Altered Protein Kinase C Regulation of Phosphoinositide-Coupled Receptors in Deoxycorticosterone Acetate–Salt Hypertensive Rats

Angelino Calderone, Laurence Oster, Pierre Moreau, Jean-Lucien Rouleau, Duncan J. Stewart, Jacques de Champlain

Abstract This study examined the contribution of phosphatidylinositol metabolism and the efficacy of protein kinase C-mediated desensitization in the exaggerated α_{1b}-adrenergic receptor–mediated inositol phosphate response in the aorta of the deoxycorticosterone acetate (DOCA)-salt rat model of hypertension. The basal accumulation of inositol phosphates and the basal incorporation of [3H]myo-inositol in the phosphatidylinositol lipid pool were significantly higher in the aorta of these hypertensive rats. A positive correlation (r = 0.88, P < 0.01) was demonstrated between basal inositol phosphate levels and the [3H]myo-inositol-labeled phosphatidylinositol lipid pool. In hypertensive rats, α_{1b}-adrenergic receptor–mediated inositol phosphate production in response to phenylephrine was significantly higher compared with normotensive rats. Despite the normalization of phenylephrine-mediated inositol phosphate production to the [3H]myo-inositol–labeled phosphatidylinositol lipid pool, the α_{1b}-adrenergic response remained significantly higher in the hypertensive rats. Phorbol ester activation of protein kinase C attenuated to a lesser extent phenylephrine-mediated inositol phosphate production (40%) in the aorta of hypertensive rats compared with the 80% attenuation observed in the aorta of normotensive rats. This desensitization was inhibited in both groups by the protein kinase C inhibitor staurosporine. The blunted desensitization of the α_{1b}-adrenergic receptor by protein kinase C activation was not associated with a decrease in protein kinase C activity in the hypertensive rats, because aortic strips from these animals were more responsive to phorbol ester activation than aortic strips from normotensive animals. Moreover, the in vivo administration of staurosporine reduced mean arterial pressure to a greater extent in the hypertensive rats. In the same vascular tissue of these hypertensive rats, endothelin-1 receptor–mediated inositol phosphate production was significantly reduced, and in contrast to the normotensive rats, in which a 50% decrease was observed, the endothelin-1 receptor was unresponsive to protein kinase C–mediated desensitization. From these results one can conclude that during the development of DOCA-salt hypertension, an increase in both basal phosphatidylinositol turnover and α_{1b}-adrenergic receptor reactivity could contribute to an enhanced vascular smooth muscle tone. These observations provide further evidence for an important role of the sympathetic nervous system and for the existence of an impaired regulation of the α_{1b}-adrenergic reactivity of vascular tissues in the development and/or maintenance of hypertension in DOCA-salt-treated rats. (Hypertension. 1994;23[part 1]:722-728.)

Key Words • protein kinase C • receptors, adrenergic, α_{1} • endothelins • hypertension, experimental • deoxycorticosterone

In both human and animal models of hypertension, an enhanced sympathoadrenal tone leading to a chronic state of elevated circulating plasma catecholamines has been reported. In this setting, numerous studies have demonstrated a diminished β-adrenergic vasodilator response, reflected by a decrease in agonist-mediated cyclic AMP production. By contrast, vascular smooth muscle reactivity to α_{1}-adrenergic stimulation was enhanced and associated with an increase in both inositol 1,4,5-trisphosphate levels and Ca^{2+} influx. Thus, this apparent dichotomy in the agonist-mediated desensitization of α_{1}- and β-adrenergic receptors would result in an α_{1}–adrenergic receptor dominance in vascular smooth muscle cells, thereby potentially contributing to the development of hypertension.

