Role of endothelium-derived relaxing factors in the renal response to vasoactive agents in hypothyroid rats

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Moreno, Juan Manuel, Rosemary Wangensteen, Juan Sainz, Isabel Rodríguez-Gomez, Virginia Chamorro, Antonio Osuna, and Félix Vargas. Role of endothelium-derived relaxing factors in the renal response to vasoactive agents in hypothyroid rats. Am J Physiol Endocrinol Metab 285: E182–E188, 2003. First published March 25, 2003; 10.1152/ajpendo.00558.2002.—This study analyzed the role of nitric oxide (NO) and endothelium-derived hyperpolarizing factor (EDHF) in the abnormal renal vascular reactivity of hypothyroid rats. Renal responses to vasoconstrictors [VC: phenylephrine (PHE) and ANG II] and vasodilators [VD: ACh, sodium nitroprusside (SNP), and papaverine (PV)] were studied in kidneys from control and hypothyroid rats under normal conditions and after NO or EDHF blockade. NO was blocked by the administration of Nω-nitro-l-arginine methyl ester (L-NAME) and EDHF by the administration of tetraethylammonium (TEA) or by an increased extracellular K⁺. The response to VC was also evaluated after endothelium removal. Hypothyroid kidneys showed reduced responsiveness to PHE and a normal response to ANG II. L-NAME and TEA administration produced an increased sensitivity to PHE and to ANG II in control preparations. L-NAME also increased the response to PHE in hypothyroid kidneys, but the differences between control and hypothyroid kidneys were maintained. TEA administration did not change the response to either VC in hypothyroid preparations. In endothelium-removed preparations, TEA was unable to increase pressor responsiveness to VC. Hypothyroid kidneys showed reduced responsiveness to ACh and SNP and normal response to PV. The differences between hypothyroid and control preparations in the responses to ACh and SNP were maintained after l-NAME or increased K⁺. In conclusion, this study shows that 1) the attenuated response to PHE in hypothyroidism is not related to an increased production of endothelium-derived relaxing factors NO and EDHF; 2) the response to VC in hypothyroid preparations is insensitive to EDHF blockade; and 3) hypothyroid preparations have a reduced reactivity to the NO donor, and NO-independent vasodilatation remains unaffected.

hypothyroidism; nitric oxide; endothelium-derived hyperpolarizing factor; vasoconstriction; vasodilatation

HYPOTHYROIDISM IS ACCOMPANIED by marked cardiovascular manifestations. Thus hypothyroidism is characterized by arterial hypotension with reduced cardiac output and increased vascular resistance (14, 15). The hypothyroid state produces important changes in the responsiveness to vasoconstrictors (VC) and vasodilators (VD) in conductance and resistance vessels (11, 22, 25). In addition, hypothyroidism prevents and reverses some models of experimental hypertension (2, 28).

It is well known that the endothelium can modulate vascular smooth muscle tone via the synthesis and release of several endothelium-derived relaxing factors (9). Nitric oxide (NO) and the endothelium-derived hyperpolarizing factor (EDHF) are the main mediators of endothelium-dependent renal vasodilatation (30); the former seems to play a major role in large conducting arteries, and EDHF appears to be of primary importance in resistance vessels (5, 10). NO synthesis is inhibited by the analog Nω-nitro-l-arginine methyl ester (l-NAME) (13). EDHF is an unidentified diffusible substance that relaxes vascular smooth muscle through hyperpolarization via an opening of K⁺ channels (3, 24). Tetraethylammonium (TEA) and high potassium concentrations have been used to inhibit EDHF activity (1).

The endothelium has been postulated to serve as a pressure and flow sensor; both pressure and flow trigger the release of endothelium-derived relaxing factors (20). In this respect, endothelial denudation augmented the responsiveness to VC in the isolated kidney (8, 29), and increased responsiveness was also produced by the administration of l-NAME or TEA (29), indicating that NO and EDHF modulate the response to VC in the isolated perfused kidney.

