Systemic inhibition of nitric oxide and prostaglandins in volume-induced natriuresis and hypertension

JAMES D. KRIER AND JUAN CARLOS ROMERO
Department of Physiology and Biophysics, Mayo Medical School, and Division of Hypertension, Mayo Clinic, Rochester, Minnesota 55905

Krier, James D., and Juan Carlos Romero. Systemic inhibition of nitric oxide and prostaglandins in volume-induced natriuresis and hypertension. Am. J. Physiol. 274 (Regulatory Integrative Comp. Physiol. 43): R175–R180, 1998.—Nitric oxide (NO) synthesis inhibition with N6-nitro-L-arginine methyl ester (L-NAME) (10 µg·kg−1·min−1 iv), cyclooxygenase inhibition with medofenamate (Medo; 5 mg/kg iv bolus), and combination of drugs (L-NAME + Medo) were used to investigate the roles of NO and prostaglandins (PG) in the hemodynamic and natriuretic responses to isotonic saline volume expansion (VE; 5% body wt over 60 min) in anesthetized dogs. Before VE, L-NAME (n = 6), Medo (n = 6), and L-NAME + Medo (n = 6) produced significant increments in mean arterial pressure (MAP) of 12 ± 2, 15 ± 3, and 17 ± 3 mmHg, respectively. VE did not change MAP in Medo-treated dogs, but produced a significant elevation in the control dogs (14 ± 6 mmHg), in L-NAME-treated dogs (17 ± 6 mmHg), and in dogs pretreated with L-NAME + Medo (12 ± 5 mmHg). VE alone induced marked natriuretic responses in the control (38 ± 9 to 562 ± 86 µmol/min), L-NAME (31 ± 9 to 664 ± 65 µmol/min), and Medo groups (41 ± 10 to 699 ± 51 µmol/min). However, this natriuretic response was attenuated in dogs pretreated with L-NAME + Medo (12 ± 4 to 185 ± 52 µmol/min). These results indicate that 1) blockade of both NO and PGs has significant diminishing effects on volume-induced natriuresis, 2) NO blockade alone impairs volume-induced natriuresis in a manner that requires further increases in MAP to restore the natriuresis, and 3) PG blockade alone does not curtail volume-induced natriuresis. N6-nitro-L-arginine methyl ester; pressure-natriuresis sensitivity

IT HAS BEEN SHOWN that nitric oxide (NO) plays an important role in the regulation of sodium excretion (1, 2, 18, 23, 26, 27), which is also influenced by the synthesis of prostaglandins (PGs) (22, 24, 28). However, the relative importance of each of these two vasodilators in different physiological conditions has not been well defined. Of particular interest is the observation that the partial inhibition of NO synthesis with N6-nitro-L-arginine methyl ester (L-NAME) at doses that are not large enough to alter mean arterial pressure produces a volume-dependent hypertension (17, 23, 29, 33). In these studies performed in normotensive rats and dogs pretreated with L-NAME, blood pressure was significantly increased during sodium overload, although these results were not confirmed by Fernández-Rivas et al. (9), who demonstrated that elevations in blood pressure produced by L-NAME were not affected by increased salt intake. Yamada et al. (33) for example, showed that chronic NO inhibition promotes salt-dependent hypertension at low doses of L-NAME. However, the hypertension induced by higher doses of L-NAME may be aggravated by salt overload but cannot be prevented by salt restriction. In addition, the blockade of PG synthesis does not appear to induce volume-dependent hypertension per se, but may enhance the salt sensitivity of NO inhibition (25). This assumption comes from the observation of Salazar et al. (25), who showed that the impaired sodium excretion induced by the intrarenal infusion of NO inhibitors during volume expansion (VE) was considerably potentiated by the concomitant blockade of PGs. The blockade of PG alone has been demonstrated to have no effects on decreased sodium reabsorption during VE (19).

The present study was undertaken to elucidate the relative role of the systemic synthesis of NO and PGs in protecting against the hypertensinogenic effect of VE. For this purpose, we performed a 5% extracellular VE (ECVE) at the rate that can induce a significant increase in mean arterial blood pressure (MAP) in the normal animal (31). The role played by NO and PGs in the antihypertensive mechanisms activated by VE was assessed by blocking their formation with L-NAME or medofenamate (Medo) or both of these substances given simultaneously. We also attempted to determine in this study the manner in which each of these inhibitors alters the rate of urinary sodium excretion in relation to the observed modification of MAP.