In vascular smooth muscle, pharmacologic evidence supports the existence of distinct α_{1}-adrenergic receptor subtypes. The α_{1b}-adrenergic receptor subtype, selectively antagonized by WB4101, has been shown primarily to mediate Ca^{2+} influx through the activation of dihydropyridine-sensitive channels, whereas the α_{1a}-adrenergic receptor subtype, selectively antagonized by chloroethyl clonidine, couples to phosphatidylinositol metabolism. However, the contribution of either the α_{1a} or α_{1b}-adrenergic receptor subtype to the enhanced vascular smooth muscle reactivity to norepinephrine during the development of hypertension remains to be elucidated. Studies have shown that these two α_{1}-adrenergic receptor subtypes are differentially regulated after an acute exposure to agonist. In the rabbit aorta, preincubation with norepinephrine for 2 hours selectively attenuated the α_{1b}-adrenergic receptor response, and this desensitization was shown to require the activation of protein kinase C. Despite this pattern of agonist-mediated desensitization, studies have reported an enhanced...
norepinephrine-mediated inositol phosphate production in vascular smooth muscle cells in animal models of hypertension associated with elevated plasma catecholamine levels.\textsuperscript{1,4} Thus, these data suggest that an impairment in the agonist-mediated desensitization of the $\alpha_\text{adrenergic}$ receptor could contribute to the exaggerated $\alpha_\text{adrenergic}$ response in vascular tissue during the development of hypertension.

An enhanced norepinephrine-mediated phosphoinositide turnover was reported in the myocardium and vascular smooth muscle cells of deoxycorticosterone acetate (DOCA)-salt hypertensive rats, in which elevated plasma catecholamine levels have been well documented.\textsuperscript{5} However, the delineation of the $\alpha_\text{adrenergic}$ receptor subtype involved in the increased reactivity to sympathetic stimulation remains unknown. Therefore, we designed this study to determine which $\alpha_\text{adrenergic}$ receptor subtype is involved in the exaggerated norepinephrine-mediated phosphoinositide turnover observed in the aorta of the DOCA-salt rat. Moreover, because numerous studies have demonstrated protein kinase C-mediated desensitization of $\alpha_\text{adrenergic}$ receptors, we examined the sensitivity of this mechanism on the exaggerated $\alpha_\text{adrenergic}$-mediated inositol phosphate response in the aorta of the DOCA-salt rat.

Methods

Male Sprague-Dawley rats (80 to 100 g, Charles River Laboratories) were uninephrectomized under pentobarbital anesthesia (60 mg/kg IP). After 4 days of recovery, randomly selected rats received a weekly subcutaneous injection of a suspension containing 10 mg DOCA (CIBA-GEIGY) and were allowed free access to 1% saline drinking solution for 4 weeks.\textsuperscript{9} Uninephrectomized normotensive (NT) rats that had free access to tap water served as controls.

Phosphatidylinositol labeling was performed as previously described,\textsuperscript{6} with the following modifications. Rat aortic rings (NT, 7.6±1.5 mg, n=15; DOCA, 8.6±2.1 mg, n=16) were preincubated in a 95% O$_2$/5% CO$_2$ environment for 30 minutes at 37°C in a Krebs-Henseleit (KH) solution of the following millimolar concentration: NaCl 118, KCl 3.5, MgSO$_4$ 2.4, KH$_2$PO$_4$ 1.2, CaCl$_2$ 2.5, NaHCO$_3$ 25, and glucose 10; this was bubbled with 95% O$_2$/5% CO$_2$ at pH 7.4. After preincubation the rings were transferred and incubated for 4 hours at 37°C with 0.3 $\mu$Ci/mL $[^3H]$myo-inositol (17 Ci/mmol, Amersham) in KH buffer. Thereafter, the rings were rinsed with KH buffer and incubated with 50 mmol/L LiCl for 15 minutes before agonist was added.

**Pharmacology of the $\alpha_\text{adrenergic}$ Receptor**

$[^3H]$myo-inositol-labeled normal aortic rings were pretreated for 45 minutes in the presence of either the $\alpha_\text{adrenergic}$ receptor antagonist WB4101 (2 mmol/L, RBI) or the $\alpha_\text{adrenergic}$ receptor antagonist chloroethyl clonidine (RBI, 20 mmol/L) before norepinephrine (10 mmol/L) stimulation. In all other studies phenylephrine was used for the assessment of $\alpha_\text{adrenergic}$ reactivity because this agonist is devoid of a $\beta$-adrenergic effect.