Preparations from hypothyroid rats show an attenuated responsiveness to VC and especially to the α₁-adrenergic agonist PHE (11, 22). It was recently suggested that enhanced NO production by the endothelium plays a role in the hyporesponsiveness to PHE in hypothyroid rats (11). In contrast, our group found no biochemical (18) or functional (25) evidence of such increased NO activity in hypothyroidism. Moreover, hypothyroidism is able to prevent the hypertension induced by a high dose of an NO inhibitor (26), in which an increased pressor responsiveness to VC plays an essential role (27), suggesting that hypothyroidism...
may reduce the responsiveness to VC even in the absence of NO. These disparate findings raise doubts regarding the participation of endothelial mediators in the abnormal responsiveness to vasoactive agents in hypothyroidism.

Functional abnormalities of endothelium-derived relaxing factors play an important role in cardiovascular and endocrine diseases (5, 9). Accordingly, we analyzed the hypothesis that NO and/or EDHF may participate in the abnormal responsiveness to VC and VD in the renal vascular bed of hypothyroid rats. To explore the contribution of these mediators, we used TEA or high potassium concentrations to inhibit EDHF activity, and l-NAME to inhibit NO synthesis.

MATERIAL AND METHODS

Animals

This investigation conformed to the Guide for the Care and Use of Laboratory Animals, published by the US National Institutes of Health (NIH Publication no. 85–23, revised 1985). Male Wistar rats initially weighing 180–200 g were maintained on standard chow and tap water ad libitum except where stated. The animals were divided into two groups: control and hypothyroid rats (n = 28 each group). Hypothyroidism was induced by the continuous administration of 0.03% methimazole (MTZ) via drinking water. The treatment was administered for 6 wk. The effectiveness of the treatment was assessed by comparing the serum thyroxine (T4), serum triiodothyronine (T3), mean arterial pressure (MAP), heart rate (HR), pulse pressure (PP), and the renal, ventricular, and body weights of control vs. treated rats. Blood pressure (BP) and HR were directly recorded in conscious rats (n = 7/group) through a polyethylene catheter inserted into the femoral artery and exteriorized at the dorsum of the neck by use of a TRA-121 transducer connected to a two-channel Letigraph 2000 recorder (Letica, Barcelona, Spain). BP was measured 24 h after catheter implantation, and blood samples from the arterial catheter were then taken for the determination of serum T3 and T4 levels by ELISA (Immunoassay System; Baxter, Miami, FL).

Experimental Protocols

The animals were anesthetized with pentobarbital sodium (40 mg/kg ip). The kidney was removed from the animal and perfused at a constant flow rate (5 ml·g kidney wt−1·min−1) with Tyrode solution at 37°C, as previously reported (29, 30). The kidney was placed in a chamber, maintained at 37°C, containing the perfusate lost from the renal vein that was not recirculated. Immediately after the kidney removal, the rats were killed with an overdose of pentobarbital sodium.

Experiment 1. This experiment was designed to study the effects of NO or EDHF blockade by the administration of l-NAME or TEA, respectively, on the renal response to vasconstrictors in kidneys from control and hypothyroid rats. Dose-response curves were made to PHE and angiotensin II (ANG II), in that order. These dose-response curves were performed under basal conditions or after the infusion of l-NAME (10−4 M) or TEA (3 × 10−3 M). l-NAME or TEA was infused during the 30 min of stabilization and during the dose-response curves.

Experiment 2. A second experiment was carried out to determine whether the TEA-induced increase in the pressor responsiveness to VC is due to EDHF blockade or to a direct effect on vascular smooth muscle cells. Dose-response curves to PHE and ANG II were studied in preparations with the endothelium removed in the presence and absence of TEA (3 × 10−3 M). The endothelium was removed by passing air through the isolated kidney for 4.5 min. Endothelium removal was assessed by measuring the vasodilator response to a bolus dose of 10−6 g/kg acetylcholine (ACh) in the vascular bed preconstricted with PHE (10−6 M). Preparations with a vasodilator response to ACh >10% were rejected.