METHODS

Animal Preparation

Twenty-four dogs (body weight 16–23 kg) of either sex were anesthetized with pentobarbital sodium (30 mg/kg iv) and ventilated mechanically with a Harvard respirator at a tidal volume that was determined by referring to the nomogram of Kleinman and Radford (16). A femoral artery was cannulated to collect peripheral arterial blood samples for measurement of plasma sodium and inulin concentrations and hematocrit and for continuous measurement of MAP using a pressure transducer (Pd231D, Statham, Hato Rey, PR) connected to a chart recorder (2600, Gould, Cleveland, OH). A femoral vein was cannulated for the infusion of experimental drugs, a 2%-inulin solution at a rate of 1 ml/min, and for anesthesia supplementation as needed. The cephalic vein was cannulated for VE.

A flank incision exposed the left kidney. A noncannulating transonic flow probe (Transonic Systems, Ithaca, NY) was placed on the renal artery at its origin from the aorta for continuous measurement of renal blood flow (RBF). The ureter was cannulated with PE-200 tubing for urine collection. The dogs were allowed to stabilize for 1 h before beginning the experiments.

Experimental Groups

Figure 1 illustrates the general scheme of the protocol using the following groups.

0363-6119/98 $5.00 Copyright © 1998 The American Physiological Society
Control group. In the control group (n = 6), after two 15-min clearance periods, ECVE was induced by a 5% body weight isotonic saline infusion over 60 min. Three 20-min clearances were obtained during the expansion. After VE, saline was infused at the rate of urine output to maintain homeostasis, during which a final 20-min clearance period occurred.

L-NAME group. In the L-NAME group (n = 6), the protocol is the same as group 1, except that after the control period L-NAME (Sigma Chemical, St. Louis, MO) was infused intravenously (10 µg·kg⁻¹·min⁻¹) for the remainder of the experiment at an infusion rate of 1 ml/min. A 15-min clearance period was started 30 min after L-NAME initiation.

Medo group. In the Medo group (n = 6), the protocol is identical to group 2, except that instead of L-NAME a 5 mg/kg iv bolus injection of Medo (Sigma) was given.

L-NAME + Medo group. In the L-NAME + Medo group (n = 6), the protocol is the same as groups 2 and 3, except that both L-NAME (10 µg·kg⁻¹·min⁻¹ iv) and Medo (5 mg/kg iv bolus) were given simultaneously before VE.

Analytic Methods

Glomerular filtration rate (GFR) was calculated from the clearance of inulin using the Anthrone method (10) to analyze inulin concentrations. Sodium concentrations were measured by flame photometry (IL943, Instrumentation Laboratory, Lexington, MA).

Statistical Analysis

Values are expressed as means ± SE. Data within groups were evaluated with one-way analysis of variance with repeated measures and the Fisher test, and comparisons of data between groups were performed with two-way analysis of variance and the Fisher test. P < 0.05 was considered significant.

RESULTS

Effects of the Experimental Drugs on Baseline Measurements

Table 1 shows the effects of L-NAME, Medo, and L-NAME + Medo on MAP and renal function parameters recorded 30 min after the drug administration. L-NAME, Medo, and L-NAME + Medo produced comparable significant (P < 0.001) increments in MAP of 12 ± 2, 15 ± 3, and 17 ± 3 mmHg, respectively. These changes can also be seen in Fig. 2, which also shows that the MAP of the control group remained unaltered before VE. Table 1 also shows that the animals in the L-NAME and L-NAME + Medo groups experienced significant reductions in RBF of 20 and 28%, respectively. However, Medo did not change RBF. The administration of the drugs did not alter GFR, urine flow rate (UV), or urinary sodium excretion (UNaV), although it appears that PG blockade had diminishing effects, as demonstrated by decreased UV and UNaV in the Medo and L-NAME + Medo groups.

Effects of VE

Hematocrit was measured as an indicator of plasma volume changes during isotonic saline VE. In the control group, hematocrit was reduced by 20% after VE, from 45 ± 2% at baseline to 36 ± 2% after VE. Similar hematocrit changes were observed in the L-NAME, Medo, and L-NAME + Medo groups, which fell by 20, 14, and 21%, respectively.

