Proc. Natl. Acad. Sci. USA
Vol. 81, pp. 6511–6515, October 1984
Medical Sciences

Intracellular free magnesium in erythrocytes of essential hypertension: Relation to blood pressure and serum divalent cations

(high blood pressure/nuclear magnetic resonance/mineral metabolism)

LAWRENCE M. RESNICK*, RAJ K. GUPTA†, AND JOHN H. LARAGH*

*Cardiovascular Center, New York Hospital–Cornell University Medical Center, New York, NY 10021; and †Department of Physiology and Biophysics, Albert Einstein College of Medicine, Bronx, NY 10461

Communicated by Alexander G. Bearn, July 5, 1984

ABSTRACT **Intracellular levels of free Mg^{2+} in human erythrocytes were determined by ^{31}P NMR spectroscopy in 26 fasted subjects and were correlated with blood pressures and serum levels of total magnesium (bound and free Mg^{2+}) and ionized calcium from the same subjects in a seated position. Untreated hypertensive individuals consistently demonstrated lower levels of intracellular free magnesium (192 ± 8 μM, n = 11) than either normotensive (261 ± 9.8 μM, $n = 7$, $P < 0.001$ vs. untreated hypertensive subjects) or hypertensive subjects whose blood pressure had been normalized on therapy (237 ± 7.8 μM, $n = 8$, $P < 0.005$ vs. untreated hypertensives). For all subjects, strong relationships existed between intracellular free magnesium and diastolic blood pressure ($r = -0.85$, $P < 0.001$) and systolic blood pressure ($r = -0.71$, $P < 0.001$). Significant relationships also were observed between intracellular free magnesium levels and extracellular serum levels of calcium ion ($r = -0.77$, $P < 0.001$) as well as serum concentrations of total magnesium ($r = 0.62$, $P < 0.001$). We conclude that significant depletion of intracellular free magnesium levels are apparent in erythrocytes of subjects with essential hypertension. Furthermore, the close, inverse relationship of free magnesium levels with the height of the blood pressure suggests that abnormalities of intracellular magnesium metabolism may contribute to the pathophysiology of human essential hypertension.**

Isolated observations over five decades suggest an association of magnesium metabolism with blood pressure regulation. Indeed, magnesium was first recommended as therapy of malignant hypertension as early as 1925 (1). Since that time, basic research has documented the ability of magnesium depletion to alter vascular smooth muscle tone, perhaps by affecting the intracellular disposition of calcium (2). However, clinical studies have failed thus far to demonstrate consistent abnormalities of magnesium metabolism in essential hypertension (3, 4). We have demonstrated an inverse relationship between serum magnesium levels and the concurrent activity of the renal pressor hormone, renin, in essential hypertensive subjects (5). While suggesting a link between the renin–aldosterone system and magnesium metabolism, the clinical significance of these findings remains uncertain. In attempting to further clarify the clinical relevance of magnesium to human hypertensive disease, we utilized ^{31}P NMR spectroscopy to assess intracellular erythrocyte levels of free magnesium ion and to relate our findings to levels of blood pressure and to extracellular, serum levels of ionized (unbound) calcium and total magnesium (bound and free Mg^{2+}). Our findings suggest consistent abnormalities of intracellular free magnesium levels in subjects with essential hypertension, which are closely linked to the height of blood pressure.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

METHODS

Hypertensive subjects, either untreated (n = 11) or normotensive on therapy (n = 8), and normotensive individuals on no medical therapy (n = 7) were studied between 9:00 a.m. and 12 noon at the Cardiovascular Center of The New York Hospital–Cornell University Medical Center. All subjects had fasted overnight, had blood pressures measured by their physician, and then, in the seated position, had peripheral venous blood drawn for analysis of serum ionized calcium, serum total magnesium, and erythrocyte intracellular free magnesium levels. Blood for serum ionized calcium was collected and processed anaerobically and was analyzed the same day by using a calcium-specific ion electrode (Orion SS-20), where the normal range in our laboratory is 2.35–2.65 meq/liter. Serum magnesium levels were measured by autoanalyzer technique.

