Mechanism of Acute Hypercalcemic Hypertension in the Conscious Rat

TOMAS BERL, MOSHE LEVI, MARILYN ELLIS, AND CIDIO CHAIMOVITZ

SUMMARY Acute hypercalcemia in the conscious, unanesthetized rat, achieved by a 30-minute infusion of CaCl₂ (serum calcium level, 12.8 ± 0.6 mg/dl) resulted in significant elevation of mean arterial pressure (from 112 ± 2 mm Hg to 129 ± 3 mm Hg, \( p < 0.001 \)). This pressor response was associated with a significant increase in systemic vascular resistance, from 0.45 ± 0.02 mm Hg/(ml/min)/kg body weight to 0.50 ± 0.02 mm Hg/(ml/min)/kg body weight (\( p < 0.05 \)), but it caused no alteration in cardiac index. The pressor response to acute hypercalcemia does not appear to be mediated by vasopressor hormones or attenuated by vasodepressor hormones since inhibition of the renin-angiotensin system (nephrectomy), catecholamines (central and peripheral 6-hydroxydopamine), vasopressin (vascular antagonist), prostaglandins (indomethacin), and parathyroid hormone (parathyroidectomy) did not significantly alter the pressor response to infusion of CaCl₂ in spite of similar serum calcium levels in all groups of animals. Rather, the pressor response to acute hypercalcemia seems to be mediated by a direct action of calcium ion on smooth muscle and perhaps myocardial cell contractility, since pretreatment with the calcium channel blockers verapamil or nifedipine blocked the pressor response to acute hypercalcemia. (Hypertension 7: 923-930, 1985)

KEY WORDS • hypertension mechanism • calcium blockers • acute hypercalcemia

ACUTE hypercalcemia has been well known to cause elevation of arterial blood pressure in humans and experimental animals.¹⁻⁹ Since calcium ion in vitro has been shown to increase cardiac muscle contractility¹⁰ and vascular smooth muscle contraction,¹¹ it has been presumed that the in vivo hypertensive response is mediated by a combination of increased cardiac output and increased systemic vascular resistance (SVR). Subsequent in vivo studies, however, have established that, while in the very initial phase of acute hypercalcemia there may be an increase in cardiac output,¹²,¹³ eventually the hypertensive response is associated with an increase in SVR and normal cardiac output.¹⁻⁴,¹⁶⁻⁶ It has not been established whether the increase in SVR is a direct consequence of the vascular action of calcium¹⁴⁻¹⁷ or the enhancement of the vasoconstrictor action of hormones such as angiotensin II, catecholamines, or vasopressin. In this respect, recent studies have shown that calcium regulates the synthesis and release of these vasoconstrictive hormones,¹⁸⁻²⁷ and also enhances and mediates their vascular action.²⁸⁻³⁰ Although some studies have measured the serum concentration of some of these vasoconstrictive hormones during acute hypercalcemia,³ their definite role in the pressor response to acute hypercalcemia has not been established.

Alternatively, vasodilative hormones such as prostaglandins³¹ and parathyroid hormone³² could attenuate the pressor response to acute hypercalcemia. In this respect, recent studies have shown that calcium enhances prostaglandin synthesis.³³⁻³⁵ The purposes of the present study therefore were 1) to determine the cardiovascular mechanisms of the pressor response to acute hypercalcemia in the conscious rat and 2) to evaluate a possible enhancing and vasoconstrictive role for angiotensin II, catecholamines, and vasopressin; a possible attenuating and vasodilative role for prostaglandins and parathyroid hormone; and the role of cellular calcium uptake in the pressor response.

Materials and Methods

All studies were performed on male Sprague-Dawley rats (Charles River Breeding Laboratories, Inc., Wilmington, MA, USA) weighing between 225 and 275 g. All rats had free access to standard rat chow and water until the morning of the experiment.
Effect of Acute Hypercalcemia on Mean Arterial Pressure, Cardiac Output, and Systemic Vascular Resistance

Rats were lightly anesthetized with ether for placement of polyethylene catheters (PE-50, Clay Adams Div., Becton, Dickinson & Co., Parsippany, NJ, USA) into the jugular vein for infusions and the femoral artery for blood pressure monitoring and blood sampling. Cannulation of the left ventricle through the right carotid artery was accomplished with tapered PE-350 tubing. Ventricular cannulation was confirmed by pressure wave tracing. During operation isotonic saline equivalent to approximately 0.5% of the body weight was infused to replace estimated fluid losses. Animals were then allowed to recover fully from anesthesia for 60 minutes, while they were placed in individual restraining cages (Narco Biosystems, Houston, TX, USA). Mean arterial pressure (MAP) was monitored continuously throughout the experiment with a P23Db pressure transducer (Statham Instruments, Oxford, MA, USA) connected to a chart recorder (Gilson Medical Electronics, Middleton, WI, USA).