Table 1. Hemodynamic and renal function parameters during NO and/ or PG blockade

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>L-NAME</th>
<th>Medo</th>
<th>L-NAME + Medo</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAP</td>
<td>114 ± 5</td>
<td>126 ± 8</td>
<td>111 ± 4</td>
<td>114 ± 5</td>
</tr>
<tr>
<td>Drug</td>
<td>115 ± 5</td>
<td>139 ± 8*</td>
<td>127 ± 3*</td>
<td>131 ± 5*</td>
</tr>
<tr>
<td>RBF</td>
<td>189 ± 5</td>
<td>234 ± 18</td>
<td>190 ± 30</td>
<td>172 ± 20</td>
</tr>
<tr>
<td>Drug</td>
<td>187 ± 6</td>
<td>188 ± 15*</td>
<td>176 ± 24</td>
<td>123 ± 12*</td>
</tr>
<tr>
<td>GFR</td>
<td>29.5 ± 3.4</td>
<td>35.3 ± 4.2</td>
<td>34.8 ± 4.3</td>
<td>30.3 ± 2.4</td>
</tr>
<tr>
<td>Drug</td>
<td>32.3 ± 4.8</td>
<td>37.3 ± 2.2</td>
<td>38.9 ± 7.0</td>
<td>24.6 ± 4.0</td>
</tr>
<tr>
<td>UV</td>
<td>0.13 ± 0.02</td>
<td>0.20 ± 0.07</td>
<td>0.32 ± 0.12</td>
<td>0.31 ± 0.14</td>
</tr>
<tr>
<td>Drug</td>
<td>0.14 ± 0.01</td>
<td>0.18 ± 0.04</td>
<td>0.13 ± 0.03</td>
<td>0.08 ± 0.03</td>
</tr>
<tr>
<td>UNaV</td>
<td>29.9 ± 8</td>
<td>35.5 ± 13</td>
<td>72.1 ± 29</td>
<td>62.4 ± 33</td>
</tr>
<tr>
<td>Drug</td>
<td>38.9 ± 9</td>
<td>31.3 ± 9</td>
<td>41.0 ± 10</td>
<td>12.3 ± 4</td>
</tr>
</tbody>
</table>

Values are means ± SE. L-NAME, nitro-L-arginine methyl ester; Medo, medofenamate; PG, prostaglandin; MAP, mean arterial pressure (mmHg); RBF, renal blood flow (ml/min); GFR, glomerular filtration rate (ml/min); UV, urine flow rate (ml/min); UNaV, urinary sodium excretion (µmol/min). *P < 0.05 vs. baseline.
The changes in MAP are illustrated in Fig. 2. In the control group, MAP increased significantly with VE, from 115 ± 5 to 129 ± 5 mmHg. There also was a significant elevation in MAP after VE in L-NAME-treated animals, from 139 ± 8 to 156 ± 9 mmHg. However, this expansion did not alter MAP in Meclo-treated dogs, whose MAP only changed from 126 ± 3 to 129 ± 7 mmHg.

RBF did not change significantly with VE in any group. RBF increased slightly in the control group (187 ± 6 to 194 ± 18 ml/min), but decreased in the L-NAME (188 ± 15 to 175 ± 11 ml/min), Meclo (from 176 ± 24 to 166 ± 20 ml/min), and L-NAME + Meclo groups (123 ± 12 to 108 ± 17 ml/min). However, VE produced significant elevations in GFR (Fig. 3) in the control group (from 32 ± 5 to 40 ± 3 ml/min) and the L-NAME group (from 37 ± 2 to 48 ± 2 ml/min). The observed change in GFR in the Meclo group (from 39 ± 9 to 43 ± 5 ml/min) was not statistically significant. However, this increase in GFR was blunted in the L-NAME + Meclo group (from 25 ± 4 to 27 ± 4 ml/min).

VE induced a marked natriuretic response in the control group (562 ± 86 µmol/min) that was significantly enhanced by the previous administration of L-NAME (664 ± 65 µmol/min) or Meclo (699 ± 51 µmol/min). However, the natriuretic response in animals treated with L-NAME + Meclo (185 ± 52 µmol/min) was significantly attenuated. The fractional sodium excretion changes (Fig. 4) are similar to the changes in absolute sodium excretion.