Experiment 3. A third experiment analyzed the effects of NO and EDHF blockade on the response to vasodilators in normal and hypothyroid kidneys. In PHE-preconstricted kidneys, ACh-induced renal vasodilation is produced by NO and EDHF, whereas in 80 mM K+ preconstricted kidneys, EDHF is blocked, and ACh-induced renal vasodilation is mediated solely by NO (30). In this experiment, NO was blocked by the administration of l-NAME (10−4 M) and EDHF by an increased extracellular potassium (80 mM). The renal responses to the following were studied: the endothelium-dependent vasodilator ACh, the endothelium-independent vasodilator donor of NO (14) sodium nitroprusside (SNP), and the endothelium-independent vasodilator papaverine (PV). These dose-response curves were performed in perfused kidneys preconstricted with PHE (10−6 M) or with PHE plus l-NAME (10−4 M) or in kidneys preconstricted with 80 mM KCl. To obtain the solution containing 80 mM potassium, equimolar concentrations of NaCl were replaced by KCl in the Tyrode solution. Because the preparations from hypothyroid rats showed a reduced responsiveness to PHE, it was necessary to give a threefold higher concentration of the vasooconstrictor to achieve a similar level of preconstriction (115 ± 2 mmHg) to that observed in control preparations (112 ± 2 mmHg). The changes in renal perfusion pressure (RPP) in response to vasodilators were expressed as percentages of the vasoconstriction obtained with PHE or KCl.

Drugs

The following drugs were used: pentobarbital sodium (Nembutal) purchased from Serva (Heidelberg, Germany), SNP from Merck (Darmstadt, Germany), and MTZ, acetylcholine chloride, phenylephrine hydrochloride, PV, ANG II, l-NAME, and tetraethylammonium chloride from Sigma (St. Louis, MO).

Statistical Analysis

Analysis of the nested design was carried out with groups and doses to compare dose-response and flow-pressure curves; the design had two fixed-effect factors (group and dose) and one random-effect factor (the kidney), with the latter factor nested in the group. When the different tests for factors and the group-dose interaction were significant, groups at different doses were compared. ED50 values were compared using the Wilcoxon test. The results for each biological variable were compared with one-way ANOVA. When ANOVA was significant, post hoc comparisons were made using Tukey’s method.

RESULTS

Biological Variables

The effects of MTZ administration on biological variables are presented in Table 1. Animals given MTZ for 6 wk gained significantly less weight than their age-matched controls during this period. MAP, PP, HR, renal weight, ventricular weight, and serum T3 and T4...
levels were decreased in hypothyroid rats. Thyroid weight was increased in hypothyroid rats. Therefore, rats administered MTZ for 6 wk developed the characteristic manifestations of hypothyroidism.

Response to Vasoconstrictors

Renal vasculature from hypothyroid rats showed markedly reduced responsiveness to PHE (Fig. 1, and Table 2). The concentration-response curve was characterized by a shift toward the right, with decreased responses to threshold and middle concentrations and a lower maximal response. However, renal vasculature from hypothyroid rats showed a dose-response curve to ANG II similar to that of controls.

L-NAME administration produced an increased sensitivity to PHE in control and hypothyroid preparations (Fig. 1Aa and Table 2). Dose-response curves were characterized by a greater responsiveness to threshold and middle concentrations, with a similar maximal response; consequently, the curves were shifted to the left in a nonparallel manner. The differences between control and hypothyroid kidneys were maintained after L-NAME administration. The effect of TEA administration on the dose-response curve to PHE was similar to that of L-NAME in control rats but was unable to shift this dose-response curve to the left in the hypothyroid preparations (Fig. 1Ba and Table 2). Therefore, greater differences between control and hypothyroid preparations were observed after TEA administration than under basal conditions at the low and intermediate doses of PHE.

