the normotensive placebo rats. Our data showing poor weight gain are similar to those of other groups studying DOCA-salt rats (25, 27). However, A-127722-treated DOCA-salt rats gained more weight than untreated rats. This latter finding is in contrast to previous studies in which DOCA-salt rats treated with the nonselective endothelin antagonist bosentan gained less body weight than untreated rats (25, 27). The difference in the weight gain with the two endothelin antagonists may be indicative of different roles for ETA and ET<sub>B</sub> receptors in controlling food consumption and body mass in disease states in which
circuiting ET-1 is elevated, although the precise mechanism by which ET-1 may influence weight gain is unknown.

In summary, our results with A-127722 show that chronic ETA Receptor blockade attenuates the development and maintenance of hypertension in DOCA-salt rats. Despite this, however, ETA receptor antagonism does not improve renal function after 3 wk of DOCA-salt treatment. These findings suggest that ETA receptors play a role in the hypertension associated with 3 wk of DOCA-salt treatment, but not in the reduced renal function at that time, although a role of ETA receptors in the earlier stages of the decline in renal function cannot be discounted.

Perspectives

There is increasing evidence that elevations in ET-1 production occur in response to salt loading. ET-1 appears to play an important role in the hypertension observed in DOCA-salt as well as Dahl salt-sensitive rats (20). The stimulus for ET-1 production in response to salt loading is unknown, but we believe that it may be an attempt to eliminate the salt load. ETA receptor activation clearly contributes to the elevated arterial pressure, which would enhance pressure natriuresis. Furthermore, it is known that ETB receptors are present on the epithelial cells of the inner medullary collecting duct (22) and that ET-1 can inhibit sodium reabsorption (17) at the tubular level. Thus a more direct influence of either circulating or intrarenal ET-1 on tubular sodium reabsorption may be at work. How- ever, because ETB receptors can also contribute to the renal vasconstrictor effects of ET-1 in the rat kidney, it is possible that ETB receptor activation may contribute to the decreased GFR associated with DOCA-salt treatment. This seems somewhat unlikely, however, because acute administration of an ETB receptor antagonist increases arterial pressure in this model, suggesting that ETB receptors serve to limit the hypertension through ETB-mediated vasodilation (6). This would suggest that, in hypertension associated with high salt, ETA receptor-selective antagonists may be more beneficial than nonselective endothelin antagonists.

The authors thank Drs. T. J. Opgenorth and J. Wessale of Abbott Laboratories (Abbott Park, IL) for kindly providing A-127722 and DOCA. This work was supported by a grant-in-aid from the American Heart Association, Georgia Affiliate. Address reprint requests to D. M. Pollock.

Received 16 December 1997; accepted in final form 26 March 1998.

REFERENCES