We have conventionally used the term "pressure-natriuresis sensitivity" to demonstrate the relationship that exists between sodium excretion and the changes in MAP (Fig. 5). It is derived by dividing the natriuresis, as expressed as micromoles per minute, during each clearance period by the observed changes in MAP. It can be seen that in the control group the increase in sodium excretion (from 38 ± 9 to 562 ± 86 µmol/min) is accompanied by a rise in MAP of 13 mmHg. This yields a pressure-natriuresis sensitivity of 40 meq/min of Na excreted/mmHg increase in MAP. Pressure-natriuresis is impaired in the L-NAME group before VE, evidenced by the increase in MAP from baseline (12 mmHg) without an increase in natriuresis. In addition, during VE a biphasic pressure-natriuresis sensitivity is demonstrated. In the early phase (C2 to C4) volume-induced natriuresis was impaired, as demonstrated by the further increase in MAP (16 mmHg) required for the natriuresis to go from 31 ± 9 µmol/min before VE to 260 ± 29 µmol/min (14 meq·min⁻¹·mmHg⁻¹). During the second phase (C4 to C6), natriuresis increased to 664 ± 65 µmol/min (a change of 404 µmol/min) while MAP increased 3.2 mmHg, yielding a pressure-natriuresis relationship of 126 meq·min⁻¹·mmHg⁻¹, which demonstrated that volume-induced natriuresis was not only restored but enhanced compared with the control group. In the Meclo group, pressure-induced natriuresis was attenuated before VE, that is, MAP increased 15 mmHg but UNaV decreased 31 µmol/min. However, volume-induced natriuresis was not affected, as shown by the significant facilitation of natriuresis. The increase in Na excretion (from 41 ± 10 to 699 ± 51...
animals treated with L-NAME significantly attenuated because the simultaneous administration of Meclo to natriuresis during the inhibition of NO synthesis, observed by the inhibition of PG synthesis alone. PGs, increasing MAP. In contrast, such an interference is not impaired in the sense that the normalization of the natriuretic response is achieved at the expense of impaired before VE. During VE, GFR significantly increased in the animals treated with L-NAME, VE-induced natriuresis is decreased in the L-NAME group. However, GFR did not significantly alter blood pressure in Meclo-treated dogs. These changes are puzzling because it could be interpreted that the inhibition of PGs renders a better antihypertensive protection against VE than in control animals, in which the inhibition of PGs produces a well-known decrease in the release of renin (4, 15) and thereby a fall in circulating levels of angiotensin II, may help prevent further increases in blood pressure during VE while facilitating the intrarenal transmission of hydrostatic pressure, which mediates natriuresis (21). It should be noted that Meclo did not improve the vascular response to VE, because at the end of the 60-min period of saline infusion the blood pressure of Meclo-treated dogs was similar to those recorded in the volume expanded control animals.

As mentioned above, VE in L-NAME-treated dogs produced a net increase in MAP that is comparable to that observed in control animals treated with VE alone. However, the absolute level of MAP in the L-NAME group was higher. This "volume-dependent hypertension" has also been demonstrated by other investigators (17, 23, 29, 33). The increased sensitivity to salt may not only depend on the reduction of the vasodilator effect of NO but on other vasopressor systems that are left unopposed. In fact, there are reports showing that NO inhibition is followed by a marked elevation of endothelin and plasma renin activity (PRA) (20, 30). However, these findings are controversial, because Johnson and Freeman (13, 14) reported that the inhibition of PG synthesis alone produces a well-known decrease in the release of renin (4, 15) and thereby a fall in circulating levels of angiotensin II, which mediates natriuresis (21). It should be noted that Meclo did not improve the vascular response to VE, because at the end of the 60-min period of saline infusion the blood pressure of Meclo-treated dogs was similar to those recorded in the volume expanded control animals.