Ten milliliters of heparinized venous blood was collected and used for analysis of erythrocyte intracellular free magnesium. Blood was spun at 2,000 rpm for 10 min, and the plasma was discarded. The remaining erythrocyte fraction was decanted into a 12-mm NMR tube and subsequently was analyzed by using ^{31}P NMR spectra. NMR spectra were recorded at 81 MHz with a Varian XL-200 NMR spectrometer in the Fourier transform mode with wide-band proton noise decoupling. Typical sample volumes were approximately 4 ml, and the temperature of the probe was maintained at 37°C by equilibration with purified gaseous nitrogen preheated to the appropriate temperature. Each erythrocyte spectrum was obtained after time-averaging for ≈30 min. The data were analyzed by one-way analysis of variance (ANOVA), with a subsequent modified t test (Bonferroni) for the level of significance. Pearson correlation coefficients and Student t tests were used for linear regression analyses. All results are expressed as means ± SEM.

Calculation of Free Intracellular Magnesium. ^{31}P NMR spectra of human erythrocytes demonstrate well-defined α, β, and γ-phosphoryl-group resonances of ATP (6) (Fig. 1). Their chemical shifts depend on the state of ATP complex formation with magnesium ion (7), according to the equation

$$MgATP \rightleftarrows Mg^{2+} + ATP;\ K_d^{MgATP} = \frac{[Mg^{2+}][ATP]}{[MgATP]}.$$

The quantity $\phi = [ATP]_{free}/[ATP]_{total}$, the fraction of free ATP, also can be determined directly from ^{31}P NMR spectra. The relative separation between the α- and β-phosphoryl-group resonances of ATP (chemical shift $\delta_{\alpha\beta}$) is also dependent on the fraction of ATP complexed with magnesium ion (6). Hence, a comparion of $\delta_{\alpha\beta}$ for a cell ($\delta_{\alpha\beta}^{cell}$) with that for free ATP and the MgATP complex ($\delta_{\alpha\beta}^{ATP}$ and $\delta_{\alpha\beta}^{MgATP}$,

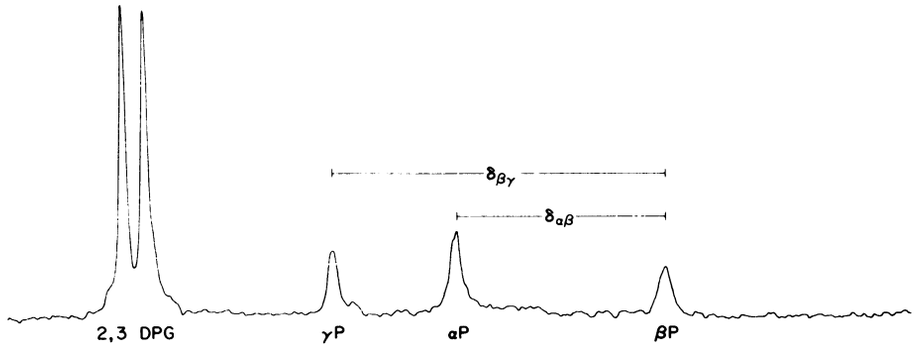

FIG. 1. Fourier transform ^{31}P NMR spectrum of erythrocytes from a normotensive subject. Heparinized blood was analyzed at 37°C and 81 MHz for ≈30 min. Spectral width of 3000 Hz is displayed. 2,3 DPG, 2,3-diphosphoglycerate; αP, βP, γP, α-, β-, and γ-phosphoryl resonances of ATP; $\delta_{\alpha\beta}$, chemical shift of αP and βP; $\delta_{\beta\gamma}$, chemical shift of βP and γP.

respectively) allows a calculation of ϕ for any unknown cell according to the formula:

$$\phi = (\delta_{\alpha\beta}^{cell} - \delta_{\alpha\beta}^{MgATP})/(\delta_{\alpha\beta}^{ATP} - \delta_{\alpha\beta}^{MgATP}).$$

Thus, free magnesium ion can be determined from the spectral data with the knowledge of the dissociation constant K_d^{MgATP} according to the formula (8):

$$[Mg^{2+}]_{free} = K_d^{MgATP}(\phi^{-1} - 1).$$

K_d^{MgATP} at 37°C and pH 7.2 was determined to be $3.8 \pm 0.4 \times 10^{-5}$ M by magnetic resonance techniques (9). Oxyhemoglobin was found not to affect the equilibrium between ATP and MgATP as indicated by the separation between the α- and β-phosphoryl group ^{31}P resonances in a sample containing a mixture of ATP and MgATP (unpublished results).