L-NAME produced a parallel shift to the left in the dose-response curve to ANG II in control preparations, whereas this dose-response curve was almost superimposed in hypothyroid kidneys after L-NAME administration (Fig. 1Ab and Table 2). TEA administration also shifted the dose-response curve to ANG II to the left in controls but was unable to modify the dose-

Table 1. Biological variables and T₄ and T₃ serum levels in control and hypothyroid rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>BW, g</th>
<th>VW, mg</th>
<th>RW, mg</th>
<th>TW, mg</th>
<th>MAP, mmHg</th>
<th>HR, beats/min</th>
<th>PP, mmHg</th>
<th>T₄, μg/dl</th>
<th>T₃, μg/dl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>402 ± 4</td>
<td>940 ± 27</td>
<td>1118 ± 40</td>
<td>40.2 ± 3</td>
<td>115 ± 2</td>
<td>380 ± 7</td>
<td>45 ± 2</td>
<td>4.5 ± 0.5</td>
<td>55 ± 6</td>
</tr>
<tr>
<td>Hypothyroid</td>
<td>255 ± 4*</td>
<td>571 ± 17*</td>
<td>830 ± 28*</td>
<td>125 ± 7.3*</td>
<td>102 ± 3*</td>
<td>315 ± 7*</td>
<td>30 ± 2*</td>
<td>0.3 ± 0.1*</td>
<td>5.9 ± 1.2*</td>
</tr>
</tbody>
</table>

*Values are means ± SE; n = 7/group. BW, body weight; VW, ventricular weight; RW, renal weight; TW, thyroid weight; MAP, mean arterial pressure; HR, heart rate; PP, pulse pressure; T₄, thyroxine; T₃, triiodothyronine. Rats were made hypothyroid by treatment with 0.03% methimazole in drinking water. *P < 0.01 compared with control group.

Fig. 1. Dose-response curves to phenylephrine (PHE, a) and angiotensin II (ANG II, b) in isolated kidneys from control and hypothyroid rats (n = 7/group) under normal conditions and after administration of N⁶-nitro-L-arginine methyl ester (10⁻⁴ M L-NAME; A) or tetraethylammonium (3 × 10⁻³ M TEA; B). RPP, renal perfusion pressure. Data are means ± SE. *P < 0.05 compared with control group; †P < 0.05 compared with its respective untreated group.
response curve to ANG II in hypothyroid kidneys (Fig. 1Bb and Table 2).

Removal of endothelium produced an increased sensitivity to VC in both groups, with the differences between them remaining (Fig. 2). TEA was unable to increase the sensitivity to VC in the endothelium-removed preparations (Fig. 2 and Table 3).

Response to Vasodilators

In isolated perfused kidneys from control and hypothyroid rats preconstricted with PHE, the bolus administration of ACh, SNP, and PV produced a dose-related decrease in RPP (Fig. 3A). Kidneys from hypothyroid rats showed a marked decrease in the responsiveness to ACh, with significant differences in the dose-response curve as well as in maximal vasodilatation. The responsiveness to SNP was significantly attenuated in hypothyroid kidneys, whereas the dose-response curve to PV was similar in control and hypothyroid preparations.

L-NAME administration attenuated the dose-response curve to ACh and increased the responsiveness to SNP in both groups (Fig. 3A). The differences between hypothyroid and control preparations in the dose-response curves to ACh and SNP were maintained after L-NAME administration. L-NAME did not affect the dose-response curve to PV in control or hypothyroid kidneys.

When the preparation was perfused with the high potassium concentration (80 mM), the dose-response curve to ACh was attenuated in both groups, with similar results to those obtained after L-NAME administration (Fig. 3B). The dose-response curve to SNP was unaffected in control and hypothyroid rats. The dose-response curve to PV was not significantly modified by the increased K+ concentration in control or hypothyroid kidneys (Fig. 3B).