**Effect of Phorbol 12-Myristate 13-Acetate on $\alpha_\text{adrenergic}$ and ET-1 Receptor Reactivity**

$[^3H]$myo-inositol-labeled aortic rings from NT and DOCA-salt rats were pretreated with the phorbol ester phorbol 12-myristate 13-acetate (PMA, 1 mmol/L Sigma Chemical Co) or the vehicle ethanol 0.1% for 1 hour before agonist stimulation (NT, n=12; DOCA-salt, n=14). The specificity of phorbol ester activation of protein kinase C was evaluated in the presence of the protein kinase C inhibitor staurosporine (1 mmol/L, Sigma) (NT, n=14; DOCA-salt, n=14). After pretreatment procedures rings were rinsed three times with 5 mL cold KH solution and incubated with 50 mmol/L LiCl for 15 minutes at 37°C in KH solution. Phosphatidylinositol hydrolysis was allowed to proceed for an additional 30 minutes in the presence of 100 mmol/L phenylephrine (Sigma) or 50 mmol/L ET-1 (IAF Biochem International).

**Isolation of Labeled Inositol Phosphates**

Aortic rings were homogenized in ice-cold chloroform/methanol (1:2). An additional 400 $\mu$L of water and chloroform was added, and samples were agitated and centrifuged. The aqueous phase was removed, and the tritiated inositol phosphate pool (inositol phosphate, inositol bisphosphate, and inositol trisphosphate) was separated by ion-exchange chromatography (AGX18 resin, Bio-Rad). The remaining organic phase was evaporated under a stream of nitrogen, and the tritiated phosphatidylinositol lipid pool was counted in a scintillation counter (Beckman Instruments). In this study the inositol phosphate pool (counts per minute) was expressed as counts per minute per milligram tissue or as a ratio of $[^3H]$myo-inositol incorporation into the phosphatidylinositol lipid pool (counts per minute) in each tissue as previously described.\textsuperscript{10}

**Preparation of Aortic Strips**

The descending thoracic aorta was excised and placed in oxygenated cold physiological saline solution of the following composition (mmol/L): NaCl 160.0, KCl 4.6, CaCl$_2$ 1.5, MgSO$_4$ 1.2, HEPES 5.0, and glucose 11.0. The aorta was cleaned of adherent fat and connective tissue and cut into helical strips 3 mm in width and 20 mm in length. Care was taken not to damage the endothelial surface. The tissue was placed in a well-oxygenated (95% O$_2$/5% CO$_2$) bath of 10 mL physiological saline solution at 37°C with one end connected to a glass movable tissue holder and the other to a tension transducer (model FT03, Grass Instruments Inc) so that isometric force measurements could be recorded on a MacLab/8 system (AD Instruments Pty Ltd). The tissue was equilibrated for 60 minutes under a resting tension of 1.2 g before experiments were begun. During this time the physiological saline solution in the tissue bath was replaced every 20 minutes. After equilibration the aortic strip was repeatedly contracted with 0.1 mmol/L phenylephrine until the contractions became reproducible. Subsequently, cumulative concentration-response curves were obtained for PMA (1 mmol/L to 3 mmol/L). In parallel studies aortic tissues were pretreated with staurosporine (0.1 mmol/L) for 30 minutes before PMA was added (1 mmol/L for 45 minutes). Time-dependent changes in tissue sensitivity were performed in parallel experiments with dimethyl sulfide, the solvent vehicle for staurosporine, or ethanol, the solvent for PMA. The final concentration of the dimethyl sulfide or ethanol in the muscle bath did not exceed 0.1%.

**In Vivo Studies With Staurosporine**

During the third week of DOCA-salt treatment, a polyethylene catheter was inserted into the femoral artery of NT (n=7) and DOCA-salt rats (n=7) under pentobarbital anesthesia. Rats were housed individually and had access to food and water or saline ad libitum for at least 24 hours. The
arterial catheter was then coupled to a Statham transducer (P23 ID) connected to a polygraph recorder (model RMP-6008M, Nihon Kohden) for measurement of mean arterial pressure (MAP). Basal MAP was measured at 5-minute intervals for 15 minutes after a stabilization period. Staurosporine (0.1 mg/kg) was then injected and MAP measured at 5-minute intervals. The maximum MAP response to staurosporine was observed within 45 minutes after administration.

Plasma Catecholamine and ET-1 Measurements

From chronically cannulated, unanesthetized, freely moving animals, blood samples were drawn and collected in tubes containing EGTA (0.25 mol/L) and reduced glutathione (0.2 mol/L, pH 7) for catecholamine measurements or 1 mg/mL EDTA for ET-1 measurements as previously described.