As mentioned above, VE in L-NAME-treated dogs produced a net increase in MAP that is comparable to that observed in control animals treated with VE alone. However, the absolute level of MAP in the L-NAME group was higher. This "volume-dependent hypertension" has also been demonstrated by other investigators (17, 23, 29, 33). The increased sensitivity to salt may not only depend on the reduction of the vasodilator effect of NO but on other vasopressor systems that are left unopposed. In fact, there are reports showing that NO inhibition is followed by a marked elevation of endothelin and plasma renin activity (PRA) (20, 30). However, these findings are controversial, because Johnson and Freeman (13, 14) reported that the inhibition of PG synthesis alone produces a well-known decrease in the release of renin (4, 15) and thereby a fall in circulating levels of angiotensin II, which mediates natriuresis (21). It should be noted that Meclo did not improve the vascular response to VE, because at the end of the 60-min period of saline infusion the blood pressure of Meclo-treated dogs was similar to those recorded in the volume expanded control animals.

As mentioned above, VE in L-NAME-treated dogs produced a net increase in MAP that is comparable to that observed in control animals treated with VE alone. However, the absolute level of MAP in the L-NAME group was higher. This "volume-dependent hypertension" has also been demonstrated by other investigators (17, 23, 29, 33). The increased sensitivity to salt may not only depend on the reduction of the vasodilator effect of NO but on other vasopressor systems that are left unopposed. In fact, there are reports showing that NO inhibition is followed by a marked elevation of endothelin and plasma renin activity (PRA) (20, 30). However, these findings are controversial, because Johnson and Freeman (13, 14) reported that the inhibition of PG synthesis alone produces a well-known decrease in the release of renin (4, 15) and thereby a fall in circulating levels of angiotensin II, which mediates natriuresis (21). It should be noted that Meclo did not improve the vascular response to VE, because at the end of the 60-min period of saline infusion the blood pressure of Meclo-treated dogs was similar to those recorded in the volume expanded control animals.

DISCUSSION

The major observation made in this study is that there is a close interaction between PGs and NO in the regulation of the hemodynamic and natriuretic responses induced by ECVE. The results indicate that in the presence of L-NAME, VE-induced natriuresis is impaired in the sense that the normalization of the natriuretic response is achieved at the expense of increasing MAP. In contrast, such an interference is not observed by the inhibition of PG synthesis alone. PGs, however, may participate in compensating part of the natriuresis during the inhibition of NO synthesis, because the simultaneous administration of Meclo to animals treated with L-NAME significantly attenuated volume-induced natriuresis.

Changes in MAP

In our study the intravenous infusion of L-NAME was followed by an increase in MAP, which is an observation that has been made by others (18) using the same dose. Furthermore, it is also known that the increase in blood pressure produced by L-NAME is not only due to the withdrawal of NO but to the activity of vasopressor systems such as angiotensin II, sympathetic activity, endothelin, etc., whose actions are left unopposed (3, 7, 12, 20, 32).

Under normal conditions, the blockade of PG synthesis does not have major consequences on blood pressure (5, 8). However, it has been shown that in anesthetized animals PGs play a significant role in counterbalancing the stimulation of sympathetic activity (24). Hence, the administration of Meclo in these animals produces significant increments in MAP.

An interesting finding of our study was to see that VE produced further increases in blood pressure of 15–20 mmHg in L-NAME-treated dogs; whereas it did not alter blood pressure in Meclo-treated dogs. These changes are puzzling because it could be interpreted that the inhibition of PGs renders a better antihypertensive protection against VE than in control animals, in which blood pressure increased by 13 mmHg. From our results, we cannot offer a definite explanation for such an effect. However, it is conceivable that the inhibition of PG synthesis, which produces a well-known decrease in the release of renin (4, 15) and thereby a fall in circulating levels of angiotensin II, may help prevent further increases in blood pressure during VE while facilitating the intrarenal transmission of hydrostatic pressure, which mediates natriuresis (21). It should be noted that Meclo did not improve the vascular response to VE, because at the end of the 60-min period of saline infusion the blood pressure of Meclo-treated dogs was similar to those recorded in the volume expanded control animals.

As mentioned above, VE in L-NAME-treated dogs produced a net increase in MAP that is comparable to that observed in control animals treated with VE alone. However, the absolute level of MAP in the L-NAME group was higher. This "volume-dependent hypertension" has also been demonstrated by other investigators (17, 23, 29, 33). The increased sensitivity to salt may not only depend on the reduction of the vasodilator effect of NO but on other vasopressor systems that are left unopposed. In fact, there are reports showing that NO inhibition is followed by a marked elevation of endothelin and plasma renin activity (PRA) (20, 30). However, these findings are controversial, because Johnson and Freeman (13, 14) reported that the inhibition of PG synthesis alone produces a well-known decrease in the release of renin (4, 15) and thereby a fall in circulating levels of angiotensin II, which mediates natriuresis (21). It should be noted that Meclo did not improve the vascular response to VE, because at the end of the 60-min period of saline infusion the blood pressure of Meclo-treated dogs was similar to those recorded in the volume expanded control animals.
those reported by Salazar et al. (25). However, in that study, the inhibitors of NO or PG synthesis were infused into the kidney while MAP was maintained constant.

