The Roles of Cell Ca$^{2+}$, Protein Kinase C and the Na$^{+}$-H$^{+}$ Antiport in the Development of Hypertension and Insulin Resistance$^{1,2}$

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ABSTRACT
There is evidence that the cytosolic free Ca$^{2+}$, protein kinase C, and the Na$^{+}$-H$^{+}$ antiport cross-communicate with one another through positive and negative feedback mechanisms, thereby maintaining cellular Ca$^{2+}$ and pH homeostasis. This triumvirate may play a role in the development of insulin resistance—a common characteristic of both essential hypertension and non-insulin-dependent diabetes mellitus. Circulating cells from patients with essential hypertension and non-insulin-dependent diabetes mellitus demonstrate elevated cytosolic free Ca$^{2+}$, increased protein kinase C activity, or both, and these perturbations are associated with augmented activity of the Na$^{+}$-H$^{+}$ antiport. If present in other cells (e.g., striated muscle cells and adipocytes), these alterations could underlie insulin resistance in essential hypertension and non-insulin-dependent diabetes mellitus.

Key Words: Protein kinase C, calcium, Na$^{+}$-H$^{+}$ antiport, non-insulin-dependent diabetes mellitus, insulin

Essential hypertension is marked by multiple alterations in metabolic regulation at the cellular and systemic levels. Precisely how these alterations are interrelated is a matter of considerable debate. A major challenge is to integrate mechanistically the diverse observations in this disease into a comprehensive thesis that provides insight into its pathophysiology. This communication focuses on insulin resistance and its relation to abnormal cellular homeostasis in essential hypertension and non-insulin-dependent diabetes mellitus (NIDDM). Both diseases are associated with an increased propensity for cardiovascular complications that may arise from common cellular alterations. These changes may be expressed in a spectrum of clinical manifestations ranging from insulin resistance without an elevation of the systemic blood pressure to frank hypertension without insulin resistance. The hypothesis presented herein links cytosolic Ca$^{2+}$, protein kinase C (PKC), and the Na$^{+}$-H$^{+}$ antiport in the pathophysiology of insulin resistance and essential hypertension. It is based on the concept that both augmented PRC activity and a rise in Ca$^{2+}$ are central to the development of essential hypertension and NIDDM. At present, the evidence supporting this idea is incomplete and requires further verification.

EPIDEMIOLOGY OF ESSENTIAL HYPERTENSION, INSULIN RESISTANCE, AND NIDDM

The finding of Modan et al. (1) that hyperinsulinemia is a common feature of hypertension, obesity, and NIDDM has strengthened previous impressions of insulin dysregulation in essential hypertension. The presence of hyperinsulinemia in essential hypertension was recognized as early as 1966 (2). However, the association between insulin resistance and essential hypertension in Caucasians was first demonstrated by Ferrannini and coworkers (3) in 1987. A number of reviews and commentaries have subsequently been published on insulin resistance and its associated dyslipidemia in essential hypertension, NIDDM, and obesity (4–13). The consensus emerging from these publications is that insulin resistance plays a major role in the pathophysiology of essential hypertension and one of its major complications, coronary heart disease. However, this idea lacks a solid foundation in that the clustering of insulin resistance with essential hypertension may reflect a mere association rather than a cause and effect relationship (5,11).

Several aspects of the epidemiology of insulin resistance, essential hypertension, and NIDDM merit specific considerations. First, hypertension is more common among patients with NIDDM and obese sub-
jects with insulin resistance than in the general population (14–16; for reviews, see references 7 and 8). Second, aggregations of coronary heart disease and hypertension are observed in families of patients with NIDDM (17,18). Third, altered glucose metabolism is observed one or two decades before NIDDM is diagnosed (19) and it is associated with increased risk for coronary heart disease (20,21). These findings suggest that not only NIDDM but also insulin resistance are associated with an increased risk for cardiovascular diseases.

The relation between insulin resistance and elevated blood pressure is, however, not universal. An apparent dissociation between insulin resistance and hypertension is expressed in Pima Indians (22,23), who are known to be predisposed to obesity and NIDDM, and in blacks (22,24), who manifest a high incidence of both NIDDM and essential hypertension (25–28). As pointed out by Ferrannini et al. (29), hyperinsulinemia and NIDDM are more common among Mexican Americans than in non-Hispanic whites, yet hypertension is less prevalent among Mexicans. Moreover, it has been recently shown that salt sensitivity is associated with a hyperinsulinemic response to oral glucose and that this response can be attenuated by salt restriction (30). Collectively, these observations bear witness to the variability in the expression of essential hypertension and insulin resistance as well as their complexity. They also suggest that pathophysiological processes associated with or promoting essential hypertension and NIDDM are influenced by genetic extraction and environmental factors.

