Increased Inositol Monophosphate Production in Cardiovascular Tissues of DOCA-Salt Hypertensive Rats

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SUMMARY The purpose of the present study was to investigate $\alpha_1$-adrenergic receptors in the heart as well as the activity and the sensitivity of the phosphoinositide pathway on tissue slices of atria, ventricles, and femoral artery of hypertensive rats treated for 4 weeks with deoxycorticosterone acetate (DOCA) and 1% saline. DOCA-salt hypertensive rats were characterized by an increased sympathoadrenal tone, as suggested by increased norepinephrine and epinephrine plasma levels. The basal activity of the phosphoinositide pathway, estimated by measuring the accumulation of inositol monophosphate in the presence of an excess of lithium, was found to be greater in atria than in ventricles and femoral artery in both normotensive and DOCA-salt hypertensive rats, but it was twofold greater in atria and ventricles of DOCA-salt hypertensive rats compared with normotensive rats. Following stimulation by norepinephrine, the production of inositol monophosphate was greater in atria and femoral artery than in ventricles in both groups. However, in DOCA-salt hypertensive rats, the production of inositol monophosphate was markedly enhanced, being about twofold greater in atria and femoral artery and about three times greater in ventricles than in tissues of normotensive rats. These differences between DOCA-salt hypertensive and normotensive animals do not appear to be associated with a difference in $\alpha_1$-adrenergic receptor number or affinity since cardiac $\alpha_1$-adrenergic receptor number was unchanged in hypertensive rats and the binding affinity to the receptor was significantly decreased in hypertensive rats compared with normotensive rats. These data therefore demonstrate an increase in basal and norepinephrine-induced inositol monophosphate production in cardiovascular tissues of DOCA-salt hypertensive rats and suggest the existence of a hypersensitivity of $\alpha_1$-adrenergic receptors or of an increased sensitivity of intracellular mechanisms responsible for the activation of the phosphatidylinositol pathway in the cardiovascular tissues of these animals. (Hypertension 12: 122-128, 1988)

KEY WORDS • sympathetic nervous system • $\alpha_1$-adrenergic receptors • norepinephrine • second messenger • experimental hypertension

THE maintenance of a normal arterial blood pressure depends on the proper control of cardiac output and peripheral vascular resistance, which are in great part modulated by the sympathetic nervous system. Moreover, the biological expression of the sympathetic activity depends mainly on the effectiveness of the adrenergic receptors to receive and transfer the neuronal signal to the effector cell. This information is transferred to the cardiac tissue through $\alpha$-adrenergic and $\beta$-adrenergic receptors, which mediate the positive inotropic and chronotropic sympathetic effects. These receptors are also present on the smooth muscle vascular cells and are an important determinant of their contractile state. At the molecular level, the $\beta$-adrenergic receptor effects of norepinephrine (NE) are mediated through activation of adenylyl cyclase, leading to the formation of cyclic adenosine 3',5'-monophosphate, whereas the NE effect on $\alpha_1$-adrenergic receptors is mediated initially by the phosphodiesteric hydrolysis of phosphatidylinositol 4,5-bisphosphate, leading to the formation of inositol 1,4,5-trisphosphate (IP$_3$) and diacylglycerol as second messengers.

It has been previously demonstrated that deoxycorticosterone acetate (DOCA)-salt hypertension is associated with an increased sympathoadrenal...
with an increase in plasma catecholamine levels, with an increased apparent NE secretion rate. A reduction in the number of β-adrenergic receptors in the heart has also been demonstrated in this experimental model, but the data on α, adrenergic receptor number and affinity remain controversial. In the present study, we investigated the capacity of the heart and vessels to produce inositol monophosphate (IP) under basal conditions and following NE activation in correlation with the number and affinity of α, adrenergic receptors in the heart to evaluate the effect of chronic sympathoadrenal activation, such as that encountered in DOCA-salt hypertension, on these membrane mechanisms.

Materials and Methods

Animal Preparation

Male Sprague-Dawley rats (weight, 80–90 g; Charles River Laboratories, St.-Constant, Quebec, Canada) were anesthetized with ether and nephrectomized unilaterally. Five days after the procedure, weekly injections of a suspension of DOCA (10 mg; CIBA-Geigy, Mississauga, Ontario, Canada) were given subcutaneously to the animals as previously described. The DOCA-salt rats were given free access to 1% saline drinking solution for 4 weeks, whereas control animals were given tap water to drink.