Statistics

Values are expressed as mean±SEM. A Student's unpaired t test was used to assess significance, which was set at a value of P<.05. A two-way ANOVA was used to assess the difference in MAP decrease after the in vivo administration of staurosporine between NT and hypertensive rats.

Results

Basal MAP and Plasma Norepinephrine and ET-1 Levels

After 4 weeks of DOCA-salt treatment, MAP was significantly increased (Table). Circulating plasma norepinephrine levels were elevated, and ET-1 plasma levels remained unchanged in the DOCA-salt rats compared with the NT rats (Table).

Pharmacology of the α1-Adrenergic Receptor

Previous studies have shown that the α1- adrenergic receptor demonstrates a high affinity for the antagonist WB4101, with a Kᵣ value of 0.2 to 2 nmol/L. In this study the norepinephrine stimulation of phosphoinositide turnover, expressed as a ratio of [³H]myo-inositol incorporation into the phosphatidylinositol lipid pool (IPP/PILP) (basal, 0.22±0.01 versus norepinephrine, 0.37±0.02; n=10), was not inhibited in the presence of 2 nmol/L WB4101 (basal, 0.19±0.02 versus norepinephrine, 0.40±0.01; n=4). By contrast, the pretreatment of NT rat aortic rings with the classic α₁-adrenergic receptor antagonist chloroethyl clonidine (20 μmol/L) completely inhibited norepinephrine-mediated phosphatidylinositol turnover (basal, 0.23±0.01 versus norepinephrine, 0.22±0.02; n=6). Thus, these pharmacologic data support previous observations of phosphoinositide turnover coupling to the α₁-adrenergic receptor in vascular smooth muscle cells.

Basal and Agonist-Mediated Stimulation of Phosphatidylinositol Turnover in Normotensive and DOCA-Salt-Treated Rats

The basal accumulation of inositol phosphates was higher (100%) in the aorta of DOCA-salt rats than in tissue from NT rats (Fig 1). This increase in basal inositol phosphate levels was associated with an exaggerated α₁-adrenergic response in the presence of phenylephrine, whereas ET-1 stimulation of inositol phosphate was decreased, although not significantly, in aorta of DOCA-salt rats (Fig 1). The [³H]myo-inositol labeling of the phosphatidylinositol lipid pool was found to be significantly higher in tissues from DOCA-salt rats than from NT animals (Fig 2). In fact, the basal formation of inositol phosphates in the aorta of both NT and hypertensive rats was found to correlate positively (r=0.88, P<.01; Fig 3) with the degree of [³H]myo-inositol incorporation in the phosphatidylinositol lipid pool. Accordingly, when the basal inositol phosphate pool was normalized as a ratio with the labeled phosphatidylinositol lipid pool, basal inositol phosphate production in the aorta of hypertensive rats was similar to that in NT rats (Fig 4). The phenylephrine-induced inositol phosphate production remained significantly higher, whereas the ET-1 response was significantly reduced in the aorta of DOCA-salt rats after the correction for the [³H]myo-inositol-labeled phosphatidylinositol lipid pool.

The Role of Protein Kinase C in Agonist-Mediated Receptor Desensitization

In NT animals, pretreatment of aortic rings with the phorbol ester PMA (1 μmol/L) for 1 hour before agonist stimulation reduced the subsequent phenylephrine- and ET-1-mediated formation of the inositol phosphate pool by 80% and 50%, respectively (Fig 5). The addition of staurosporine (1 μmol/L) inhibited the PMA-mediated desensitization of both phenylephrine and ET-1 responses and also potentiated both phenyl-

| Hemodynamic and Hormonal Changes in the Development of DOCA-Salt Hypertension |
|------------------|------|------------|------|---|
|                  | NT   | n          | DOCA-HT | n  |
| MAP, mm Hg       | 100±4| 7          | 186±7*  | 7  |
| Plasma norepinephrine, ng/mL | 0.122±0.008 | 6       | 0.243±0.040* | 6  |
| Plasma ET-1, pg/mL | 2.65±0.50  | 5        | 2.12±0.50    | 4  |

DOCA indicates deoxycorticosterone acetate; NT, normotensive rats; DOCA-HT, DOCA hypertensive rats; MAP, mean arterial pressure; and ET-1, endothelin 1. Values are mean±SEM; n is number of rats. *P<.01 vs NT.