RESULTS

Clinical and laboratory features of all subjects are listed in Table 1. Whereas no significant age differences were observed when subjects were divided according to their blood pressures, there were more males among the untreated hypertensive subjects and more females within the treated hypertensive subgroup (8/11 vs. 2/8 males). Untreated hypertensive subjects had blood pressures (165 ± 7/102 ± 3 mm of Hg) significantly higher than normotensive controls (125 ± 8/74 ± 3; significance level, 0.01/0.001) and diastolic pressures higher than treated hypertensive subjects (significance level, 0.001). Treated hypertensive individuals had blood pressures (150 ± 8/83 ± 1 mm of Hg) considered to be adequate on present medical regimens. Medical therapy in treated hypertensive subjects consisted of a variety of pharmacologic agents, including spironolactone (1), beta blockers (2), hydrochlorothiazide (2), nifedipine (2), and prazosin (1). Serum levels of electrolytes did not distinguish hypertensive subjects, either treated or untreated, from normotensive controls, and both serum ionized calcium and total magnesium were within normal limits for the reporting laboratory (ionized calcium, 2.35–2.65 meq/liter; total magnesium, 1.6–2.4 meq/liter).

Intracellular free magnesium levels were clearly and significantly different among the different patient groups (Table 1 and Fig. 2). Normotensive controls had levels of intracellular free magnesium, 261 ± 9.8 μM, that were similar to published results with ^{31}P NMR techniques (6). In comparison, untreated hypertensive subjects had intracellular free magnesium levels (192 ± 8 μM) that were significantly lower than those of normotensive controls (significance level, 0.001). Furthermore, drug-treated hypertensive subjects, whose blood pressures were significantly lower than those of untreated hypertensive subjects, had intracellular free magnesium levels (237 ± 7.8 μM) significantly greater than those of the untreated hypertensive group (significance level, 0.005) and were indistinguishable from levels in normotensive controls.

Remarkable relationships also were observed between intracellular free magnesium levels and the concurrent blood pressure for all subjects. Systolic blood pressure correlated negatively with intracellular free magnesium levels ($r = -0.71$, $P < 0.001$), and diastolic blood pressure was even more strongly and inversely linked to intracellular free magnesium levels ($r = -0.85$, $P < 0.001$) (Fig. 3). Furthermore, intracellular free magnesium levels also were directly related to serum magnesium ($r = 0.62$, $P < 0.001$) and inversely related to the serum ionized calcium ($r = -0.77$, $P < 0.001$) (Fig. 4). No significant relationships were observed, however, between blood pressures and either serum magnesium ($r = -0.21$, systolic, P = not significant; $r = -0.23$, diastolic, P = not significant) or serum ionized calcium ($r = 0.32$, systolic, P = not significant; $r = 0.37$, diastolic, $P < 0.1$).

DISCUSSION

In our initial attempts to assess intracellular free Mg^{2+} levels in human hypertension, three related observations were made: (*i*) individuals with essential hypertension had consistently lower levels of intracellular free magnesium than did normotensive control subjects (Table 1 and Fig. 2); (*ii*) eight

Table 1. Clinical and laboratory data

Group	*n*	BP mm of Hg	Age, yr	M/F ratio	Total Mg, meq/liter	Ca^{2+}, meq/liter	$\delta_{\alpha\beta}^{cell}$, Hz	ϕ	Mg^{2+}, μM
NL	7	125 ± 8 / 74 ± 3	50 ± 6	4/3	1.92 ± 0.04	2.48 ± 0.02	700.1 ± 0.8	0.129 ± 0.004	261 ± 9.8
HiBP	11	165 ± 7*/102 ± 3†	51 ± 4	8/3	1.82 ± 0.04	2.52 ± 0.03	707.9† ± 1.3	0.168† ± 0.006	192† ± 8.0
HiBP-Rx	8	150 ± 8 / 83 ± 1‡	58 ± 3	2/6	1.89 ± 0.04	2.53 ± 0.03	702.1 ± 0.8	0.139 ± 0.004	237 ± 7.8