Inositol Monophosphate Assay

Slices (about 10 mg) from the right atrium, left atrium, right ventricle, left ventricle, and femoral or mesenteric artery were incubated for 30 minutes at 37 °C in Krebs-Henseleit buffer (118 mM NaCl, 4.7 mM KCl, 2.5 mM CaCl2, 1.2 mM NaH2PO4, 1.2 mM MgCl2, 25 mM NaHCO3, 11 mM glucose, pH 7.4) saturated with 95% O2 and 5% CO2. Slices were then transferred to 3-ml vials containing 300 μl of buffer with 0.3 μM [3H]inositol ([3H]inositol (17.1 Ci/mmol; New England Nuclear) were incubated in a total volume of 0.15 ml for 35 minutes at 25 °C. The incubation was stopped by the addition of 2 ml of ice-cold buffer followed by rapid filtration under reduced pressure through Whatman GF/B glass fiber filters (Clifton, NJ, USA). The filters were washed twice with 2 ml and twice with 3 ml of ice-cold buffer. Nonspecific binding was defined as the binding remaining in the presence of 10 μM of phentolamine (CIBA-Geigy). Saturation was studied with 10 concentrations of [3H]inositol in the range of 0.075 to 2 nM. The apparent Kd and maximum binding values were analyzed according to the method of Scatchard. The protein concentration was measured by the method of Lowry et al., using bovine serum albumin as standard.

Plasma Catecholamine Measurement

One day before the experiment, the rats were anesthetized with halothane (Ayerst Laboratories), 1.5 to 2.0% (vol/vol) in a mixture of 95% O2, 5% CO2. The right femoral artery was cannulated with polyethylene tubing (Becton Dickinson, Parsippany, NJ, USA) for recording of blood pressure, through a Statham strain gauge transducer (Model P233D6, Oxnard, CA, USA) connected to a polygraph recorder (Model RMP-6008M, Nihon Kohden Kogyo, Tokyo, Japan), and for sampling of blood for catecholamine assays. The plasma NE and epinephrine concentrations were determined by means of the radioenzymatic assay of Peuler and Johnson. In brief, this technique is based on the enzymatic conversion of NE, epinephrine, and dopa-
mine to their tritiated metabolites, normetanephrine, metanephrine, and methoxytyramine, in the presence of catechol-o-methyl-transferase and tritiated S-adenosylmethionine as the methyl donor. After purification through organic extractions, the tritiated metabolites were separated by thin-layer chromatography and counted in a Beckman liquid scintillation counter.

Statistical Analysis

Paired or unpaired Student's t test was used when applicable to determine the statistical significance of the results. Differences were considered significant at a p level below 0.05.

Results

The mean arterial pressure, catecholamine levels, and cardiac weight/body weight ratio of DOCA-salt HT and normotensive (NT) rats are shown in Table 1. Four weeks after treatment was begun, blood pressure, circulating NE and epinephrine levels, and the ventricular weight to body weight ratio were significantly increased in DOCA-salt-treated rats. Moreover, the average body weight of DOCA-salt HT rats was significantly lower than that of control animals.

In NT rats, the incubation of tissue slices with NE significantly enhanced the breakdown of PI in the right and left atria, as reflected by the marked increases in the accumulation of IP compared with basal activity (Figure 1). The potentiation in the IP production observed by the addition of NE in the incubation media appears to be mediated specifically by α1-adrenergic receptors, since this effect was totally blocked by the addition of prazosin (Table 2). In DOCA-salt HT rats the basal activities of both atria were significantly higher than those in NT rats. Moreover, the activation by NE resulted in markedly greater accumulations of IP in both atria of DOCA-salt HT rats compared with NT rats.

In NT rats the basal activity in both ventricles (Figure 2) was about 50% lower than that in the atria. Moreover, in contrast to the atria, the addition of NE significantly increased IP only in the right ventricle of NT rats (see Figure 2). The basal activity was significantly greater in both ventricles of DOCA-salt HT rats, and the addition of NE induced much larger increases in IP accumulation in both ventricles of DOCA-salt HT rats than in NT rats. However, the accumulation of IP in the left ventricle was less pronounced than that in the right ventricle of DOCA-salt HT rats after NE stimulation.

The NE activation caused a greater response in the femoral artery of NT rats than in the left ventricle (Figure 3). In the DOCA-salt HT rats, the IP formation by the femoral artery was markedly potentiated after NE activation. Similar observations were also made in the mesenteric artery (see Figure 5). However, in contrast to cardiac tissues, no differences were observed between basal IP accumulations in vessels of NT control and DOCA-salt HT rats.

Differential increases of IP accumulation above baseline values after incubation with NE for 1 hour are illustrated in Figure 4. This graph shows clearly the enhanced accumulation of IP in response to NE activation in the right atrium, left atrium, right ventricle, and femoral artery of DOCA-salt HT rats. When IP accumulation was measured at various times after the beginning of the incubation with NE, an enhanced response could be observed in cardiac and vascular tissues of DOCA-salt HT rats at earlier times after starting the incubation, and these differences became progressively greater over time (Figure 5).

No significant difference in basal and NE-induced IP accumulation was found in tissues of NT rats.