DISCUSSION

Rats given MTZ for 6 wk developed manifestations characteristic of a hypothyroid state, and these were accompanied by abnormal vascular reactivity to VC and VD in the isolated kidney. The dose-response curve to PHE in isolated kidneys from hypothyroid rats was decreased relative to the controls. However, a normal

Table 2. ED50 and maximal response in endothelium-intact preparations

<table>
<thead>
<tr>
<th>Groups</th>
<th>ED50, −log g</th>
<th>Maximal Response</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenylephrine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>6.09 ± 0.03</td>
<td>215 ± 8.1</td>
</tr>
<tr>
<td>Control + L-NAME</td>
<td>6.52 ± 0.04</td>
<td>203 ± 5.5</td>
</tr>
<tr>
<td>Control + TEA</td>
<td>6.60 ± 0.07</td>
<td>205 ± 8.3</td>
</tr>
<tr>
<td>Hypothyroid</td>
<td>5.92 ± 0.04</td>
<td>156 ± 4.4</td>
</tr>
<tr>
<td>Hypothyroid + L-NAME</td>
<td>6.18 ± 0.05</td>
<td>167 ± 6.1</td>
</tr>
<tr>
<td>Hypothyroid + TEA</td>
<td>6.04 ± 0.04</td>
<td>162 ± 9.7</td>
</tr>
<tr>
<td>Angiotensin II</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>7.69 ± 0.07</td>
<td>105 ± 7.6</td>
</tr>
<tr>
<td>Control + L-NAME</td>
<td>8.42 ± 0.02</td>
<td>148 ± 6.5</td>
</tr>
<tr>
<td>Control + TEA</td>
<td>8.39 ± 0.05</td>
<td>136 ± 7.5</td>
</tr>
<tr>
<td>Hypothyroid</td>
<td>8.04 ± 0.11</td>
<td>117 ± 7.9</td>
</tr>
<tr>
<td>Hypothyroid + L-NAME</td>
<td>8.15 ± 0.07</td>
<td>103 ± 4.6</td>
</tr>
<tr>
<td>Hypothyroid + TEA</td>
<td>8.25 ± 0.05</td>
<td>88 ± 7.2</td>
</tr>
</tbody>
</table>

Data (−log g) are expressed as means ± SE. Concentration of phenylephrine and angiotensin II causing ED50 of maximal response and maximal response in isolated perfused kidneys from control and hypothyroid rats. *P < 0.05 compared with respective control group.

Table 3. ED50 and maximal response in endothelium-removed preparations

<table>
<thead>
<tr>
<th>Groups</th>
<th>ED50, −log g</th>
<th>Maximal Response</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenylephrine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>6.62 ± 0.03</td>
<td>185 ± 8.5</td>
</tr>
<tr>
<td>Control + TEA</td>
<td>6.60 ± 0.04</td>
<td>180 ± 12.1</td>
</tr>
<tr>
<td>Hypothyroid</td>
<td>6.49 ± 0.04</td>
<td>157 ± 10.9</td>
</tr>
<tr>
<td>Hypothyroid + TEA</td>
<td>6.20 ± 0.05*</td>
<td>152 ± 11</td>
</tr>
<tr>
<td>Angiotensin II</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>8.01 ± 0.1</td>
<td>134 ± 20.9</td>
</tr>
<tr>
<td>Control + TEA</td>
<td>8.40 ± 0.12</td>
<td>136 ± 13.2</td>
</tr>
<tr>
<td>Hypothyroid</td>
<td>8.02 ± 0.06</td>
<td>126 ± 16.4</td>
</tr>
<tr>
<td>Hypothyroid + TEA</td>
<td>8.12 ± 0.07</td>
<td>116 ± 17.7</td>
</tr>
</tbody>
</table>

Data (−log g) are expressed as means ± SE. Concentration of phenylephrine and angiotensin causing ED50 of maximal response and maximal response in isolated perfused kidneys from control and hypothyroid rats. *P < 0.05 compared with respective control group.