BP, blood pressure; NL, normotensive subject; HiBP, untreated hypertensive subject; HiBP-Rx, hypertensive subject on therapy.
*Bonferroni significance level of 0.01 vs. normotensive subjects.
†Bonferroni significance level of 0.001 vs. normotensive subjects.
‡Bonferroni significance level of 0.001 vs. untreated hypertensive subjects.

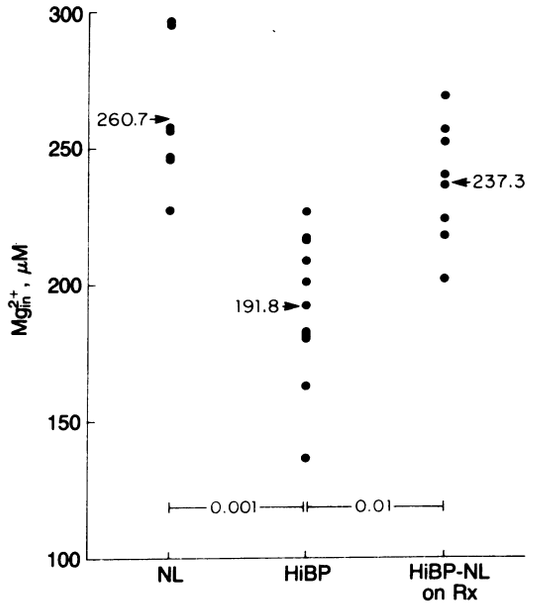

FIG. 2. Intracellular free magnesium (Mg^{2+}_{in}) levels among patient groups. NL, normotensive; HiBP, untreated hypertensive; HiBP-NL on Rx, hypertensive subjects with normalized blood pressure on therapy. Mean values for each group are indicated by arrows. Significance levels between indicated groups are based on the modified *t* test (Bonferroni) and one-way analysis of variance.

hypertensive individuals, who on therapy had achieved normalization of their blood pressure, had levels of intracellular free magnesium that were significantly higher than those of untreated hypertensive subjects and indistinguishable from those of normotensive control subjects (Fig. 2); and (*iii*) a continuous inverse relationship was observed between intracellular free magnesium and the height of the blood pressure (Fig. 3). These findings strongly suggest a physiologic relationship between intracellular magnesium metabolism and blood pressure regulation and pathophysiologically suggest intracellular magnesium depletion to be common in human essential hypertension.

^{31}P NMR has been demonstrated to be an effective technique for the measurement of intracellular free magnesium in a variety of tissues (10). Because $\delta_{\alpha\beta}$, the relative chemical shift of the α- and β-phosphoryl groups of ATP, can be measured with a precision of ≤ 2 Hz, the calculated levels of intracellular free magnesium are quite reproducible (Fig. 1). Furthermore, K_d^{MgATP} varies $\leq 30\%$ over the pH range 7–8, and $\delta_{\alpha\beta}^{MgATP}$ and $\delta_{\alpha\beta}^{ATP}$ are essentially unaffected by variations in pH within the pH range 7–8. Therefore, an accurate knowledge of intracellular pH is not necessary for determining free magnesium levels by this procedure. Although ^{31}P NMR has given values significantly lower than other techniques (11), recent confirmation of NMR-measured intracellular free magnesium levels in Ehrlich ascites tumor cells has been reported by Cittadini and Scarpa, who used a null-point method with the ionophore A32187 (12). The value of K_d^{MgATP} used also has been debated. However, recent studies unambiguously support the lower value of the MgATP dissociation constant used here, $\approx 3.8 \times 10^{-5}$ M (at 37°C) (13–15). Regardless, however, of the absolute level of K_d^{MgATP} and, hence, of calculated free intracellular magnesium, it is (*i*) the difference between untreated hypertensive individuals and both normotensive and treated hypertensive subjects and (*ii*) the continuous inverse correlation between diastolic pressure and intracellular free magnesium levels that remain significant.