**CAUSE OF INSULIN RESISTANCE**

Insulin resistance reflects the diminished capacity of tissues such as skeletal muscle, fat, and the liver to respond to insulin. Obesity is frequently complicated by insulin resistance due to overstimulation of insulin secretion by excess feeding. The resulting chronic hyperinsulinemia down-regulates insulin receptors, decreases coupling efficiency between the receptors and glucose transport, and perhaps influences other intracellular pathways (31–36). In contrast, increased blood insulin in essential hypertension and NIDDM reflects true insulin resistance, probably arising from primary abnormalities in the cellular response to the hormone. The resulting hyperinsulinemia therefore represents a homeostatic response to overcome the target tissue resistance. The distinction between primary and secondary insulin resistance is particularly difficult in established disease states when the metabolic criteria reflecting the action of insulin relate to an original resistance to the hormone in target tissues, a secondary response to hyperinsulinemia, or both. Moreover, essential hypertension, obesity, and NIDDM often coexist, thereby further complicating the elucidation of the factors underlying insulin resistance (7). These factors may be both genetic and environmental in their origin. Another problem relates to the different and sometimes opposite effects of insulin on various cells. For instance, insulin stimulates Ca²⁺-ATPase in hepatic plasma membranes and renal basolateral membranes (37–39) but inhibits the enzyme in adipocyte membranes (40). Thus, the expression of insulin resistance in tissues may differ, reflecting the diverse effects of the hormone.

At the cellular level, insulin resistance can be expressed in abnormalities ranging from diminished density of insulin receptors to reduced numbers of insulin-dependent glucose carriers. The earliest event in the cellular action of insulin is its binding to specific receptors on the plasma membrane, comprising α and β subunits (for reviews, see references 41 and 42). The next step is tyrosine kinase-mediated phosphorylation of the β subunits (43–45). Lower density of insulin receptors in target cells is observed in both obesity and NIDDM (46–49). However, only cells from patients with insulin resistance also show reduced insulin receptor kinase activity (47–51) and a consequent decrease in sensitivity of glucose transport to insulin; this alteration is unrelated to the reduction in the density of insulin receptors (52). Such observations point to a defective coupling between insulin binding and the cellular expression of insulin action. Rare conditions of extreme insulin resistance appear to arise from mutations of the insulin receptor gene, resulting in a lack of cleavage of the insulin receptor α and β subunits and inhibition of tyrosine kinase activity (53–56; for a review, see reference 57). Inactivation of tyrosine kinase was also produced in vitro by mutation of the ATP binding site of the receptor (58).

Insulin resistance in NIDDM may also result from impaired cellular response downstream to the tyrosine kinase (for a review, see reference 59), including insulin-mediated signal transduction and the final activation of the glucose transporters (for the nature and regulation of these transporters, see references 60–62). Garvey et al. (63) observed a depletion of the glucose transporters and a consequent reduction of their insulin-evoked translocation to adipocyte plasma membrane of patients with NIDDM. Moreover, these investigators showed that in vitro exposure of adipocytes to high levels of glucose and insulin impaired the translocation of the glucose transporters to the cell membrane without a reduction in the total cellular number of these transporters (63). It was shown more recently that metformin, which is widely used in Europe in the treatment of NIDDM, potentiates the insulin-evoked translocation of glucose transporters from the intracellular pool to the
plasma membrane with no effect on insulin receptor
kinase activity (64). Together, these findings suggest
that insulin resistance is expressed by abnormalities
of key processes from the binding of insulin to its
receptors to the final expression of insulin action—
the activity of glucose transporters.
Approximately 80% of the insulin-induced periph-

eral utilization of glucose takes place in skeletal

muscle. It is therefore logical to assume that skeletal

muscle is a major element in insulin resistance. This

was shown to be the case in NIDDM (for a review, see
reference 59) and recently in patients with essential
hypertension (65). Skeletal muscle comprises slow-
twitch, oxidative fibers with great insulin sensitivity
(type I), fast-twitch oxidative-glycolytic fibers of in-

termediate insulin sensitivity (type IIA), and fast-
twitch, glycolytic fibers, which are the least respon-
sive to the hormone (IIB) (for a review, see reference
66). The relative distribution of these fibers may be
genetically determined (67) and correlates with in-
sulin sensitivity and vascular resistance (68,69). In-
sulin sensitivity of type I fibers may also relate to
their higher number of insulin-binding sites (70),
greater capillary density, and shorter diffusion dis-
tance from these capillaries to target cells (71,72).
The latter features may render glucose and insulin
more accessible to these muscle fibers.