Fig. 2. Dose-response curves to PHE (A) and ANG II (B) in isolated kidneys from control and hypothyroid rats (n = 7/group) after removal of endothelium and treated and nontreated with TEA (3 × 10−3 M). Data are means ± SE. *P < 0.05 compared with control group.
reactivity to ANG II was observed in the hypothyroid kidneys. The hypothyroid kidneys also showed an attenuated reactivity to ACh and to SNP with a normal responsiveness to PV. The aim of this study was to determine whether NO and EDHF are involved in these abnormalities in the renal vascular reactivity of hypothyroid rats.

Vascular responsiveness to PHE and other \(\alpha\)-adrenoceptor agonists was found to be decreased in several preparations from hypothyroid animals (4, 11, 19, 22). The inhibitory effect of hypothyroidism on PHE-induced contraction was restored by T\(_4\) replacement therapy (11, 15), suggesting that the actions of antithyroid drugs are dependent on the induction of the hypothyroid state and are not due to a direct action of the drug or to other unrelated factors.

Endothelial denudation augmented the responsiveness to VC in the isolated kidney (8, 29), and increased responsiveness was also produced by the administration of l-NAME or TEA (29), indicating that NO and EDHF modulate the response to VC in the isolated perfused kidney. After NO blockade, the pressor responsiveness to PHE was increased in control and hypothyroid kidneys in the present study, but the differences between the groups were maintained after l-NAME administration. Therefore, these results suggest that the hyporesponsiveness to PHE observed after oral MTZ treatment is not due to enhanced NO production by the endothelium, at least in the renal vascular bed. This observation is supported by the ability of MTZ to attenuate the pressor response to acute NO blockade and to prevent L-NAME hypertension (26), indicating that hypothyroidism attenuates the pressor responsiveness to VC via an NO-independent way.

Although the correlation between this disease state and \(\alpha_1\)-adrenoceptor density has not been well established, an important but poorly understood association between the adrenergic nervous system and thyroid dysfunctions is evident in many instances (15). Therefore, the decreased sensitivity to PHE exhibited by isolated kidneys from MTZ-treated animals may be explained by a reduction in the number of \(\alpha_1\)-adrenoceptors present in the vascular smooth muscle or by a change in their coupling efficiency. However, although it has been established that hypothyroidism may modulate the number of \(\alpha\)-adrenoceptors present in cardiac (31) and adipose tissue (7), there is currently no direct evidence to suggest that this condition has a similar effect on vascular \(\alpha_1\)-adrenoceptor density. Moreover, a defective response of the vascular smooth muscle also occurred as a consequence of chronic thyroid deficiency, as indicated by the reduced capacity of barium chloride
to adequately stimulate isolated kidneys obtained from MTZ-treated animals (22).

The renal vascular response to ANG II was normal in the kidneys from hypothyroid rats but was unaffected by the blockers of endothelial relaxing factors or by endothelium removal. It is well known that ANG II stimulates NO production in many preparations (17). The enhanced dose-response curve to ANG II produced by the NO inhibitor in control preparations (see Fig. 1) indicates that NO counteracts the pressor effect of ANG II in the isolated perfused rat kidney. The inability of L-NAME to modify the ANG II dose-response curve in hypothyroid preparations indicates a reduced ANG II-induced NO release in the hypothyroid kidney. This observation is in consonance with the reduced responsiveness of hypothyroid kidneys to ACh (see Fig. 3), an endothelium-dependent vasodilator that triggers the release of NO (9). Moreover, the unexpected normal response to ANG II in the hypothyroid kidneys might also result from a reduced response to NO, as indicated by the attenuated response to the NO donor (12) SNP, which would not counterregulate the pressor response to ANG II. However, additional experiments are required to determine the cause of this abnormal ANG II-NO interaction in the vasculature of the hypothyroid kidney.