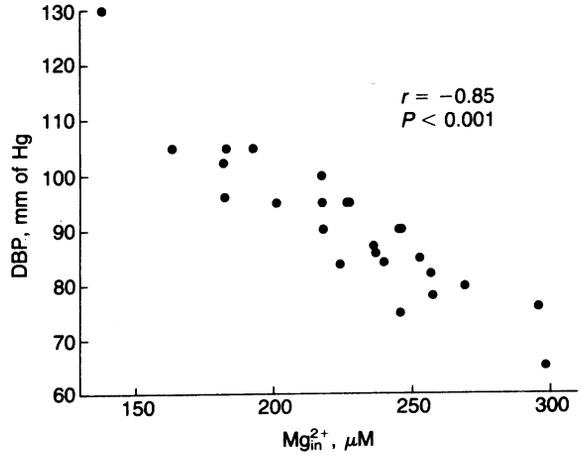

FIG. 3. Relationship of intracellular free magnesium (Mg^{2+}_{in}) to diastolic blood pressure (DBP) for all subjects. Regression analysis used the Pearson correlation coefficient and Student *t* test for level of significance.

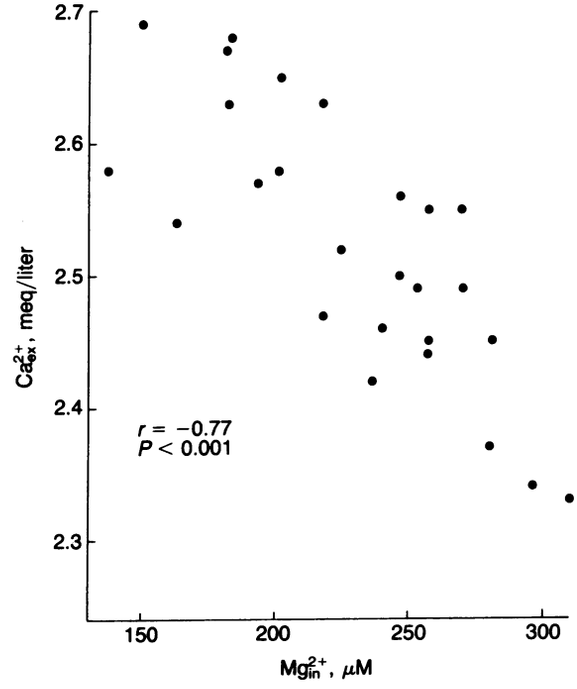

FIG. 4. Relationship of intracellular free magnesium (Mg^{2+}_{in}) to extracellular ionized calcium for all subjects. Blood samples were analyzed the same day for both parameters (see *Methods*). The Pearson correlation coefficient and Student *t* test were used for regression analysis.

Extracellular levels of both calcium and magnesium have been evaluated in previous studies of human normotensive and hypertensive populations. Higher total serum calcium levels with higher blood pressures were observed in a large population of normotensive individuals (16). The same group later reported, however, no difference in serum ionized calcium levels between hypertensive and normotensive subjects (17). Indeed, one study reports lower serum levels of ionized calcium among hypertensive individuals (18). Similarly, serum total magnesium levels have been reported to be both higher and lower in hypertensives compared with normotensives in different published studies (3, 4, 19), although the majority have tended to emphasize lower values in hypertensive subjects. We previously reported that serum levels of both ionized calcium and total magnesium in hypertensive individuals are indistinguishable from those of nor-

motensive control subjects when hypertensives were considered as a homogeneous group (5). Significant deviations were observed that correlated closely with levels of plasma renin activity, but no significant relationships were observed between the height of the blood pressure and serum concentrations of these divalent cations. Thus, neither have measurements of extracellular calcium or magnesium levels consistently distinguished hypertensive from normotensive populations, nor have reported deviations been directly related to blood pressure in any quantitative manner.

