THE CHRONIC EFFECT OF NITRENDIPINE ON HEART AND REGIONAL BLOOD FLOW IN RENOVASCU LAR HYPTERTENSIVE RATS

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Nitrendipine, a new calcium entry blocker, was administered to renovascular hypertensive rats (2K1C Goldblatt) (RHR, n = 8) and sham operated control rats (ShC, n = 8) to evaluate the effects on left ventricular hypertrophy and regional blood flow using radioactive microspheres. Nine untreated RHR and 8 untreated ShC served as control groups. After 6 weeks treatment (20 mg/kg subcutaneously, every other day), blood pressure reduced significantly in both RHR and ShC associated with a reduction in total peripheral resistance. Significant reversal of left ventricular hypertrophy was noted in RHR (1278 ± 41 to 1024 ± 19 mg, p < 0.01), but not in ShC. There was a significant relationship between blood pressure and left ventricular mass in both untreated rats (r = 0.955, p < 0.001) and treated rats (r = 0.729, p < 0.005). Nitrendipine increased coronary blood flow in RHR (430 ± 30 to 566 ± 47 ml/m/100g, p < 0.05) as well as in ShC (375 ± 15 to 508 ± 29 ml/m/100g, p < 0.05), without increasing cardiac oxygen demand. Renal blood flow was unchanged, whereas cerebral blood flow was significantly increased in both RHR (128 ± 6 to 164 ± 13 ml/m/100g, p < 0.01) and ShC (124 ± 7 to 173 ± 5 ml/m/100g, p < 0.01). Thus, long treatment of nitrendipine effectively regressed cardiac hypertrophy toward normal. Nitrendipine reduced total peripheral resistance; however, the effects on regional blood flow were not uniform among various organs.

The hemodynamic pattern in most patients with chronic hypertension is characterized by an increase in total peripheral resistance. Recently, the calcium entry blockers have been shown to be the effective agents for treatment of hypertension and such a choice seems a logical one since they diminish arteriolar tone with subsequent reduction in systemic vascular resistance. Such action resembles that of a number of other drugs, old and new, which are defined as vasodilators. However, calcium entry blockers are unique in that they have potent direct effects on the myocardium and the coronary circulation. Furthermore, drugs that have been categorized as belonging to this class differ from each other both in potency and regarding the site of action. Their chemical structures are also different, and hence, when used clinically, different pharmacological and therapeutic potentials can be easily expected.

This study was designed to evaluate the consequences of chronic administration of nitrendipine, a new calcium entry blocker, on hypertrophied heart, coronary blood flow, cerebral blood flow and renal blood flow in renovascular hypertensive rats.

Key words:
- Calcium antagonist
- Renovascular hypertension
- Coronary flow
- Renal flow
- Cerebral flow

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MATERIALS AND METHODS

Animal Model and Blood Pressure Control:
Renovascular hypertension was produced in 17 male Sprague-Dawley rats aged 40–50 days, weighing 150–175g, by placing a silver clip (0.2 mm internal width) on the left renal artery under light ether anesthesia, leaving the contralateral kidney untouched (2K1C Goldblatt, RHR). Sixteen rats from the same batch subjected to a sham operation without application of clips served as controls (ShC). Following the development of hypertension, blood pressure was determined once a week by the tail cuff method. Only those rats whose blood pressure rose to \( \geq 160 \text{ mmHg} \) were considered hypertensive. At 4 weeks after the operation administration of nitrrendipine was started. Since nitrrendipine is insoluble in water, it was dissolved in peanut oil (20 mg/ml) and injected subcutaneously (20 mg/kg) every other day for 6 weeks. Preliminary experiment with another series of both RHR and ShC showed that blood pressure began to fall within 15 minutes and reached to its lowest level about 3 hours after the injection. The systolic blood pressure was still decreased by 40–50 mmHg in RHR and 15–20 mmHg in ShC 48 hours after the injection.

In actual experiments the treatment groups consisted of 8 RHR and 8 ShC. Nine other RHR and 8 other ShC were left untreated and received injections of peanut oil alone every other day. All rats were housed, handled and fed in exactly the same way. Their blood pressures were determined by the tail cuff method once a week during the treatment, 48 hours after the most recent injection. 24 hours after the last injection of nitrrendipine or peanut oil, hemodynamic studies were performed.