After a 60-minute equilibration period, hypercalcemia was induced in 10 rats by the infusion of 0.27 mmol of CaCl₂ in 1 ml over approximately 30 minutes delivered with a calibrated syringe pump (Model 351; Sage Instruments Div., Orion Research, Cambridge, MA, USA). The amount of CaCl₂ infused in our studies was lower than that employed to induce acute hypercalcemia in parathyroidectomized rats, because our animals were not parathyroidectomized. The infusion rate increased mean serum calcium concentration to 12.8 mg/dl, as measured in the arterial blood. Seven rats were given an equal volume of an isotonic NaCl solution over a 30-minute period. In addition, since the CaCl₂ infusion is hypertonic, five rats were infused with an equal volume of hypertonic NaCl (1000 mOsM/kg H₂O). Blood samples (0.5 ml) were drawn just before and at the end of the infusion period. The volume was replaced with equal volumes of 0.9% saline. The blood was analyzed for calcium (atomic absorption, Perkin-Elmer, Norwalk, CT, USA). At the end of the 30-minute infusion period, cardiac index (CI) and SVR were determined by a radioactive microsphere technique described by Hsu et al. and adapted for use in our laboratory. The microspheres were 8.8 ± 0.9 μm in diameter and were labeled with strontium-85 (3M Co., Minneapolis, MN, USA). Radioactivity was counted in a Biogamma Radiation counting system (Beckman Instruments, Fullerton, CA, USA). We have previously shown that these microspheres are not susceptible to arteriovenous shunting as renal venous blood collected for 2 minutes following microsphere injection revealed significant radioactivity.

Calculations were performed as follows: 

\[ CI (\text{mlliliters per minute per kilogram body weight}) = \frac{\text{counts per minute injected/femoral blood (counts per minute) \times femoral blood flow rate/body weight}}{\text{SVR (millimeters of mercury divided by milliliters per minute per kilogram of body weight)}} = \frac{\text{MAP}}{\text{CI}}. \]

Role of Vasconstrictive Hormones on the Pressor Response to Acute Hypercalcemia

To assess whether activation of the sympathetic nervous system is involved in enhancing the pressor response to acute hypercalcemia, studies were undertaken in six rats pretreated with 6-hydroxydopamine (6-OHDA), 200 mg/kg, given intravenously in two sequential doses 24 hours apart. This maneuver has been shown to essentially deplete peripheral catecholamines within 7 days after the second injection. Another group of six rats was treated in the same manner using the blank solution for 6-OHDA instead. On Day 8, both groups of animals were infused with CaCl₂, as described before and MAP was measured. Central catecholamines were depleted by treating five rats with 6-OHDA, 200 μg in 50 μl of saline given intravenicularly. Another group of four rats was treated in the same manner using the blank solution for 6-OHDA instead. The animals were allowed to recover, and the CaCl₂ was given 7 days later. In a third group of three rats, both peripheral and central catecholamines were depleted by the combination of intravenous and intraventricular 6-OHDA injection. Another group of three rats was treated in the same manner using the blank solution for 6-OHDA instead. On the eighth day the rats were given the CaCl₂ infusion.

A possible role for vasopressin in enhancing the pressor response to acute hypercalcemia was evaluated in rats pretreated with the selective vascular antagonist of vasopressin BL III 19, d(CH₂)₅DDAVP (kindly donated by Dr. M. Manning, Toledo, OH, USA), 5 mg/kg, given intravenously. This dose completely prevented the pressor response to 10 mM of exogenous vasopressin in every animal studied. The animals were then infused with CaCl₂ (n = 7) or NaCl (n = 5), and MAP was measured, as described above.