Table 1. Mean Arterial Pressure, Circulating Catecholamine Levels, and Ventricular Weight to Body Weight Ratios in Control and DOCA-Salt Hypertensive Rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Body wt (g)</th>
<th>Ventricular wt/body wt (mg/g)</th>
<th>Mean arterial pressure (mm Hg)</th>
<th>NE (pg/ml)</th>
<th>E (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (n=14)</td>
<td>265 ± 8</td>
<td>2.88 ± 0.07</td>
<td>110 ± 20</td>
<td>156 ± 19</td>
<td>64 ± 7</td>
</tr>
<tr>
<td>DOCA-salt (n=14)</td>
<td>172 ± 6*</td>
<td>4.63 ± 0.14*</td>
<td>200 ± 15*</td>
<td>294 ± 38†</td>
<td>150 ± 26†</td>
</tr>
</tbody>
</table>

Values represent means ± SEM for 14 rats. NE = norepinephrine; E = epinephrine.

*p < 0.001, †p < 0.025, compared with control values.
INOSITOL MONOPHOSPHATE IN DOCA-SALT HYPERTENSION

and de Champlain

TABLE 2. Receptor Specificity of Norepinephrine-Stimulated [3H]Inositol Monophosphate Formation in Cardiovascular Tissues of Normotensive Rats

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Basal Activity (cpm/10 mg wet weight)</th>
<th>NE stimulation</th>
<th>NE + prazosin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Right atria</td>
<td>94 ± 9</td>
<td>389 ± 31*</td>
<td>74</td>
</tr>
<tr>
<td>Left atria</td>
<td>75 ± 9</td>
<td>324 ± 45*</td>
<td>60</td>
</tr>
<tr>
<td>Right ventricle</td>
<td>54 ± 3</td>
<td>89 ± 9*</td>
<td>42</td>
</tr>
<tr>
<td>Left ventricle</td>
<td>48 ± 4</td>
<td>66 ± 5†</td>
<td>43</td>
</tr>
<tr>
<td>Femoral artery</td>
<td>71 ± 10</td>
<td>163 ± 32†</td>
<td>66</td>
</tr>
</tbody>
</table>

Tissue slices from right and left atria, right and left ventricles and the femoral artery, labeled with tritiated inositol were incubated with norepinephrine (NE) with or without 0.1 μM of prazosin. The values represent the means ± SEM obtained from 12 experiments for basal and NE activation and two experiments for the treatment with prazosin.

*p < 0.001, †p < 0.05, compared with basal values.

receiving 1% saline to drink for 4 weeks compared with tissues of NT rats receiving tap water to drink (data not shown).

The study of [3H]prazosin-specific binding to membranes prepared from atria and ventricular cardiac tissues showed that the number of α1-adrenergic receptors, as illustrated by the maximum binding capacity, was similar in atria and ventricles of NT and DOCA-salt HT rats (Table 3). However, the affinity, as illustrated by the $K_d$, was significantly reduced in the ventricles of DOCA-salt HT rats, whereas the affinity was normal in atria of DOCA-salt HT rats (Figure 6).

Discussion

Based on the measure of the accumulation of IP, the present data suggest that the basal activity and the reactivity to NE of the PI pathway are enhanced in the heart as well as in the arteries of DOCA-salt HT rats. The importance of these findings on the heart function and on the contractility of the arteries in the development and maintenance of DOCA-salt HT is still unclear. IP$_3$ has been demonstrated to release Ca$^{2+}$ from intracellular stores,$^{13}$ and the importance of the intracellular Ca$^{2+}$ concentration is well established in the contractility of the smooth muscle cell. However, in cardiac tissue, the role of IP$_3$ as an intracellular Ca$^{2+}$-mobilizing agent is still controversial.$^{14}$ On the one hand, Movsesian et al.$^{15}$ have postulated that diacylglycerol rather than IP$_3$ is responsible for the induction of mechanisms that indirectly affect intracellular Ca$^{2+}$ concentration through activation of protein kinase C in the heart. On the other hand, others have observed that the production of IP$_3$ is enhanced in cardiac cells under conditions that trigger positive inotropic effects, such as those following α1-adrenergic receptor or electrical stimulation.$^{16,17}$ Moreover, it has also been shown that the addition of IP$_3$ to cardiac cell sarcoplasmic reticulum induces the release of Ca$^{2+}$.$^{14,18,19}$ Thus suggesting that IP$_3$ may be closely involved in the excitation-contraction coupling of the heart cells.