Fig 1. Bar graph shows basal formation of inositol phosphates (IPP) and during activation with phenylephrine (PHE, 100 μmol/L) or endothelin-1 (ET-1, 10 nmol/L) in aorta of deoxycorticosterone acetate–salt hypertensive (DOCA-HT, n=15 aortic rings) and normotensive (NT, n=16 aortic rings) rats. Values are mean±SEM. *P<.01 vs NT.
ephrine and ET-1 receptor responsiveness by 140% and 41% respectively, compared with untreated rings.

In aortic rings of DOCA-salt rats the phenylephrine-mediated formation of inositol phosphates was attenuated to a lesser extent (40%) after PMA pretreatment than in tissue from NT rats (80%) (Fig 5, top). Moreover, PMA pretreatment did not further reduce ET-1 responsiveness in the aorta of hypertensive rats (Fig 5, bottom). In the presence of staurosporine the partial attenuation of the α1-adrenergic response by PMA was completely blocked, as observed in NT animals (Fig 5, top). However, in contrast to the NT rats, the presence of staurosporine did not potentiate the phenylephrine- or ET-1-mediated inositol phosphate formation in the aorta of the DOCA-salt rats (Fig 5, bottom).

The In Vitro Responsiveness of Aortic Rings From Normotensive and Hypertensive Rats to Phorbol Ester Activation

PMA induced concentration-dependent contractions of aortic strips in NT and DOCA-salt rats (Fig 6). Ethanol, the vehicle for PMA, did not affect basal tension (data not shown). Aortic rings from DOCA-salt rats were more responsive to PMA compared with aortic rings from NT rats.

![Fig 2](image_url)  
**Fig 2.** Bar graph shows incorporation of \([\text{H}]\)myo-inositol in the phosphatidylinositol lipid pool (PILP) in aorta of deoxycorticosterone acetate–salt hypertensive (DOCA-HT, n=15 aortic rings) and normotensive (NT, n=16 aortic rings) rats in the absence or presence of phenylephrine (PHE, 100 μmol) or endothelin-1 (ET-1, 10 nmol). Values are mean±SEM. *P<.01 vs NT.

![Fig 3](image_url)  
**Fig 3.** Plot shows correlation between basal production of inositol phosphates (IPP) and incorporation of \([\text{H}]\)myo-inositol into the phosphatidylinositol lipid pool (PILP) in rat aorta (r=.88, P<.01). Values from normotensive (NT, n=24 aortic rings) and deoxycorticosterone acetate–salt hypertensive (DOCA-HT, n=30 aortic rings) rats are pooled in this figure.

![Fig 4](image_url)  
**Fig 4.** Bar graph shows ratio of \([\text{H}]\)inositol phosphates (IPP) over \([\text{H}]\)myo-inositol incorporation in the phosphatidylinositol lipid pool (PILP) under basal conditions and during activation with phenylephrine (PHE, 100 μmol) or endothelin-1 (ET-1, 10 nmol) in aorta of deoxycorticosterone acetate–salt hypertensive (DOCA-HT, n=15 aortic rings) and normotensive (NT, n=16 aortic rings) rats. Values are mean±SEM. *P<.01 vs NT.

![Fig 5](image_url)  
**Fig 5.** Bar graphs show changes in accumulation of the ratio of \([\text{H}]\)inositol phosphates (IPP) over \([\text{H}]\)myo-inositol incorporation in the phosphatidylinositol lipid pool (IPP/PILP) during phenylephrine (PHE, 100 μmol) (top) or endothelin-1 (ET, 10 nmol) (bottom) activation of aortic rings from deoxycorticosterone acetate–salt hypertensive (DOCA-HT, n=14 aortic rings) and normotensive (NT, n=12 aortic rings) rats in the absence or presence of phorbol 12-myristate 13-acetate (PMA) and PMA plus staurosporine (STS). Values are mean±SEM. *P<.01 vs PHE (top) or ET (bottom); tP<.01 vs PMA pretreatment.
The In Vivo Effect of Staurosporine in Normotensive and DOCA-Salt Hypertensive Rats

The in vivo administration of 0.1 mg/kg staurosporine reduced MAP in a time-dependent manner in both animal groups (Fig 7). However, the staurosporine-mediated decrease in MAP was significantly greater in the DOCA-salt rats (Fig 7). In fact, the hypotensive effect of staurosporine was found to be inversely correlated with the initial MAP in those animals (r = -0.9, P < .001, Fig 8).