Hemodynamic determination:
Cardiac output and organ blood flow distribution were determined in conscious rats using left atrial injection of radioactive microspheres. The precise technique and validity of our microsphere method have been described in detail previously.\(^7,8\) In brief, a catheter was placed into the left atrium 4 to 5 days prior to the hemodynamic determination. Rats were anesthetized with pentobarbital sodium (30 mg/kg ip) and ventilated via an intratracheal tube with room air. After a left thoracotomy, a PE-10 tubing was placed into the left atrium through its appendage. The catheter was filled with heparin and tunneled subcutaneously so as to be exteriorized on the back of the neck. The chest was closed after lung expansion. One day before the studies, a PE-50 tubing was cannulated into the aorta through a right femoral artery for blood pressure determination and reference blood sample withdrawal. The tip of the catheter was placed below the level of renal arteries.

During these periods, the rats were trained to sit quiet on small boxes so that it was subsequently possible to obtain measurement in the unrestrained conscious rats. On the day of the measurement, rats were placed on their boxes and the hemodynamic measurements were performed 45–60 minutes later. At that time, both blood pressure and heart rate were stabilized.

The microspheres employed in this study had a diameter of 15 \( \mu \text{m} \) and were labelled with \(^{85}\text{Sr}\) or \(^{141}\text{Ce}\) (3M Company, Minnesota) and their order was randomized. Each was suspended in 70% glucose solution containing 0.05% of Tween 80 as an anti-aggregant agent. This solution was shown in preliminary experiments to have no significant effects on blood pressure or heart rate in conscious rats. The above solution containing the microsphere was dispersed mechanically and ultrasonically for 15 minutes prior to each injection; 0.03 ml of suspension was withdrawn in a PE-50 tubing and counted in a gamma-well counter (Packard Model 578). The tubing was then connected to the left atrial catheter and the microspheres were injected into the left atrium over a period of 10 seconds which was then followed by 0.2 ml saline. It was estimated that 60,000–65,000 microspheres were injected during each study. The reference blood samples were withdrawn from the aortic cannula beginning 10 seconds before the injection and continued for 70 seconds at a rate of 1.03 ml/min. This rate of withdrawal assured a microsphere number in the blood greater than 400\(^9\) so that the variability related to the random distribution of the particles was minimal. Blood pressure and heart rate were monitored before and after the injection of microspheres to ensure that the injection and blood withdrawal did not affect these parameters.

At the conclusion of the experiment, the rats were sacrificed with a large dose of sodium pentobarbital. The heart, the kidneys and the brain were removed immediately afterwards. In the heart, the atria and the free wall of the right ventricle were dissected away from the left.
ventricle and the septum. The left ventricle (LV) reported hereafter includes, therefore, the free wall of the LV plus the whole interventricular septum. These tissues were carefully cleaned, blotted dry and weighed on a precision balance.

Tissue samples were placed in plastic tubes and counted for 5 minutes along with the blood samples withdrawn from the femoral catheter in a gamma-well counter. Cardiac output and regional blood flow were computed after appropriate corrections for decay, backgound and overlapping. The total microsphere dose injected was estimated as the difference in counts in the tubing before and after the injection.

Values given are means ± standard error. The data were statistically analyzed by analysis variance. p < 0.05 was considered significant.

RESULT

Blood Pressure Control and Reversal of Cardiac Hypertrophy (Fig. 1, Table I):

Systolic blood pressures just before treatment were 216 ± 6.9 mmHg in untreated RHR (n = 9), 209 ± 7.4 mmHg in treated RHR (n = 8), 128 ± 5.1 mmHg in untreated ShC (n = 8) and 134 ± 3.6 mmHg in treated ShC (n = 8). The hypotensive response following nitrendipine was rapid and maintained in both RHR and ShC during the 6 weeks, treatment. However, elevated blood pressure of RHR was not fully normalized at this dose.

As expected, LV mass was significantly heavier in untreated RHR than in untreated normotensive rats, whether expressed in absolute terms or relative to body weight. A significant reduction in cardiac weight was observed in RHR after 6 weeks, treatment of nitrendipine. However, the regression of hypertrophy was incomplete, since the LV weight to body weight ratio in treated RHR was still significantly higher (p < 0.01) than that in untreated ShC.