It is therefore possible to postulate that an increase in the activity and reactivity of the PI pathway in the cardiovascular tissues of DOCA-salt HT rats may potentiate the sympathetic effects on the heart and arteries of these animals, thus probably contrib-

FIGURE 2. Accumulation of [3H]inositol monophosphate under basal conditions and following norepinephrine (NE) stimulation in 10-mg slices from the right and left ventricles of control and DOCA-salt hypertensive (HT) rats. The bars illustrate the means ± SEM. Numbers in parenthesis indicate numbers of animals in each group. Asterisks indicate the significance compared with values in normotensive animals.

FIGURE 3. Accumulation of [3H]inositol monophosphate (IP) under basal conditions and following norepinephrine (NE) stimulation in 10-mg slices from the femoral artery. The bars illustrate the means ± SEM for 14 rats. Asterisk indicates the significance compared with values in normotensive animals. HT = hypertensive.
IP PRODUCTION BY NE STIMULATION

FIGURE 4. Differential increase above basal activity following norepinephrine (NE) stimulation in atria, ventricles, and femoral artery of control and DOCA-salt hypertensive (HT) rats. The bars illustrate the means ± SEM for 14 normotensive and 14 DOCA-salt HT rats. IP = inositol monophosphate. Asterisks indicate the significance compared with values in normotensive animals.

Our findings of an increased IP accumulation in the heart following NE stimulation are consistent with previous observations made in the rat atria,22 ventricles,23,24 cardiomyocytes,15,25 and arteries.26-28 In our study, the enhanced IP production by NE activation seemed to be mediated by \( \alpha_2 \)-adrenergic receptors, since the \( \alpha_1 \)-antagonist prazosin was found to block the NE-induced responses in atria, ventricles, and femoral artery.

The accumulation of IP in the atria of NT rats was found to be about fourfold greater than that in the ventricles. The reasons for a greater IP formation in the atria under NE stimulation are unclear. In fact, the atria was reported to contain fewer \( \alpha_1 \)-adrenergic receptors than the ventricles.22 Thus, a difference in receptor density cannot account for this differential responsiveness. Moreover, studies on cardiomyocytes23 and on atria22 have shown that the activation of muscarinic receptors enhanced PI pathway activity by only 35%, compared with a more than twofold enhancement of the activity by \( \alpha_2 \)-adrenergic receptor agonists despite a higher density of muscarinic receptors.29 This response...
may also differ between animal species, as suggested by Brown and Brown, who reported that, although $\alpha_\text{adrenergic}$ receptor stimulation by phenylephrine resulted in an important $^{32}\text{P}$ incorporation in rat atria, this treatment failed to induce any response in mouse atria.

The differences in PI pathway activity or reactivity between tissues of NT and DOCA-salt HT rats could not be linked to the degree of $\alpha_\text{adrenergic}$ receptor occupancy and affinity for NE. The observation that the $\alpha_\text{adrenergic}$ receptor numbers were identical in atria and ventricles of DOCA-salt HT and NT rats shows a clear dissociation between the NE activation of the PI pathway and the number of $\alpha_\text{adrenergic}$ receptors in the heart. In addition, the activation of the PI pathway appeared to be independent of the binding affinity to the $\alpha_\text{adrenergic}$ receptors, since the affinity was similar in the atria of both groups of animals and was even reduced in the ventricles of DOCA-salt HT rats. Our observations are therefore in agreement with those of others who have demonstrated that the receptor-mediated activation of PI metabolism is not always linearly related to $\alpha_\text{adrenergic}$ receptor density in vascular tissues. One possible explanation for this discrepancy has been suggested by Chiu et al. who proposed the existence of two populations of $\alpha_\text{adrenergic}$ receptor recognition sites, one coupled exclusively to receptor-operated Ca$^{2+}$ channels and the other coupled to the PI pathway. Moreover, Heagerty et al. have also reported that the maximum hydrolysis of PI in control rats was observed at much higher NE concentrations than that needed to elicit maximal smooth muscle contraction, thus supporting the hypothesis of a dissociation between receptor occupation and PI pathway activation.

In summary, our results suggest the existence of a marked alteration in the PI cycle in the cardiovascular tissues of DOCA-salt HT rats that seems to exist beyond the $\alpha_\text{adrenergic}$ receptor site. Although it is difficult at this point to link these changes to the development of DOCA-salt–induced hypertension, it is nevertheless possible to postulate that these alterations could potentiate the sympathetic tone on the heart and vessels, thus contributing to the development of an increased vascular reactivity as well as to the end-organ damage associated with this form of experimental hypertension.

Acknowledgments

The authors are grateful for the expert technical assistance of Mrs. Jo-Anne Le Guerrier, Mrs. Diane Papin, and Mrs. Lise Farley; for the editorial assistance of Mrs. Solange Lecerc; and for the statistical assistance of Mr. Bernard de Champlain.

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Increased inositol monophosphate production in cardiovascular tissues of DOCA-salt hypertensive rats.
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Hypertension. 1988;12:122-128
doi: 10.1161/01.HYP.12.2.122
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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