THE ROLES OF Ca2+ AND PKC IN INSULIN ACTION
AND INSULIN RESISTANCE

The roles of Ca2+ and PKC in insulin action have
been matters of substantial controversy. This review
will focus on studies showing that Ca2+ and PKC may
be involved in the insulin-evoked signal transduc-
tion. It is noteworthy that not all studies support this
concept. For instance, Klip (73), Kelly (74), and their
respective coworkers failed to show involvement of
Ca2+ in insulin action on muscle cells and adipocytes.
Klip and Ramml (75) also showed that down-regu-
lation of PKC did not impair insulin-dependent glucose
takeup in muscle cells. The reasons for differences
between these investigations and others are not clear
(for reviews, see references 76 and 77).
Ca2+ involvement in insulin action may be ex-
pressed at various levels of cellular metabolism (78–80).
The insulin receptor contains a high-affinity
Ca2+-binding site and its phosphorylation is Ca2+-
dependent (81). It also has a calmodulin domain (82).
In adipocyte plasma membrane, insulin increases
both Ca2+ binding (83) and the high-affinity binding
sites for calmodulin, and via its receptor kinase, it
also stimulates calmodulin phosphorylation (84–86).
In turn, calmodulin enhances insulin-evoked phos-
phorylation of the insulin receptor β subunit (85).
To reconcile findings of an insulin-mediated increase in
binding sites for calmodulin with the inhibitory effect
of the hormone on Ca2+-ATPase in adipocytes (41,87),
it was proposed that the former process renders cal-
modulin less available for interaction with the Ca2+-
ATPase (84).
Glucose transport in adipocytes is Ca2+ dependent
(88,89) and so is liver glucose-6-phosphatase activity
(90). The concept that Ca2+ is the intracellular signal
for insulin-mediated processes had been suggested
by several studies (for a review, see reference 91).
However, only recently have investigators, using in-
tracellular Ca2+ probes, demonstrated that insulin
can evoke a rise in Ca2+ in adipocytes (92,93). Draznin
and coworkers have proposed that insulin-mediated
glucose transport effectively functions only within
an optimal range of Ca2+ and that elevated basal Ca2+
can produce insulin resistance (94,95). They have
further suggested that insulin resistance in obesity
and NIDDM is related to high basal Ca2+ in adipocytes
(93,96) and showed that it can be reversed in vitro
by treatment with the Ca2+ channel blocker verapa-
mil (93). Support for this concept has emerged from
other studies. Taylor et al. (88) found that verapamil,
Ni2+ (a general Ca2+ channel blocker), and TM88
inhibited insulin-dependent glucose transport. Kelly
et al. (74) could not demonstrate a relation between
a rise in Ca2+ in adipocytes and insulin resistance; how-
ever, they did observe a strong correlation be-
 tween Ca2+ influx and inhibition of insulin-depend-
ent glucose transport. More recently, Eckle and co-
workers (97) observed that the Ca2+ channel blocker
nifedipine attenuated the induction of insulin resis-
tance in primary cultures of cardiac myocytes. Thus,
although further research is needed to establish the
mechanisms by which Ca2+ produces insulin resis-
tance, the evidence suggests that a rise in Ca2+ or a
local increase of Ca2+ at the submembrane domain is
involved in insulin resistance.
A substantial body of observations indicates that
PKC plays a role in the insulin-evoked cellular re-
sponse. These observations include the following: (1)
insulin increases diacylglycerol (DAG) levels and
stimulates PKC in myocytes and adipocytes through
acceleration of the translocation of the enzyme to the
plasma membrane and by an increase in its maximal
reaction velocity (V_{max}) (98–102); (2) phorbol esters,
which stimulate PKC by binding to the DAG site of
the enzyme, produce an insulin-like effect by phos-
phorylating the β subunit of the insulin receptor
(103–107); (3) inhibition of PKC blunts the insulin-
evoked cellular response (108); and (4) in B3H-1
myocytes, sulfonylurea directly stimulates glucose
transport (109) in association with the activation of
PKC (110). It is noteworthy, however, that insulin
stimulates glucose transport through both PKC-de-
dependent and PKC-independent pathways (108). The
mechanisms that may mediate the rise in DAG and
the consequent stimulation of PKC have been re-
cently reviewed by Farese (77).
Although the primary involvement of PKC and Ca\textsubscript{i} in insulin action is a matter of debate, there is no controversy regarding the role of PKC in regulatory feedback loops that mediate cellular homeostasis after exposure to a variety of agonists (for a recent review, see reference 111). Among PKC-mediated functions are negative feedbacks on: (1) the binding of insulin to its binding sites (112), (2) the phosphorylation of the insulin receptors (104), and (3) the insulin-evoked stimulation of phospholipase C (113). These effects of PKC are not limited to insulin, because the enzyme also modulates through feedback processes other hormones and growth factors expressing tyrosine kinase activity, e.g., epidermal growth factor (114,115; for a review, see reference 116). This modulation may also be important in the \beta-adrenergic–mediated decline in the binding of insulin and epidermal growth factor to their respective receptors (117) and in the inhibition of the receptor-mediated stimulation of adenylate cyclase (118). Such findings suggest an intriguing explanation for insulin resistance. Insulin resistance could arise when PKC is stimulated by insulin-independent mechanisms, which in turn would blunt the response to insulin. Recent studies suggest that this may well be the case.