There was a significant positive correlation between the level of systolic blood pressure and the degree of LV hypertrophy in the untreated group (r = 0.955, p < 0.001) as well as in the

| TABLE I | REVERSAL OF LEFT VENTRICULAR HYPERTROPHY BY NITRENIDIPINE ADMINISTRATION |
|----------|-----------------|-----------------|-----------------|-----------------|
|          | Untreated ShC   | Treated ShC     | Untreated RHR   | Treated RHR     |
| Sys. BP (mmHg) | 132 ± 3.1     | 112 ± 2.8*      | 209 ± 7.7**     | 159 ± 5.7**§    |
| Body wt (g)   | 401 ± 8.6      | 414 ± 9.5       | 373 ± 15.6      | 367 ± 15.8      |
| Ventricular wt | 930 ± 16       | 978 ± 40        | 1278 ± 41**     | 1024 ± 19§      |
| Absolute (mg) | 2.33 ± 0.06    | 2.37 ± 0.07     | 3.46 ± 0.15**   | 2.82 ± 0.09**§  |

Values are mean ± SE.
ShC = sham operated rats; RHR = renovascular hypertensive rats; Sys. BP = systolic blood pressure (tail cuff method) at the end of administration
Significantly different from untreated ShC: *p < 0.05, **p < 0.01
Significantly different from untreated RHR: §p < 0.01

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treated group \((r = 0.729, p < 0.005)\).

Systemic Hemodynamic Effects (Table II):
Total peripheral resistance (TPR) was significantly elevated in untreated RHR compared with untreated ShC. Prolonged control of blood pressure with nitrendipine was accompanied by a significant decrease in TPR in both RHR and ShC. Cardiac output (CO) was almost the same in untreated RHR and untreated ShC. CO was slightly increased in treated ShC, but increased significantly in treated RHR. Heart rate was similar between untreated ShC and untreated RHR and was not changed by the treatment of nitrendipind. The index of the external LV work was obtained as the product of the mean arterial pressure and CO (ml/m-mmHg x 10^-3). This index was significantly increased in untreated RHR compared to untreated ShC, whereas nitrendipine did not change the external LV work in either ShC or RHR.

Coronary Circulation (Table III):
Left ventricular blood flow (LVF) per unit mass at rest was similar in both untreated ShC and untreated RHR. Following 6 weeks, treatment with nitrendipine, LVF was increased significantly in both ShC and RHR. This was associated with a significant reduction in coronary vascular resistance (CVR) in LV both in treated ShC and treated RHR.

Renal Circulation (Table IV):
Renal blood flow (RBF) in ShC was assessed as an average flow in the right and left kidneys, whereas in RHR, right (unclipped) and left (clipped) RBF were calculated separately. Right RBF normalized by 100g tissue in untreated RHR was significantly decreased compared to the RBF in untreated ShC. This was associated with a significant increase in renal vascular resistance (RVR) in untreated RHR. As expected, in untreated RHR, absolute RBF in the
clipped kidney was markedly decreased compared to that in the contralateral kidney and RVR was significantly increased in the clipped side. However, RBF normalized by tissue weight was essentially similar in the right and left kidneys in untreated RHR. Nitrendipine did not change RBF in either ShC or RHR. RVR tended to decrease in treated RHR, but the difference did not attain statistical significance.

Cerebral Circulation (Table V):
Cerebral blood flow (CerBF) normalized by 100g of tissue was similar in untreated ShC and untreated RHR, whereas cerebral vascular resistance (CerVR) was significantly increased in untreated RHR compared to untreated ShC. Nitrendipine produced a significant increase in CerBF in both ShC and RHR. This was associated with a significant reduction in CerVR both in treated ShC and treated RHR.

**DISCUSSION**
Nitrendipine reduced blood pressure in both RHR and normotensive controls. Although there was a tendency for blood pressure to rise slightly in the 5th and 6th week of therapy in RHR as compared to levels measured in the first week, the hypotensive effect of nitrendipine was rapid and was maintained during the whole six weeks, treatment period.
LV weight was significantly heavier in the untreated hypertensive group than in normotensive rats. The development of ventricular hypertrophy in RHR was closely related to the degree of rise in blood pressure as previously demonstrated. Reversals of LV hypertrophy induced by hypertension have been reported in both experimental animal models and humans treated with various antihypertensive agents. We also have observed in another