Since previous studies have revealed that calcium inhibits renin production but enhances angiotensin II-mediated vasoconstriction, a possible role for the renin-angiotensin system in enhancing the pressor response to acute hypercalcemia was evaluated in 11 rats who had undergone bilateral nephrectomy, thereby removing the source of renin. In these experiments, rats were lightly anesthetized with ether, a midline abdominal incision was made, both kidneys were identified, the renal artery and vein were ligated, both kidneys were removed, and the incision was then closed. The animals were then allowed to recover for 24 hours. On the following day the animals were infused with either CaCl₂ (n = 5) or NaCl (n = 6) and MAP was measured as described above.

Role of Vasodilative Hormones on the Pressor Response to Acute Hypercalcemia

A possible role for prostaglandins in attenuating the pressor response to acute hypercalcemia was evaluated in 10 rats pretreated with the cyclooxygenase inhibitor indomethacin. The drug was given intraperitoneally at a dose of 5 mg/kg. This dose has been shown to inhibit renal prostaglandin synthesis by 80% within 6 hours of administration. Within 6 hours of indomethacin...
administration, the rats were infused with either CaCl$_2$ ($n = 6$) or NaCl ($n = 4$) and MAP was measured.

A possible role for parathyroid hormone in attenuating or enhancing the pressor response to acute hypercalcemia was evaluated in four rats who were surgically parathyroidectomized. The adequacy of parathyroidectomy was established in each animal by demonstrating significant decreases in serum calcium levels, to below 7 mg/dl. Once this was shown, the animals’ serum calcium levels were maintained by supplementing the drinking water with CaCl$_2$, 1 g/dl. The short-term CaCl$_2$ infusion was performed 5 to 7 days after parathyroidectomy. To achieve a level of serum calcium comparable to that of nonparathyroidectomized rats, 0.36 mmol of CaCl$_2$ was infused in these animals. This was given also over a 30-minute period in a 1 ml volume. Since parathyroid hormone probably does not influence blood pressure primarily by volume mediated mechanism, the NaCl infusion was not performed in these animals.

**Effect of Calcium Channel Blockers on the Pressor Response to Acute Hypercalcemia**

To assess whether the cellular uptake of calcium was causative in the observed pressor response to CaCl$_2$ infusion, the effect of pretreatment with the calcium blocker verapamil was studied.$^{45-47}$ In these studies, animals received either verapamil, 50 μg/kg/min ($n = 8$), or a carrier solution ($n = 5$) intravenously for 30 minutes. This pretreatment time was employed because preliminary studies have shown that these agents cause a decrease in blood pressure over the first 30 minutes but remain stable thereafter. Then animals were infused with CaCl$_2$ for 30 minutes while the infusion of verapamil was continued. To further assess the mechanism whereby verapamil blocked the pressor effect of hypercalcemia (see Results), in seven normocalcemic and 13 acutely hypercalcemic rats, the effect of verapamil on CI and SVR was determined by the radioactive microsphere technique already described. The systemic hemodynamics were also measured in six acutely hypercalcemic rats receiving a dissimilar calcium channel blocker, nifedipine, 7.5 μg/kg/min.

**Statistics**

A two-tailed paired $t$ test was used to compare results in the same animal; the unpaired $t$ test was used to compare effects in two different groups of rats; and one-way analysis of variance with Scheffe’s method was used for multiple group comparisons.$^{48}$ A $p$ value less than 0.05 was considered significant. All data are expressed as means ± SEM.