In our study, the changes in systemic pressure explain the observed changes in sodium excretion, which increased during VE in all groups, although the increase was significantly attenuated in the animals treated with L-NAME + Medo. In accordance with these results, it was necessary to block, simultaneously, PGs and NO to have a significant decreased response in sodium excretion during VE. This response was not only due to the failure to increase GFR but also to an increase in tubular reabsorption of sodium because fractional sodium excretion was reduced.

The analysis of the increase in MAP that was necessary to eliminate a given amount of sodium (pressure-natriuresis sensitivity) reveals some important characteristics about the specific role that NO and PGs play in regulating sodium excretion.

First, it should be noted from Fig. 5 that the increase in the basal levels of blood pressure that follows the administration of Medo alone or L-NAME alone or L-NAME + Medo was not accompanied by any significant change in urine sodium excretion. This is not surprising because the overall increase in blood pressure recorded in these groups was relatively small (13–15 mmHg), and in addition, each of these drugs (L-NAME or Medo) has been shown to produce a marked decrease in pressure-induced natriuresis (6, 11, 27). This indicates that the mild increase in sodium excretion that could have been induced by a moderate elevation of blood pressure was offset by the antinatriuretic effect of the drugs.

Under these conditions, it was found that, during a blockade of PG synthesis, VE produced a very significant increase in sodium excretion that did not "require" practically any elevation of blood pressure. This pressure-natriuresis sensitivity (233 µmol·min⁻¹·mmHg⁻¹) is much higher than that observed in control animals (42 µmol·min⁻¹·mmHg⁻¹). Although the sensitivity is quite different in both groups, the increase in natriuresis was steady at the onset of VE. These patterns of response to VE contrast with the biphasic response seen after inhibiting the synthesis of NO with L-NAME. Under these conditions, VE was followed by an elevation of blood pressure, which was accompanied by a sluggish natriuretic response (14 µmol·min⁻¹·mmHg⁻¹). However, after the blood pressure had increased further, the continuous VE triggered a response that resembled that seen during the activation of PGs. This is further evidenced by the response observed when Medo was given in addition to L-NAME. In this latter case, VE yielded a pressure-natriuresis sensitivity of 14 µmol·min⁻¹·mmHg⁻¹, equal to the response in the L-NAME group. However, in contrast to the L-NAME only-treated group, the natriuresis that occurred after the increase in blood pressure was completely abolished with the addition of PG inactivation.

In summary, there appears to be a close relationship between NO and PG in mediating the effects of VE. Inhibition of the cyclooxygenase system does not appear to impair the hemodynamic and excretory responses to VE. In contrast, blockade of NO synthesis impairs volume-induced natriuresis, because to be manifested, an increased blood pressure and subsequent stimulation of PGs are required. However, as long as PGs are available, renal function and volume homeostasis can be maintained. When both NO and PG production are curtailed, the ability to handle a sodium load is compromised.

**Perspectives**

It is well known that the renal synthesis of NO and PG is important for the regulation of sodium excretion, but the specific role played by each of these substances during the natriuresis induced by either an increase in blood pressure or after VE with sodium overload remains unknown. This study shows that volume-induced natriuresis is mainly mediated by NO, but is not affected by the inhibition of PG synthesis. The blockade of NO synthesis diminishes volume-induced natriuresis and produces salt sensitivity because blood pressure is markedly increased. Under these conditions, PG synthesis may be activated, thus restoring sodium balance back to normal through the activation of PG. This sequence of events is important when interpreting the pathophysiology of salt sensitivity in hypertensive patients.

Address for reprint requests: J. C. Romero, Mayo Clinic, Dept. of Physiology, 200 First St., NW, Rochester, MN 55905.

Received 12 May 1997; accepted in final form 7 October 1997.

**REFERENCES**