The activation of PKC takes place through the translocation of PKC from the cytosol to the plasma membrane, where it interacts with DAG and assumes its active mode. The activity of PKC is therefore an expression of a dynamic equilibrium between the membrane-bound PKC and its soluble apoenzyme, the cytosolic PKC. Ca\textsubscript{i} is a key factor in the PKC translocation from the cytosol to the plasma membrane, "priming" the enzyme for interaction with DAG (119,120). Thus, both DAG and Ca\textsubscript{i} act synergistically in PKC activation (121–123) and a rise in their levels, singly or in combination, could enhance PKC activity through its translocation to the plasma membrane. In this regard, independent of insulin, elevated glucose can also stimulate PKC (124) through a \textit{de novo} synthesis of DAG (125–127). This process is likely to promote insulin resistance, and it can be reversed by PKC inhibition (128).

\textbf{THE Na\textsuperscript{+}–H\textsuperscript{+} ANTIPORT Ca\textsubscript{i}, PKC LINK AND ITS ROLE IN NIDDM AND ESSENTIAL HYPERTENSION}

The Na\textsuperscript{+}–H\textsuperscript{+} antiport is a key transport system engaged in the regulation of the pH\textsubscript{i}. Stimulation of the Na\textsuperscript{+}–H\textsuperscript{+} antiport is associated with cellular growth, the mitogenic response, fertilization, platelet activation, Na\textsuperscript{+} reabsorption by the kidney, cellular volume regulation, and numerous other cellular functions. These include the vascular smooth muscle (VSM) response to vasoactive agents and growth factors as well as the response of various cells to insulin (for reviews, see references 129–132). PKC-dependent pathways and Ca\textsubscript{i} are important mediators for the activation of the Na\textsuperscript{+}–H\textsuperscript{+} antiport. The pH\textsubscript{i} threshold (set point) for the activation of the Na\textsuperscript{+}–H\textsuperscript{+} antiport (XI) is usually lower than the pH\textsubscript{i} of resting cells. Hence, the Na\textsuperscript{+}–H\textsuperscript{+} antiport is quiescent under basal pH\textsubscript{i} and its activity progressively increases as the cytosol is acidified below the XI. One way to activate this transport system is to acidify the cell to a pH\textsubscript{i} lower than the XI. Another way is to induce an alkaline shift in the XI. The latter can be accomplished by a number of agonists that activate PKC and raise the Ca\textsubscript{i}. The alkaline shift in the XI reflects the increased affinity of the antiporter to cytosolic protons, and recent evidence indicates that this process is mediated by phosphorylation of the antiporter carrier protein (133).