**Results**

**Effect of Acute Hypercalcemia on Mean Arterial Pressure, Cardiac Output, and Systemic Vascular Resistance**

Intravenous infusion of CaCl$_2$, for 30 minutes resulted in a significant elevation of serum calcium concentration (from 9.1 ± 0.3 mg/dl to 12.8 ± 0.6 mg/dl; $p < 0.001$). This increment in serum calcium concentration was observed in all subsequent groups of rats given CaCl$_2$. As shown in Figure 1, in 10 rats this degree of hypercalcemia resulted in significant increase in MAP (from 112 ± 2 mm Hg to 129 ± 3 mm Hg; $p < 0.001$). A possible role for volume expansion in this pressor response was evaluated by treating seven animals with an equal volume of NaCl; the rise in MAP was less significant (from 111 ± 6 mm Hg to 115 ± 3 mm Hg; $p < 0.05$). In fact, the change in MAP in the hypercalcemic animals (17 ± 1 mm Hg) was markedly and significantly greater when compared with the change in MAP in the NaCl-treated normocalcemic animals (4 ± 1 mm Hg; $p < 0.001$). An increment in pressure was not observed in animals serving as time controls and not receiving any infusion, which demonstrates that the increment in pressure was not due to hemodynamic changes that could be associated with recovery from anesthesia. Likewise, the infusion of a hypertonic NaCl solution (1000 mOsM/kg) increased MAP by only 3 ± 1 mm Hg. As shown in Table 1, the pressor response to hypercalcemia was associated with a significant increase in SVR: the SVR in the hypercalcemic animals was 0.50 ± 0.02 mm Hg/(ml/min)/kg body weight, whereas it was 0.45 ± 0.02 mm Hg/(ml/min)/kg body weight in the NaCl-treated normocalcemic animals ($p < 0.05$). There was no significant difference in the CI: the CI in the hypercalcemic animals was 261 ± 7 ml/min/kg body weight, whereas in the NaCl-treated normocalcemic animals it was 258 ± 4 ml/min/kg body weight.

**Role of Vasoconstrictive Hormones on the Pressor Response to Acute Hypercalcemia**

The role of the central and peripheral catecholamines in the pressor response to acute hypercalcemia was evaluated in animals whose central, peripheral, or both central and peripheral catecholamine stores were chemically depleted by administration of 6-OHDA. As is shown in Table 2, the administration of CaCl$_2$ to
TABLE 1. Effect of NaCl, CaCl2, and Calcium Channel Blockers on Mean Arterial Pressure, Cardiac Index, and Systemic Vascular Resistance in the Conscious Rat

<table>
<thead>
<tr>
<th>Experimental setting</th>
<th>MAP (mm Hg)</th>
<th>CI (ml/min/kg BW)</th>
<th>SVR (mm Hg/ml/min)/kg BW</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaCl (n = 7)</td>
<td>115±3</td>
<td>258±4</td>
<td>0.45±0.02</td>
</tr>
<tr>
<td>CaCl2 (n = 10)</td>
<td>129±3*</td>
<td>261±7</td>
<td>0.50±0.02*</td>
</tr>
<tr>
<td>NaCl + verapamil (n = 7)</td>
<td>101±2†</td>
<td>286±6†</td>
<td>0.36±0.01†</td>
</tr>
<tr>
<td>CaCl2 + verapamil (n = 13)</td>
<td>99±1†</td>
<td>263±6†</td>
<td>0.38±0.01†</td>
</tr>
<tr>
<td>CaCl2 + nifedipine (n = 6)</td>
<td>102±3†</td>
<td>268±7†</td>
<td>0.38±0.01†</td>
</tr>
<tr>
<td>F</td>
<td>40.70</td>
<td>3.99</td>
<td>22.47</td>
</tr>
</tbody>
</table>

Values are means ± SEM. MAP = mean arterial pressure; CI = cardiac index; BW = body weight; SVR = systemic vascular resistance. *p < 0.001, †p < 0.01, ‡p < 0.05, compared with NaCl-treated rats; §p < 0.05, compared with verapamil-pretreated rats receiving NaCl.

animals whose central catecholamine stores were depleted increased MAP (from 119 ± 5 mm Hg to 138 ± 5 mm Hg; p < 0.001). This pressor response was similar to that seen with the administration of CaCl2 to vehicle-treated controls in which MAP increased (from 114 ± 5 mm Hg to 130 ± 6 mm Hg; p < 0.01). In fact, the resultant change in MAP in the 6-OHDA-treated and vehicle-treated animals (19 ± 1 mm Hg and 16 ± 3 mm Hg respectively) was not significantly different.

The systemic administration of 6-OHDA (Table 2) caused a decrement in MAP in all rats; however, the response to CaCl2 was unaltered. Specifically, the administration of CaCl2 to animals whose peripheral catecholamine stores alone were depleted increased MAP (from 102 ± 2 mm Hg to 119 ± 2 mm Hg; p < 0.001), to a similar extent as that seen with the administration of CaCl2 to vehicle-treated controls, in whom MAP increased (from 114 ± 5 mm Hg to 130 ± 6 mm Hg; p < 0.001). The resultant change in MAP in the 6-OHDA-treated and vehicle-treated animals (19 ± 1 mm Hg and 16 ± 3 mm Hg respectively) was not significantly different.