Discussion

This study demonstrates that in the presence of αβ-adrenergic receptor antagonists, norepinephrine-mediated phosphatidylinositol turnover in the normal rat aorta was mediated by the αβ-adrenergic receptor subtype. Moreover, the exaggerated phenylephrine response observed in the DOCA-salt rats may be associated with a reduced efficacy of protein kinase C to desensitize the αβ-adrenergic receptor, as demonstrated by the lesser inhibitory effect of PMA treatment on phenylephrine-mediated inositol phosphate production. This occurred despite an increase in total protein kinase C activity, as demonstrated by the greater contractions of DOCA-salt aortic rings to phorbol ester in vitro and by the greater in vivo hypotensive effect of staurosporine treatment in the hypertensive rats. In contrast, endothelin-mediated inositol phosphate production was found to be decreased in the aorta of these hypertensive rats, and contrary to the observation in the aorta of NT rats, ET-1 receptors were found to be completely unresponsive to PMA-mediated desensitization.

In the aorta of the NT rat, pharmacologic evidence has demonstrated that norepinephrine-mediated phosphoinositide turnover is coupled to the αβ-adrenergic receptor subtype. In the DOCA-salt rat, the physiological consequences of an exaggerated αβ-adrenergic-mediated phosphoinositide turnover on vascular smooth muscle reactivity remain speculative. In rat vas deferens, the αβ-adrenergic receptor is coupled to phosphoinositide turnover, whereas αα-adrenergic receptors selectively activate contraction by promoting Ca2+ influx through dihydropyridine-sensitive Ca2+ channels. Presently, no evidence exists that suggests a participation of αα-adrenergic receptors in the modulation of vascular smooth muscle tone in the hypertensive rat. In contrast, the αα-adrenergic receptor has been shown to contribute to the phasic component of the rat aortic contractile response to epinephrine. Moreover, the suggested coupling of the αα-adrenergic receptor to protein kinase C activation and the study by Galizzi and colleagues demonstrating that protein kinase C may be involved in the regulation of the vascular smooth muscle dihydropyridine-sensitive calcium channel provide further evidence of a role for the αα-adrenergic receptor in the regulation of vascular smooth muscle tone and the development of hypertension in the DOCA-salt rat.

Basal inositol phosphate levels were significantly higher in the aorta of DOCA-salt rats and were associated with a greater incorporation of [3H]myo-inositol in the phosphatidylinositol lipid pool compared with aorta from NT rats. In fact, a positive correlation (r = .88, P < .01) was observed between the level of basal inositol phosphates and [3H]myo-inositol labeling of the phosphatidylinositol lipid pool. Moreover, Conrad and colleagues have shown that a decrease in [3H]myo-inositol labeling of the phosphatidylinositol lipid pool was associated with a decrease in basal inositol phosphate level in the aorta of gravid rats. Together, these data suggest that the uptake of myo-inositol can regulate basal inositol phosphate levels. More importantly, if a similar relation is also present in resistance vessels, a change in myo-inositol uptake could contribute to the modulation of vascular smooth muscle tone and pressor responsiveness in DOCA-salt rats.
In the aorta of DOCA-salt rats, phenylephrine-mediated inositol phosphate production was nearly 2.5-fold higher than in aorta of NT rats. When the inositol phosphate pool was normalized to the [3H]myo-inositol-labeled phosphatidylinositol lipid pool, the reactivity of the α₁-α₁-adrenergic receptor signaling pathway remained significantly higher in the hypertensive rats. This hyperreactivity of the α₁-α₁-adrenergic receptor persisted despite the presence of elevated plasma catecholamines, a condition that has been shown to promote both α₁- and β-adrenergic receptor desensitization. Previous studies have demonstrated that agonist-mediated desensitization of the α₁-α₁-adrenergic receptor involves the activation of protein kinase C. The protein kinase C inhibitor staurosporine not only inhibited the PMA-mediated decrease in α₁-α₁-adrenergic receptor reactivity but also potentiated the phenylephrine-induced production of inositol phosphates by 140% above control. In aortic rings from hypertensive animals, phorbol ester pretreatment reduced the enhanced α₁-α₁-adrenergic response by only 40%. In the presence of staurosporine, the partial PMA-mediated attenuation of the phenylephrine response in the DOCA-salt rats was inhibited but not potentiated, as observed in aortic rings from NT animals.