In itself, a rise in Ca\textsubscript{i} can stimulate the Na\textsuperscript{+}–H\textsuperscript{+} antiport independent of PKC (134–138). This stimulation occurs through an alkaline shift in the XI, augmented activity of the Na\textsuperscript{+}–H\textsuperscript{+} antiport at the pH\textsubscript{i} domain in which the antiport is active, or both (136,137). In platelets, cyclic nucleotides diametrically oppose agonist-evoked Ca\textsubscript{i} response at various levels, including the inhibition of agonist-receptor interaction and phospholipase C activation (139–141), the blunting of Ca\textsuperscript{2+} mobilization from intracellular organelles (142–144), and the stimulation of the extrusion of Ca\textsuperscript{2+} (145). In these cells, cyclic nucleotides antagonize the agonist-evoked alkaline shift in the XI by blunting the Ca\textsubscript{i} response (137). In fact, the XI is exquisitely sensitive to perturbations in Ca\textsubscript{i} (136,137). Hence, cyclic nucleotides share with PKC several important characteristics that attenuate the agonist-mediated Ca\textsubscript{i} response, including stimulation of the Ca\textsuperscript{2+} pump (146–149). However, PKC and cyclic nucleotides differ in that PKC also stimulates the Na\textsuperscript{+}–H\textsuperscript{+} antiport, whereas at least in platelets, cyclic nucleotides inhibit the Na\textsuperscript{+}–H\textsuperscript{+} antiport by blocking the agonist-mediated Ca\textsubscript{i} response.

Because at least some of the action of insulin is mediated through PKC and Ca\textsubscript{i}, it is expected that the hormone would stimulate the Na\textsuperscript{+}–H\textsuperscript{+} antiport. Several studies have shown that this is the case, particularly in skeletal muscle, which is a major target of insulin action (150–153). This process is associated with direct or secondary stimulation of the Na\textsuperscript{+} pump (154–159; for a review, see reference 160) and hyperpolarization of the plasma membrane (151,161).

What, then, is the relevance of an agonist-mediated or a Ca\textsubscript{i}-evoked alkaline shift in the XI to cellular function in general and to the insulin response in particular? Using platelets as a cellular paradigm, my colleagues and I have proposed that in vivo the agonist-evoked alkaline shift in the XI is essential for maintaining the basal pH\textsubscript{i} (136,137). The agonist-mediated rise in Ca\textsubscript{i} would stimulate the Ca\textsuperscript{2+} pump,
which is a $\text{Ca}^{2+}/\text{H}^+$ exchanger (for reviews, see references 162 and 163). The agonist-mediated rise in $\text{Ca}_\text{i}$ is also likely to be associated with increased metabolic activity of the cell. Both factors would promote cellular acidification. An alkaline shift in the $\text{Xi}$ would activate the $\text{Na}^{+}-\text{H}^+$ antiport under basal pH, concurrently with the rise in $\text{Ca}_\text{i}$. The cell thus takes full advantage of the $\text{Na}^{+}-\text{H}^+$ antiport, avoiding the necessity for substantial acidification to stimulate this system in order to extrude the acid load. Thus, the activation of the $\text{Na}^{+}-\text{H}^+$ antiport through an alkaline shift in $\text{Xi}$ would blunt the tendency for cellular acidification associated with a rise in $\text{Ca}_\text{i}$ and other perturbations that increase cellular metabolic activity. Under physiological conditions, in concert with the $\text{Na}^{+}$-dependent and $\text{Na}^{+}$-independent $\text{Cl}^-$/ $\text{HCO}_3^-$ exchangers, the $\text{Na}^{+}-\text{H}^+$ antiporter thus serves to control the basal pH (164–167). This task is of particular importance in vivo, when cells are constantly subjected to fluctuating levels of hormones and growth factors that alter the $\text{Ca}_\text{i}$ and cellular metabolic activity.

It is clear that $\text{Ca}_\text{i}$, PKC, and the $\text{Na}^{+}-\text{H}^+$ antiport are linked in a web of cellular processes designed to carry out agonist-mediated cellular messages with minimal interruptions of other cellular activities that are pH$_\text{i}$ and $\text{Ca}_\text{i}$ dependent (Figure 1). It is no wonder that the interactions among these variables offer a clue to the nature of insulin resistance.

Because platelets are easily accessible and share multiple characteristics with VSM cells, they have been used extensively to study cellular mechanisms in NIDDM and essential hypertension. Increased platelet activity is commonly observed in NIDDM, and platelets play a role in the vascular complications of this disease (168–171). Thus, the elucidation of the causes for platelet hyperactivity may promote an understanding of the cellular perturbations in NIDDM. This is particularly relevant to the regulation of $\text{Ca}_\text{i}$, which is central to platelet activation. Platelets exhibit specific insulin receptors (172–174) that are down-regulated in NIDDM (174). Whether these platelet receptors are coupled with intracellular signals is a matter of controversy (175–177). We have found that insulin has no measurable effect on platelet $\text{Ca}_\text{i}$ and pH$_\text{i}$ profiles (J. Cho and A. Aviv, unpublished data). This lack of effect provides a unique opportunity to evaluate $\text{Ca}_\text{i}$ and pH$_\text{i}$ homeostasis in NIDDM without the confounding effect of hyperinsulinemia. Alterations in these systems in platelets would therefore reflect the metabolic status in NIDDM independent of insulin, albeit the question still remains whether such perturbations are primary or merely secondary responses to an abnormal metabolic milieu in NIDDM.