Finally, the administration of CaCl2 to animals with both central and peripheral catecholamine stores depleted increased MAP (from 100 ± 3 mm Hg to 113 ± 2 mm Hg; p < 0.02); again, this pressor response was similar to that observed with the administration of CaCl2 to vehicle-treated controls, whose MAP increased (from 117 ± 4 mm Hg to 134 ± 3 mm Hg; p < 0.01). The resultant increase in MAP in the 6-OHDA-treated and vehicle-treated animals (13 ± 2 mm Hg and 17 ± 2 mm Hg respectively) was not significantly different.

The role of vasopressin in the pressor response to acute hypercalcemia was evaluated in 11 animals pretreated with the vascular antagonist of vasopressin, d(CH2)5DDAVP, before CaCl2 was administered (Figure 2). The administration of CaCl2 to seven animals pretreated with the vascular antagonist of vasopressin resulted in a significant increase in MAP (from 114 ± 3 mm Hg to 132 ± 3 mm Hg; p < 0.001), whereas the administration of NaCl to similarly treated animals did not result in a significant change in MAP (from 119 ± 4 mm Hg to 117 ± 3 mm Hg). The increase in MAP caused by CaCl2 administration in these rats pretreated with the arginine vasopressin vascular antagonist was not different from that in animals not receiving the antagonist.

The role of the renin-angiotensin system was studied...
in 5 nephrectomized rats (Figure 3). The CaCl₂ infusion in these rats resulted in a significant rise in MAP (from 124 ± 7 mm Hg to 145 ± 6 mm Hg; p < 0.005). A possible role for volume expansion in this pressor response was evaluated by infusing an equal volume of NaCl in six rats, which resulted in a significant rise in MAP (from 118 ± 6 mm Hg to 125 ± 7 mm Hg; p < 0.02). However, the increment in MAP in the CaCl₂-treated rats (21 ± 2 mm Hg) was markedly and significantly greater than the increase in MAP in the NaCl-treated rats (7 ± 2 mm Hg; p < 0.001).

**Role of Vasodilative Hormones on the Pressor Response to Acute Hypercalcemia**

The role of prostaglandins in the pressor response to acute hypercalcemia was evaluated in animals that were pretreated with indomethacin followed by administration of CaCl₂ 6 hours later (Figure 4). In the prostaglandin-inhibited animals, CaCl₂ administration resulted in a significant increase in MAP (from 106 ± 3 mm Hg to 124 ± 3 mm Hg; p < 0.001), whereas NaCl administration did not result in any significant change in MAP (from 110 ± 2 mm Hg to 110 ± 2 mm Hg) in these rats. The resultant increase in the CaCl₂-treated compared with that in NaCl-treated prostaglandin-inhibited rats (17.5 ± 1 mm Hg) was not significantly different than the increment in MAP observed in the non-prostaglandin-inhibited animals (17 ± 1 mm Hg).

The role of parathyroid hormone in the pressor response to acute hypercalcemia was evaluated in animals who were parathyroidectomized. The prestudy serum calcium concentration was 7.5 mg/dl in four such rats, but since they received a greater CaCl₂ load, they achieved an experimental serum calcium concentration comparable to that of the other groups (13.4 mg/dl). In the parathyroidectomized animals, CaCl₂ administration resulted in a significant increase in MAP (from 108 ± 1 mm Hg to 129 ± 4 mm Hg; p < 0.02). The change in MAP (21 ± 4 mm Hg) did not, however, significantly differ from the increment in MAP seen in nonparathyroidectomized animals (17 ± 1 mm Hg).

**Effect of Calcium Channel Blockers on the Pressor Response to Acute Hypercalcemia**

Figure 5 shows that in eight verapamil-pretreated rats the administration of CaCl₂ failed to cause a rise in MAP (113 ± 3 mm Hg before and 113 ± 4 mm Hg after CaCl₂). In contrast, in five vehicle-pretreated animals, CaCl₂ administration resulted in significant increases in MAP (from 116 ± 4 mm Hg to 137 ± 6 mm Hg; p < 0.001). This increase occurred in spite of achieving similar serum calcium concentrations in both groups of animals (13.7 vs 14.1 mg/dl respectively). The administration of verapamil resulted in a decrease in MAP; therefore, to infuse CaCl₂ at approximately comparable levels of MAP in verapamil-treated and vehicle-treated animals, the rats employed for the verapamil infusion in this section of the study had somewhat higher pre-verapamil treatment levels of MAP in verapamil-treated and vehicle-treated animals, the rats employed for the verapamil infusion in this section of the study had somewhat higher pre-verapamil treatment MAP (125 mm Hg). Nonetheless two of the rats still had MAP of about 100 mm Hg, which was comparable to that of subsequently studied verapamil-pretreated rats. As was the case for the group as a whole, these rats did not have an increase in MAP in response to CaCl₂.