The decreased efficacy of PMA to completely attenuate the exaggerated phenylephrine response in DOCA-salt rats could result from a decrease in protein kinase C activity. However, this does not seem to be the case because aortic rings from DOCA-salt rats were more responsive to phorbol ester activation than aortic rings from NT rats. In the presence of staurosporine, the PMA-induced contraction was abolished in both normotensive and hypertensive aortic rings. Moreover, a greater hypotensive effect was observed in the DOCA-salt rats after the in vivo administration of staurosporine. This hypotensive effect of staurosporine was found to be inversely correlated to basal MAP, suggesting an increased protein kinase C activity in the resistance vascular bed of these hypertensive rats. This conclusion is supported in a study by Turla and Webb demonstrating an increase in protein kinase C activity in the mesenteric artery of the DOCA-salt rat model of hypertension. Thus, these data provide evidence for a role for protein kinase C in the development of and/or maintenance of hypertension in this model. Moreover, the observation that elevated protein kinase C activity was less effective in attenuating the phenylephrine response suggests an alteration in α₁-α₁-adrenergic receptor sensitivity to protein kinase C inhibitory action. This reduced sensitivity of the α₁-α₁-adrenergic receptor signaling pathway to protein kinase C regulation could contribute to the lack of agonist-mediated desensitization of these receptors in the presence of elevated plasma catecholamine levels.

Although this study demonstrates an increase of total protein kinase C activity in aorta of DOCA-salt rats, the status of the various protein kinase C isoforms remains unknown. Recent studies have demonstrated that protein kinase C isoforms exhibit different cellular distribution patterns and Ca²⁺ sensitivity. In addition, only a small portion of the total cellular protein kinase C population has been shown to translocate to the plasma membrane after the addition of an agonist. These differences suggest a physiological specificity among the various protein kinase C isoforms, and it remains to be shown whether those protein kinase C isoforms involved in vascular smooth muscle contraction also participate in the homologous desensitization of the α₁-α₁-adrenergic and other receptors coupled to phosphoinositide turnover. It is thus possible that the decreased efficacy of protein kinase C to attenuate the α₁-α₁-adrenergic receptor response to phenylephrine in the aorta of hypertensive rats despite an apparently enhanced total protein kinase C activity could be related to the selective decrease in the content and/or activity of a particular protein kinase C isoform involved in the homologous regulation of the α₁-α₁-adrenergic receptor. This latter possibility could explain the lack of potentiation of phenylephrine-mediated inositol phosphate production in the presence of staurosporine, as observed in the DOCA-salt rats.