Three groups have recently reported that basal $\text{Ca}_\text{i}$ or $\text{Ca}_\text{i}$ poststimulation by several agonists is elevated in the platelets of patients with NIDDM (178–180). We have confirmed these observations in black females with NIDDM (Z. Zentay and A. Aviv, unpublished data). Furthermore, we also showed that the activity of the $\text{Na}^{+}-\text{H}^+$ antiport was positively correlated with blood pressure, irrespective of diabetes, i.e., subject with or without NIDDM demonstrated a higher $\text{Na}^{+}-\text{H}^+$ antiport activity at a higher blood pressure. Other studies showed that the guanylate cyclase activity (181) and cAMP levels (182) are lower in the platelets of patients with NIDDM; these alterations would promote a greater agonist-mediated $\text{Ca}_\text{i}$ response. Collectively, such observations strongly support the concept of elevated $\text{Ca}_\text{i}$ and augmented $\text{Na}^{+}-\text{H}^+$ antiport activity in the platelets of patients with NIDDM, particularly when it is associated with elevated blood pressure. Because these changes are unlikely to relate to hyperinsulinemia, they either reflect the abnormal environment of NIDDM or represent a possible cause for insulin resistance. Findings in the platelets of essential hypertensives, who manifest insulin resistance without the profound metabolic abnormalities observed in NIDDM, suggest the latter possibility.

Erne et al. (183) reported in 1984 that $\text{Ca}_\text{i}$ in unstimulated platelets positively correlated with blood pressure. Subsequent studies yielded similar findings in stimulated and unstimulated platelets (184–188). These data were complemented by the observations that: (1) the $V_{\text{max}}$ for $\text{Ca}^{2+}$-ATPase in platelet membranes is lower in essential hypertensives, suggesting a reason for elevated basal $\text{Ca}_\text{i}$, in their platelets (189); and (2) the level of platelet cAMP is also reduced in essential hypertension—a perturbation that predisposes to exaggerated agonist-evoked $\text{Ca}_\text{i}$ response (190).

In a seminal investigation, using an indirect method for the measurement of the $\text{Na}^{+}-\text{H}^+$ antiport, Livne and coworkers (191) showed that the activity

![Diagram](image-url)
of this transport system was higher in platelets from hypertensives. Others subsequently documented similar findings (192, 193). We examined the Na\(^+\)-H\(^+\) antiport directly by monitoring the pH, and focused on the measurements of XI in human platelets (194). Our results implicate race-, gender-, and blood pressure–related variations as factors that influence the XI. Platelets from male and black subjects exhibited alkaline shifts in XI compared with female and white subjects, respectively. Furthermore, white males showed an increased alkaline shift in the XI associated with hypertension. Similar findings were recently documented by Livne et al. in hypertensive white patients (195).

According to the thesis proposed here, platelets that exhibit an alkaline shift in XI are more likely to show elevated Ca\(^{2+}\). However, concurrent measurements of Ca\(^{2+}\) and the Na\(^+\)/H\(^+\) antiport in platelets from hypertensives have not been reported. A recent study showed that Ca\(^{2+}\) is higher in platelets from male than female subjects (188). This observation is in agreement with the relatively alkaline XI in platelets from males (184). In addition, increased Ca\(^{2+}\) and augmented Na\(^+\)/H\(^+\) antiport activity were shown in white blood cells of essential hypertensives (196–199). The analogy between these observations in platelets and leukocytes from essential hypertensives to those from patients with NIDDM is clear. These data suggest perturbations in Ca\(^{2+}\) and pH, regulation in essential hypertension and NIDDM that are independent of hyperinsulinemia.