The mechanism whereby verapamil blocked the pressor response to acute hypercalcemia was investigated in another group of rats by measuring the CI and SVR in verapamil-pretreated or nifedipine-pretreated normocalcemic rats, which were then infused with CaCl₂ or NaCl.
As can be seen in Table 1, pretreatment with the calcium blocker verapamil in NaCl-treated (i.e., normocalcemic) rats resulted in a significant decrease in SVR, from 0.45 ± 0.02 mm Hg/(ml/min)/kg body weight to 0.36 ± 0.01 mm Hg/(ml/min)/kg body weight; \( p < 0.05 \). This marked decrement in peripheral resistance caused a reflex increase in CI (from 258 ± 4 ml/min/kg body weight to 286 ± 6 ml/min/kg body weight; \( p < 0.05 \)) but did not prevent a significant decrement in MAP (from 115 ± 3 to 101 ± 2 mm Hg; \( p < 0.05 \)). Since these rats were not selected as the ones described in Figure 5 the MAP in rats given the channel blockers was about 100 mm Hg. As was the case with the verapamil-pretreated rats, in these animals the administration of CaCl\(_2\) to either verapamil-pretreated (\( n = 13 \)) or nifedipine pretreated (\( n = 6 \)) rats caused no increase in mean systemic pressure. Unlike rats not treated with calcium channel blockers, these rats sustained only a small nonsignificant increase in SVR, from 0.36 ± 0.01 mm Hg/(ml/min)/kg body weight to 0.38 ± 0.01 mm Hg/(ml/min)/kg body weight, but sustained a significant decrement in CI (\( p < 0.05 \)). Therefore, in the verapamil-pretreated or nifedipine-pretreated animals, a mild increase in SVR following CaCl\(_2\) administration resulted in a significant reflex decrease in CI.

**Discussion**

Acute hypercalcemia has previously been shown to cause elevation of arterial blood pressure in humans and experimental animals.\(^{14,15}\) Since calcium ion in vitro has been shown to increase myocardial contractility and velocity of myocardial fiber shortening\(^{10}\) and also induce smooth muscle contraction,\(^{11}\) it was initially presumed that the hypertensive response to short-term elevation in serum calcium concentration was mediated by a combination of both increased cardiac output and increased SVR. Similar to the hemodynamic findings in these previous studies, in the present study acute hypercalcemia lasting 30 minutes induced in awake, unanesthetized rats resulted in a significant increase in MAP, which was matched by a parallel and significant rise in SVR as CI remained constant. The absence of an adaptive hemodynamic process in acute hypercalcemia, in the present and previous studies, does in fact suggest an additive role for cardiac output in the hypertensive response to acute hypercalcemia.

Previous in vivo and in vitro studies have revealed that calcium tends to inhibit renin production\(^{22-24,26}\) but to enhance angiotensin II-mediated vasoconstriction\(^{30,43}\); the net effect of the renin-angiotensin system in the observed hypertension was therefore not predictable. Although there are pharmacological inhibitors of the system, in the present studies the role of the renin-angiotensin system was evaluated in nephrectomized rats, therefore eliminating the source of renin. Plasma renin activity is essentially zero 24 hours after nephrectomy. The hypertensive response to acute hypercalcemia was not different in the nephrectomized rats, especially when the volume-mediated blood pressure rise was taken into account, which therefore rules out a substantial contribution of the renin-angiotensin system. It must be acknowledged, however, that a mechanism mediated by extrarenal renin such as the one that may be present in blood vessels\(^{49}\) cannot be entirely excluded. Furthermore, while nephrectomy may have consequences other than removal of the renin-angiotensin system, our control animals were similarly nephrectomized.