By comparison, our data suggest a close quantitative relationship between intracellular free magnesium levels and hypertension not appreciated heretofore in studies of extracellular ion levels. This notion is supported by previous *in vitro* and whole-animal studies and by certain clinical observations. Altura, Altura, and co-workers in a series of studies have demonstrated for a variety of vascular beds in different species, that magnesium levels strongly influence vascular tone and vascular responsiveness to pressor agents (20). Furthermore, these authors have recently shown that dietary magnesium depletion in rats decreases levels of serum magnesium, decreases luminal diameter of peripheral resistance vessels, and increases blood pressure (21). Similarly, Berthelot and Esposito reported that varying dietary magnesium levels in the spontaneously hypertensive rat enhanced or retarded development of high blood pressure when animals were fed low or high magnesium-containing diets, respectively (22). Clinically, magnesium was first shown to lower blood pressure in patients with malignant hypertension as early as 1925 and by 1942 was also shown to lower pressure in some but not all chronic hypertensive individuals (23). The hypotensive efficacy of oral magnesium supplementation in essential hypertensive subjects on diuretic therapy also was recently reported (24). Our own group has demonstrated that short-term magnesium therapy in essential hypertensive subjects preferentially lowered blood pressure in patients with higher levels of plasma renin activity.‡ Significantly, these hypertensive subjects with higher renin levels had lower initial levels of serum magnesium, similar to our previous larger population study, where lower magnesium levels were also observed among subjects with higher renin levels (5).

The above studies support our present, new data. Intracellular free magnesium levels correlated significantly with extracellular magnesium levels ($r = 0.62$, $P < 0.001$), although it was intracellular magnesium levels that were so closely related to diastolic blood pressure ($r = -0.85$, $P < 0.01$), rather than extracellular concentrations ($r = -0.23$, P = not significant). The question naturally arises, how might intracellular magnesium depletion result in the increased peripheral resistance characteristic of diverse forms of hypertensive disease?

Despite the significant inverse relationship between serum magnesium and plasma renin activity previously reported, insufficient numbers of subjects within differing renin subgroups have been evaluated to establish a relationship with intracellular free magnesium. Furthermore, little is known about the biochemistry of cellular magnesium homeostasis, although magnesium is involved in a wide variety of biochemical processes including ATP and phosphoryl-group-transfer reactions, as well as thiamin-dependent reactions. Recently a specific magnesium transport system has been described (26), and magnesium transport appears to be linked to sodium although independent of calcium and calmodulin (27). However, the ability of magnesium to influence blood pressure may be more likely related to its influence on intracellular calcium metabolism. Magnesium is a weak antagonist of calcium entry into vascular smooth muscle (28), and magnesium may induce vasodilatation comparable to that of calcium-channel inhibitors (29). Indeed, magnesium also may potentiate verapamil-induced vasodilatation (30). The intracellular content and disposition of calcium also is influenced by magnesium, and calcium binding at intracellular sites may be antagonized by magnesium. Thus, magnesium shifts the force–calcium relationship of muscle contraction to the right, possibly suggestive of decreased calcium binding to myofilament regulatory sites (31). Furthermore, magnesium stimulates sarcoplasmic reticulum calcium influx and inhibits calcium dependent-efflux (31). Magnesium also may retard calcium efflux into the extracellular space, perhaps related to intracellular sequestration (2). We observed a similar reciprocal relationship between intracellular free magnesium levels and extracellular ionized calcium (see Fig. 4).

Therefore, because smooth muscle tension development is directly linked to available cytosolic free calcium, one would hypothesize that lower levels of intracellular free magnesium would be associated with higher intracellular free calcium levels in proportion to the degree of intracellular magnesium depletion and, thus, be associated with proportionately higher blood pressures. This hypothesized reciprocal relationship between intracellular free magnesium and blood pressure is exactly what was observed here (Table 1 and Figs. 2 and 3). This hypothesis is also supported by recent measurements of intracellular free calcium in normal and hypertensive human platelets (25). Regardless of hypothesized mechanisms, however, and the many further studies these initial results encourage, we believe our observations remain quite significant and suggest intracellular magnesium as a biochemical regulator of the physiology and pathophysiology of blood pressure control in man.

‡Resnick, L. M. & Laragh, J. H. (1983) Endocrine Society 65th Annual Meeting, June 8–10, 1983, San Antonio, TX, abstr. 358.