THE ROLE OF INSULIN IN ESSENTIAL HYPERTENSION

The support for a role for insulin in the development of essential hypertension is derived from conceptually simple observations. Insulin increases sympathetic activity (200–202), and plasma norepinephrine levels tend to rise in essential hypertension (for a review, see reference 203). Hyperinsulinemia is frequently associated with increased sympathetic activity (200, 204), whereas epinephrine can induce insulin resistance (205). In addition, insulin increases renal Na\(^+\) reabsorption (206; for a review, see reference 207) leading to extracellular volume expansion. However, the involvement of insulin in hypertension has been seriously challenged of late by the work of Hall and coworkers (208, 209), showing that chronic (up to 4 wk) insulin infusion in the dog caused a transient Na\(^+\) retention but did not produce an increase in the blood pressure. Additionally, chronic insulin infusion neither induced elevations of plasma catecholamines nor potentiated the hypertensive effect of norepinephrine. The role of insulin in essential hypertension was also questioned by the finding of Tsutsu et al. (210) that patients with insulinomas do not develop blood pressure elevations. These observations should, nonetheless, be put into perspective, because the dog may be an inappropriate model for insulin-evoked hypertension in humans and because the hypertensive effect of this hormone might be expressed only when coupled with a normal or elevated blood glucose, which is uncommonly the case in patients with insulinomas. Yet, the implications of the studies by Hall, Tsutsu, and their coworkers are weighty and they leave unresolved the question of the role of insulin in the evolution of essential hypertension.

Perhaps the answer to this question rests in the function of insulin as a growth factor rather than in its vasoactive or Na\(^+\)-retaining properties. As a growth factor, insulin would exert a hypertensive effect through protracted stimulation of VSM cells and other cellular elements in the vascular wall. This effect may therefore be seen only after years of hyperinsulinemia. Because insulin stimulates VSM cell proliferation (211, 212), the resulting gradual obliteration of the vascular lumen would contribute to increased peripheral vascular resistance associated with established essential hypertension.

The trophic effect of insulin may resolve a vexing dilemma concerning hyperinsulinemia, insulin resistance, and their roles in the pathophysiology of essential hypertension. How can insulin produce a deleterious effect in essential hypertension and NIDDM when target tissues demonstrate resistance to its action? One possibility is that not all tissues exhibit resistance to the hormone in essential hypertension or NIDDM. Another explanation may well rest in the observation that separate receptors exist for growth and metabolic actions of insulin or insulin-like factors (213). Chronic hyperinsulinemia can therefore be associated with both insulin resistance and enhanced growth or proliferation of cellular elements of the vascular wall. Such a concept converges on the original premise, i.e., at the cellular level, a major perturbation associated with both essential hypertension and NIDDM is a rise in Ca\(^{2+}\), coupled with augmented activities of PKC and the Na\(^+\)-H\(^+\) antiport. This triumvirate participates in the cellular action of both vasoactive agents and growth factors (134, 214–219; for reviews, see references 129–132, 220). In fact, some vasoactive agents that act through Ca\(^{2+}\), PKC, and the Na\(^+\)-H\(^+\) antiport may function as growth factors. Thus, if the damage of insulin in essential hypertension is mediated via its trophic effect, it is likely to involve Ca\(^{2+}\), PKC, and the Na\(^+\)-H\(^+\) antiport. It should be emphasized, nonetheless, that elevated Ca\(^{2+}\) and the augmented activities of PKC and the Na\(^+\)-H\(^+\) antiport are more likely to underlie the cause of insulin resistance rather than its consequence. Moreover, the hyperglycemia associated with insulin resistance can in itself influence tissue
growth and composition through modulating the expression of genes responsible for the biosynthesis of extracellular matrix components (221).

Ca, PKC, THE Na⁺-H⁺ ANTIPORT, INSULIN RESISTANCE, AND ESSENTIAL HYPERTENSION

Insulin resistance can now be incorporated into the paradigm linking Ca, with the Na⁺-H⁺ antiport in the evolution of essential hypertension (222). Originally, this model proposed that elevated Ca, and augmented activity of the Na⁺-H⁺ antiport would produce hypertension due to: (1) increased contractility of the VSM; (2) hypertrophy of the vascular media; and (3) in the case of salt-sensitive hypertension, increased Na⁺ reabsorption by the kidney. The specific cause for Ca, elevation may vary, because a rise in Ca, can arise from multiple causes. These include: (1) increased sensitivity to vasoactive agents and growth factors, expressed in enhanced Ca⁺⁺ entry through receptor-operated channels, increased Ca⁺⁺ mobilization from intracellular organelles, or both; (2) accelerated Ca⁺⁺ influx through voltage-gated Ca⁺⁺ channels; and (3) decreased capacity of Ca⁺⁺ extrusion or reduced sequestration into intracellular organelles by Ca⁺⁺ transport systems, e.g., the Ca⁺⁺-pump and the Na⁺/Ca⁺⁺ exchanger. However, implicit to this model is the concept that at the cellular level, processes responsible for vasoconstriction and salt sensitivity are in fact the same as those promoting the vascular and cardiac hypertrophy of long-standing hypertension. A rise in Ca, not only produces vasoconstriction, but in association with the stimulation of PKC and augmented Na⁺-H⁺ antiport activity, it exerts a trophic effect on the cardiovascular system. Such perturbed cellular dynamics also emerge as a pivotal element in insulin resistance.