Protein kinase C has also been shown to participate in the homologous desensitization of the ET-1 receptor. In the present study, PMA decreased ET-1-mediated production of inositol phosphates, and staurosporine prevented the inhibitory effect of PMA and potentiated the agonist-mediated inositol phosphate production in the aorta of NT rats similarly to that observed for the α₁-α₁-adrenergic receptor. In contrast to the observed hyperreactivity of the α₁-α₁-adrenergic receptor in the aorta of the hypertensive rat, the reactivity of the ET-1 receptor was desensitized, as reflected by a threefold decrease in ET-1-mediated inositol phosphate production (normalized for [3H]myo-inositol-labeled phosphatidylinositol lipid pool). In addition, it was observed that phorbol ester activation of protein kinase C did not further reduce the ET-1 receptor response to agonist and that protein kinase C inhibition by staurosporine did not potentiate or restore the sensitivity of ET-1 receptors. Therefore, despite a similar pattern of protein kinase C-mediated inhibition observed for both the α₁-α₁-adrenergic and ET-1 receptors in the NT rat, these latter findings of a decreased ET-1 receptor-mediated inositol phosphate production in the presence of an exaggerated α₁-α₁-adrenergic response in the DOCA-salt rat suggest the possibility of multiple pathways regulating phosphoinositide-coupled receptors. In support of this hypothesis, recent studies have demonstrated that agonist-mediated downregulation of the ET-1 receptor can occur in the absence of protein kinase C, whereas protein kinase C plays a major role in the agonist-mediated downregulation of the rabbit aortic smooth muscle α₁-α₁-adrenergic receptor. Therefore, in the DOCA-salt rat, the decrease in ET-1 receptor-mediated inositol phosphate production and the reduced inhibitory action of PMA as well as the lack of potentiation by staurosporine may be a consequence of ET-1 receptor downregulation, despite an elevated protein kinase C activity. In fact, a decrease in ET-1 receptor-mediated inositol phosphate production was associated with an important reduction in ET-1 binding sites in both the aorta and mesenteric arteries during the early stages of DOCA-salt-mediated hypertension. However, we and others have shown that both the decrease in ET-1 receptor reactivity and receptor downregulation occurred despite normal circulating plasma ET-1 levels in
the DOCA-salt rat,\(^2\) suggesting that plasma ET-1 levels may not be a representative index of endothelin release from endothelial cells. However, it is tempting to speculate that the local release of ET-1 may have been enhanced at an earlier stage in the DOCA-salt rat, thereby promoting ET-1 receptor downregulation, but this possibility still remains to be demonstrated.

In conclusion, in the DOCA-salt rat model of hypertension, the greater \(^{[3]H}\)myo-inositol incorporation in the aortic phosphatidylinositol lipid pool, which positively correlated to basal inositol phosphate levels, suggests that an increase in basal phosphatidylinositol turnover could modulate vascular smooth muscle tone. Second, the exaggerated reactivity of the \(\alpha\)-adrenergic receptor-inositol phosphate transmembrane signaling pathway in the aorta of the DOCA-salt rat was associated with a decreased efficacy of protein kinase C to completely desensitize the phenylephrine response. Because this abnormality was associated with an enhanced total protein kinase C activity, the reduced efficacy of protein kinase C to desensitize the \(\alpha\)-adrenergic receptor reactivity may be a consequence of an altered sensitivity of the receptor to protein kinase C action. However, in the aorta of the DOCA-salt rat, the functional status of the numerous protein kinase C isoforms remains unknown. Additional studies are required to assess whether those protein kinase C isoforms involved in vascular smooth muscle contraction also participate in agonist-mediated desensitization of the \(\alpha\)-adrenergic- or other phosphoinositide-coupled receptors. Nonetheless, alterations in the inhibitory modulation of protein kinase C-mediated inositol phosphate turnover could play a major role in the development and maintenance of hypertension in the DOCA-salt rat. In contrast to the hyperreactivity of the \(\alpha\)-adrenergic receptor, the activity of the phosphoinositide-coupled receptor ET-1 was blunted in the DOCA-salt rat. This finding of a reduced ET-1 response in both the early\(^{2}\) and latter stages of DOCA-salt hypertension suggests that ET-1 does not play a primary role in the development and/or maintenance of hypertension in this model. Furthermore, the differential pattern of \(\alpha\)-adrenergic and ET-1 receptor regulation in this model implies the existence of additional pathways that can selectively modulate the activity of the various phosphoinositide-coupled receptors.

Acknowledgments

This work was supported by grants from the Medical Research Council of Canada (MRC) and the Heart and Stroke Foundation of Canada. Dr J.-L. Rouleau is a scholar of Les Fonds de la recherche en santé du Québec. Dr J. de Champlain is a C. C. Edwards Foundation Career Investigator. Dr D.J. Stewart is an MRC scholar. P. Moreau is a recipient of an MRC studentship. L. Oster is a recipient of a postdoctoral fellowship from the Heart and Stroke Foundation of Canada. The authors wish to thank Diane Papin and Jo-Anne LeGuerrier for technical assistance as well as Carole Champagne for her editorial help in the preparation of this manuscript.

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Hypertension. 1994;23:722-728
doi: 10.1161/01.HYP.23.6.722

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0194-911X. Online ISSN: 1524-4563

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