1. Blackfan, K. D. & Hamilton, B. (1925) *Boston Med. Surg. J.* **193,** 617–628.
2. Altura, B. M. & Altura, B. T. (1981) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **40,** 2672–2679.
3. Walker, B. S. & Walker, E. B. (1936) *J. Lab. Clin. Med.* **21,** 713–720.
4. Albert, D. G., Morita, Y. & Iseri, L. T. (1958) *Circulation* **17,** 761–764.
5. Resnick, L. M., Laragh, J. H., Sealey, J. E. & Alderman, M. H. (1983) *N. Engl. J. Med.* **309,** 888–891.
6. Gupta, R. K., Benovic, J. L. & Rose, Z. B. (1978) *J. Biol. Chem.* **253,** 6172–6176.
7. Cohn, M. & Hughes, T. R., Jr. (1962) *J. Biol. Chem.* **237,** 176–181.
8. Gupta, R. K. & Yushok, W. D. (1980) *Proc. Natl. Acad. Sci. USA* **77,** 2487–2491.
9. Gupta, R. K., Benovic, J. L. & Rose, Z. B. (1978) *J. Biol. Chem.* **253,** 6165–6171.
10. Gupta, R. K., Gupta, P., Yushok, W. D. & Rose, Z. B. (1983) *Physiol. Chem. Phys. Med. NMR* **15,** 265–280.
11. Tsien, R. Y. (1983) *Annu. Rev. Biophys. Bioeng.* **12,** 91–116.
12. Cittadini, L. & Scarpa, A. (1983) *Arch. Biochem. Biophys.* **227,** 202–208.
13. Garfinkel, L. & Garfinkel, D. (1984) *Biochemistry* **23,** 3547–3552.
14. Gupta, R. K., Gupta, P., Yushok, W. D. & Rose, Z. B. (1983) *Biochem. Biophys. Res. Commun.* **117,** 210–216.
15. Gupta, R. K., Gupta, P. & Moore, R. D. (1984) *Annu. Rev. Biophys. Bioeng.* **13,** 221–246.
16. Kesteloot, H. & Geboers, J. (1982) *Lancet* **i,** 318–815.
17. Kesteloot, H., Schaftinger, E. V., VanHoof, R. & Geboers, J. (1983) *Circulation* **68,** III–90.
18. McCarron, D. A. (1982) *N. Engl. J. Med.* **307,** 226–228.
19. Petersen, B., Schroll, M., Christiansen, C. & Transbol, I. (1977) *Acta Med. Scand.* **201,** 31–34.
20. Altura, B. M. & Altura, B. T. (1978) *Blood Vessels* **15,** 5–16.
21. Altura, B. M., Altura, B. T., Gebrewold, A., Ising, H. & Günther, T. (1984) *Science* **223,** 1315–1317.

22. Berthelot, A. & Esposito, J. (1983) *J. Am. Coll. Nutr.* **4,** 343–353.
23. Winkler, A. W., Smith, P. K. & Hoff, H. E. (1942) *J. Clin. Invest.* **21,** 207–216.
24. Dyckner, T. & Wester, P. O. (1983) *Br. Med. J.* **286,** 1847–1849.
25. Erne, P., Bolli, P., Bürgissen, E., Bühler, F. R. & Bolli, P. (1983) *N. Engl. J. Med.* **310,** 1084–1088.
26. Maguire, M. E. & Grubbs, R. D. (1983) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **42,** 300.
27. Güthen, T., Vormann, J. & Förster, R. (1984) *Biochem. Biophys. Res. Commun.* **119,** 124–131.
28. Karaki, H., Hatano, K. & Weiss, G. B. (1983) *Pflügers Arch.* **398,** 29–32.
29. Ji, B. H., Erne, P., Kiowski, W., Bühler, F. R. & Bolli, P. (1983) *J. Hypertension* **1** Suppl. 2, 368–371.
30. Phillips, R. J. W. & Robinson, B. F. (1984) *Clin. Sci.* **66,** 39P.
31. Stephenson, W. E. (1981) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **40,** 2662–2666.