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**TABLE IV EFFECTS OF NITRENIDPINE ON RENAL HEMODYNAMICS**

<table>
<thead>
<tr>
<th></th>
<th>Untreated ShC</th>
<th>Treated ShC</th>
<th>Untreated RHR</th>
<th>Treated RHR</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBF (ml/m)</td>
<td>14.9±1.50</td>
<td>12.1±0.77</td>
<td>13.9±1.50</td>
<td>14.4±1.57</td>
</tr>
<tr>
<td>(ml/m/100g)</td>
<td>939±38</td>
<td>748±56</td>
<td>637±68**</td>
<td>657±68**</td>
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<tr>
<td>%CO</td>
<td>10.7±0.60</td>
<td>7.7±0.60</td>
<td>10.5±1.21</td>
<td>9.1±1.00</td>
</tr>
<tr>
<td>RVR (mmHg/ml/m)</td>
<td>7.1±0.4</td>
<td>7.7±0.5</td>
<td>13.4±2.3*</td>
<td>10.1±1.5</td>
</tr>
</tbody>
</table>

Values are mean ± SE.
ShC = sham operated rats; RHR = renovascular hypertensive rats; Rt K = right kidney;
Lt K = left kidney; RBF = renal blood flow; %CO = RBF in percent of cardiac output;
RVR = renal vascular resistance
Significantly different from untreated ShC: *p < 0.05, **p < 0.01
Significantly different from Rt K in untreated RHR: §p < 0.01

**TABLE V EFFECTS OF NITRENIDPINE ON CEREBRAL HEMODYNAMICS**

<table>
<thead>
<tr>
<th></th>
<th>Untreated ShC</th>
<th>Treated ShC</th>
<th>Untreated RHR</th>
<th>Treated RHR</th>
</tr>
</thead>
<tbody>
<tr>
<td>CerBF (ml/m/100g)</td>
<td>124±7</td>
<td>173±5*</td>
<td>128±6</td>
<td>164±13§</td>
</tr>
<tr>
<td>%CO</td>
<td>0.94±0.07</td>
<td>1.10±0.04</td>
<td>1.07±0.09</td>
<td>1.10±0.07</td>
</tr>
<tr>
<td>CerVR (mmHg/ml/m)</td>
<td>80.7±4.5</td>
<td>52.6±3.0*</td>
<td>116.0±5.7*</td>
<td>77.1±7.2§</td>
</tr>
</tbody>
</table>

Values are mean ± SE.
ShC = sham operated rats; RHR = renovascular hypertensive rats; CerBF = cerebral blood flow; %CO = CerBF in percent of cardiac output; CerVR = cerebral vascular resistance
Significantly different from untreated ShC: *p < 0.01
Significantly different from untreated RHR: §p < 0.01
series of investigation that regression of LV hypertrophy in RHR could be produced by either surgical cure (nephrectomy) or medical treatment (captopril).\textsuperscript{15}

In the present study, although the elevated blood pressure was not fully normalized by nitrendipine, significant regression of cardiac hypertrophy was noted in treated RHR (−18.5%). These observations are similar to those reported by Kazda et al.\textsuperscript{16} As in the case of development of LV hypertrophy, reversal of cardiac hypertrophy was influenced predominantly by the pressure load in RHR.\textsuperscript{15} LV weight to body weight ratio in treated RHR was still significantly higher than that of untreated normal control. This lack of complete reversal of LV hypertrophy in treated RHR could be related to the incomplete normalization of the blood pressure levels. However, at least in part, an increased sympathetic activity might have exerted an influence on full regression of ventricular hypertrophy, since in our previous study the development of some reflex increase in sympathetic tone was suggested in both RHR and normotensive controls treated with nitrendipine.\textsuperscript{17,18} Although heart rate was not significantly altered by chronic drug treatment in this study, a negative chronotrophic effect of this agent might interfere with the reflex increase in heart rate.