Vascular reactivity to VC is modulated by K+ channels. Thus openers of K+ channels inhibit leukotrienes C4 and D4 or ANG II-induced vasoconstriction (12, 16), and TEA induces a dose-dependent increase in the vascular sensitivity to norepinephrine and ANG II (21). However, it has been postulated that, under normal physiological conditions, the probability of K+ channels being in an open state is very low, because the intracellular concentration of ATP will be buffered by creatine phosphate at a level in excess of that required to close the channels (6). The fact that the administration of TEA caused an increased responsiveness to VC in our system indicates that some of the K+ channels were in an open state through the release of EDHF, because TEA was unable to modify the reactivity to VC in endothelium-removed preparations. These observations confirm previous findings by our group (29) indicating that EDHF present in the vasculature is involved as a modulator of the renal response to VC in the isolated perfused rat kidney.

The administration of TEA was unable to significantly modify the pressor response to PHE or ANG II in the kidneys from hypothyroid rats. This failure may be produced by a reduced production of EDHF in response to VC, or, alternatively, the hypothyroidism may determine a greater number of K+ channels in a closed state as a compensatory response to the defective contractile system in the vascular smooth muscle of hypothyroid rats (22). The present study is, to our knowledge, the first to investigate the actions of hypothyroidism on vascular K+ channels. Further studies are required to determine the mechanism responsible for the observed effects.

The kidneys from hypothyroid rats showed a reduced vasodilator response to ACh and to SNP, consistent with previous observations by our group (25), but the response to PV was not affected, in line with findings by Takiguchi et al. (23) in the mesenteric vasculature. Moreover, the blockade of NO or EDHF by the administration of L-NAME or increased extracellular potassium, respectively, produced an adequate inhibition of ACh-induced vasodilation. All of the results above indicate that, in hypothyroid preparations, the two endothelial components of ACh-induced vasodilation in the renal vascular bed, NO and EDHF (30), are present but have a reduced reactivity or lower production. The reduced reactivity to the NO donor is consistent with this observation. However, the NO-independent vasodilation was unaffected in the hypothyroid kidney. In addition, the recently reported reduced vascular activity of NO synthase in the aortic tissue from hypothyroid rats (18) might also contribute to the attenuated response to ACh in hypothyroid preparations, reducing the total amount of endothelial NO produced.

In conclusion, the results of the present study indicate that the attenuated renal pressor responsiveness to PHE in the hypothyroid state is not related to an increased activity of endothelium-derived relaxing factors NO or EDHF. Evidence was also provided of abnormalities in EDHF release or K+ channels in hypothyroid preparations, given that the dose-response curve to VC was not increased by TEA administration. Our results also indicate that hypothyroid preparations have a reduced reactivity to the NO donor SNP and that the NO-independent vasodilation is unaffected.

**Perspectives**

An impaired release of endothelial mediators of vasodilation plays an important role in the genesis of the functional vascular abnormalities that appear in hypertension, atherosclerosis, or diabetes. The present study analyzed the role of endothelial relaxing factors in the abnormal vascular reactivity of hypothyroidism. The data reported herein provide evidence that the attenuated renal pressor responsiveness to PHE in the hypothyroid state is not secondary to an increased production or activity of the endothelium-derived relaxing factors NO or EDHF. This study also shows that the response to VC in hypothyroid preparations is insensitive to K+ channel blockade. This abnormality in K+ channels, together with the reduced reactivity to NO, may be compensatory responses to the defective contractile system in the vascular smooth muscle of hypothyroid rats. The present study is, to our knowledge, the first to evaluate the actions of hypothyroidism on vascular K+ channels and therefore opens new perspectives for the assessment of the cardiovascular abnormalities of thyroid disorders.

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