In the present study the role of catecholamines was
evaluated in catecholamine-depleted rats. Peripheral, central, or combined peripheral and central catecholamine stores were eliminated by pretreating rats with 6-OHDA as previously described.45-47 The hypertensive response to acute hypercalcemia was not different in the catecholamine-depleted rats, which therefore makes a substantial contribution of catecholamines unlikely. However, since 6-OHDA does not entirely eliminate the source of catecholamines originating from the adrenal medulla,41 a possible contributory role for catecholamines cannot be entirely ruled out. In this respect, other investigators have recently shown increased plasma epinephrine and norepinephrine levels following infusions of calcium gluconate in humans.3 However, of interest in that study, blood pressure was significantly elevated at 1 hour, long before plasma epinephrine levels became elevated at 2 hours. We attempted to determine a role for catecholamines in the pressor response to acute hypercalcemia in adrenalec-tomized rats receiving physiological replacement of glucocorticoids and mineralocorticoids whose central and peripheral catecholamines were then depleted by appropriate administration of 6-OHDA. The combination of 6-OHDA treatment with adrenalectomy rendered the animals very hypotensive (MAP, 60 mm Hg), which precluded the performance of meaningful studies. Our studies, therefore, cannot completely rule out a contribution of systemic catecholamines in the pressor response to calcium infusions, as studies directed to answer this question appear not to be performable in vivo. Finally, a role for vasopressin in mediating the hypertensive response to acute hypercalcemia was evaluated in animals pretreated with a vascular antagonist of vasopressin. The resulting hypertensive response to acute hypercalcemia was not different in these animals either. Therefore, the studies suggest that a potential substantial role for vasopressor hormones such as the renin-angiotensin system, catecholamines, and vasopressin in mediating the hypertensive response to acute hypercalcemia in the rat is unlikely.

In the present study a potential role of vasodepressor hormones such as the prostaglandins31 and parathyroid hormone32 in attenuating the hypertensive response to acute hypercalcemia was also investigated. In rats pretreated with the cyclooxygenase inhibitor indomethacin the hypertensive response to acute hypercalcemia was not altered. Similarly, in parathyroidectomized rats whose serum calcium levels after CaCl₂ infusion were similar to the other group of hypercalcemic rats, the hypertensive response to acute hypercalcemia was not significantly different either. Therefore, these studies do not suggest a major role for vasodepressor hormones such as the prostaglandins and parathyroid hormone in attenuating the hypertensive response to acute hypercalcemia in the rat.

Finally, a direct role of calcium ion causing peripheral vasoconstriction in acute hypercalcemia was evaluated in rats pretreated with two dissimilar calcium channel blockers, verapamil and nifedipine.45, 47, 48 Treatment with verapamil or nifedipine resulted in a significant decrease in MAP that was associated with a significant decrease in SVR and reflex increase in cardiac output. Verapamil or nifedipine pretreatment blocked the hypertensive response to acute hypercalcemia. In these rats the short-term administration of calcium caused only a minimal and nonsignificant increase in SVR, although CI decreased to control levels. It would therefore appear that in the face of these agents neither the vasoconstrictive nor the inotropic myocardial effects of calcium are observed. Entry of calcium into the vascular cell therefore appears to be critical in the mechanism of hypertension caused by short-term increases in the concentration of the cation. However, since pretreatment with verapamil or nifedipine also blocked the pressor response to catecholamines, vasopressin, and angiotensin II, a possible role for these vasoconstrictive hormones, especially catecholamines and vasopressin whose release is enhanced in hypercalcemia, cannot be completely ruled out, but seems unlikely. It is increasingly clear, therefore, that since the effect of the agent is not specific, we cannot unequivocally conclude that the constrictive effect of hypercalcemia is due to calcium entry.

The present studies therefore indicate that acute hypercalcemia induced by a 30-minute infusion of CaCl₂ in the conscious, unanesthetized rat results in a significant rise in MAP. The increase in MAP was largely accounted for by a parallel and significant increase in SVR, since CI was maintained despite an expected reflex compensatory decrease. A major role for vasopressor hormones in mediating or enhancing and for vasodepressor hormones in attenuating the hypertensive response to acute hypercalcemia in the rat seems unlikely. Rather, the hypertensive response to acute hypercalcemia seemed to result from a direct action of calcium ion to increase SVR and maintain cardiac output, as pretreatment with two dissimilar calcium channel blockers, verapamil or nifedipine, blocked the hypertensive response to acute hypercalcemia.

Acknowledgments

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