During the development and regression of cardiac hypertrophy, histological and biochemical changes occur, although the heart does not respond similarly to all types of overload.\textsuperscript{19} There is a significant increase in myocardial collagen synthesis in renal hypertensive rats. When medical treatment prevented the development of myocardial hypertrophy, collagen concentration remained unchanged. However, when therapy was begun after hypertrophy had been established, reversal of hypertrophy was associated with an increase in collagen concentration, since collagen fibers cannot be removed so easily.\textsuperscript{20} This cardiovascular collagen as well as noncollagen protein synthesis may be modulated by beta-adrenergic receptors. Indeed, adrenergic factor is considered a potent factor in the regression of cardiac hypertrophy.\textsuperscript{21,22} On the contrary, the biochemical and molecular properties of left ventricular myosin were fully restored after complete reversal of cardiac hypertrophy.\textsuperscript{23}

Total blood flow to the hypertrophied ventricle was found to be increased progressively in proportion to the magnitude of the hypertrophy. However, LV flow expressed per gram of myocardial tissue was essentially similar between normotensive and hypertensive rats. Our finding to this effect is in accord with most of the already reported studies.\textsuperscript{24,25} A long treatment with nitrendipine resulted in marked increase in LV flow and this was associated with a significant decrease in CVR in both ShC and RHR. It is well established that the coronary blood flow is closely related to myocardial oxygen consumption. In this regard, in our observation, the arterial blood pressure was decreased without a significant change in heart rate and the external left ventricular work was not increased by nitrendipine. Thus, the observed increase in coronary flow was most likely due to a direct vasodilating effect of nitrendipine on the coronary blood vessels rather than an increase in oxygen demand.

This model of RHR allows evaluation of the flow or function of the kidney opposite the stenosis as it responds to systemic influences that result in elevated blood pressure. In this study, the vascular resistance of the unclipped kidney in RHR was greater than that of the normal kidney in ShC, as reported previously.\textsuperscript{26,27} Total RBF of the unclipped kidney in RHR was not different from that in the normotensive group. However, when corrected for kidney weight, the flow of the subject was significantly less in RHR than in ShC. These data are in agreement with the results in a same model obtained by an electromagnetic flow probe.\textsuperscript{27} The weight of the clipped kidney was much smaller than the contralateral kidney in RHR. Although in RHR total RBF was significantly decreased in the clipped kidney compared to the contralateral one, RBF normalized by tissue weight was essentially similar in both sides.

It is to be noted that, in another experiment, we found that an acute administration of nitrendipine (0.3 and 3.0 mg/kg) in RHR produced a significant reduction in RBF but long treatment with nitrendipine was not associated with any significant change in RBF in both ShC and RHR. After 6 weeks, treatment with nitrendipine, RVR tended to decrease in RHR, although the data did not attain full statistical significance. However, in spite of this small decrement in RVR, RBF was well maintained in both the clipped and the contralateral kidney in RHR. In this model of hypertension, many humoral and neurogenic factors such as adrenergic influences,
renin-angiotensin system and prostaglandin activity may play the same roles in determining the renal blood flow. Reflex sympathetic stimulation induced by the systemic reduction in arterial pressure and resulting in renal vasoconstriction seems to be the most likely explanation for the acute effect of nitrendipine. On the other hand, during long treatment, RBF might be maintained through some autoregulatory mechanism. Long-term treatment with nitrendipine in man did not change the renal hemodynamics.

The CerVR increase significantly more in untreated RHR than in untreated ShC. Yet, CerBF was very similar in the two groups. These data are in agreement with the investigations of cerebral circulation in SHR and RHR (bilateral renal artery constriction). The brain has little capacity to maintain its normal function in the absence of an adequate flow. Various humoral and neurogenic mechanisms are mobilized to maintain this flow. However, the intraluminal pressure itself appears to provide a direct stimulus for variation of arterial tone. Long treatment with nitrendipine significantly increased CerBF associated with decrease in CerVR, in spite of the reduction in arterial pressure. Although autoregulatory mechanisms may play a role as protective biological devices in response to reduction of blood pressure, the direct vasodilating effect of nitrendipine on cerebral vessels seems to be more important in these situations. The significance of increased CerBF on cerebral function was not well understood in this study.

In summary, nitrendipine effectively controlled blood pressure in 2K1C Goldblatt hypertensive rats throughout its 6 weeks of administration. Although blood pressure was not fully normalized, significant reversal of LV hypertrophy was noted in RHR. The antihypertensive effect of nitrendipine was associated with a significant decrease in TPR. However, the effects of nitrendipine on regional blood flow and VR was not uniform among various organs; nitrendipine produced a significant increase in blood flow in the heart and the brain but did not change the RBF in RHR.

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