Acting through PKC, a rise in Ca, would produce insulin resistance by blunting the effect of insulin on target tissues. The ensuing hyperinsulinemia would promote further insulin resistance. When left unchecked, the evolving spiral of decreased target tissue responses and the reaction of pancreatic β cells is likely to result in hyperinsulinemia, coupled with hyperglycemia, i.e., NIDDM. Sustained hyperglycemia would promote a more-pronounced insulin resistance by the de novo formation of DAG, which would act synergistically with Ca, to further stimulate PKC. Hyperinsulinemia, in turn, may exert a trophic influence on cardiovascular tissues independent of the metabolic effects of insulin.

The extent of involvement of specific target tissues (e.g., VSM, skeletal muscle, adipose tissue, cardiac muscle, liver, and kidney) and their homeostatic responses to blunt the aforementioned processes would dictate the final expression of the disease state. A plethora of pathophysiological manifestations may arise, ranging from fulminant NIDDM without much hypertension to essential hypertension with minimum insulin resistance. For instance, compensatory mechanisms in striated muscle may attenuate insulin resistance, whereas the same process in the VSM may slow the development of hypertension. According to this model, the drive for PKC stimulation is less in essential hypertension than in NIDDM, because NIDDM is expressed not only by hyperinsulinemia but also by hyperglycemia; the latter further enhances DAG formation. Thus, in NIDDM, there would be a tendency for further stimulation of PKC. However, even in NIDDM, there must be mechanisms by which the positive feedback loop for PKC stimulation, i.e., its translocation to the plasma membrane by a rise in Ca, and increased DAG, is interrupted. This can be accomplished by the down-regulation of the enzyme. A recent study showed that in mononuclear cells from patients with NIDDM in good glycemic control, there is a shift in the PKC from the cytosol to the plasma membrane (223), as predicted by the model. A further shift in PKC from the cytosol to the plasma membrane is exhibited by mononuclear cells from diabetic patients in poor glycemic control. However, cells from diabetics in poor glycemic control also demonstrate a decline in total cellular PKC activity coupled with a lack of PKC response to a phorbol ester, suggesting that the continuous stimulation of PKC in NIDDM results in PKC down-regulation.

PKC plays a dual role not only with respect to insulin action, but also in the control of Ca, homeostasis and vascular tone. The activation of PKC serves to sustain isometric forces of the VSM (224–226). However, the stimulation of the enzyme attenuates agonist-mediated Ca, responses through accelerating Ca⁺⁺ extrusion (146–149) and blocking the formation of inositol phosphates (227,228). Increased PKC activity would thus tend to lower the amplitude of Ca, oscillation in vivo—a state in which Ca, continuously oscillates because of exposure to fluctuating levels of a multitude of hormones, autacoids, and growth factors. A PKC-mediated reduction in Ca, would occur in concert with an alkaline shift of the Xi. However, the trade-off for this orchestrated response to lower Ca, oscillation and maintain pH, might be insulin resistance. It is this intricate and delicate balance between PKC, the Na⁺-H⁺ antiport, and other factors that produces the lasting imprint of distributed Ca, be it essential hypertension, NIDDM, or both. Theoretically, elevated Ca, may not be absolutely crucial for increased vascular tone, because alterations in the contractile apparatus of the VSM and its increased sensitivity to Ca, can also enhance vasoconstriction (for a review, see reference 229). Nonetheless, the evidence before us fingers Ca, PKC, and the Na⁺-H⁺ antiport as culprits both in insulin resistance and essential hypertension.
A number of previous investigations focus on the role of the Na⁺-H⁺ antiport in cellular alkalization. However, cellular alkalization is not synonymous with increased Na⁺-H⁺ antiport activity, and cellular resting pH may turn out to be less informative in illuminating the cellular perturbations in essential hypertension and diabetes. Agonist-mediated stimulation of the Na⁺-H⁺ antiport in a bicarbonate medium may not promote cytosolic alkalization (230,231), because the Na⁺-independent Cl⁻/HCO₃⁻ exchange serves to protect the cell from an alkaline overshoot (164–167). As previously indicated, the activation of the Na⁺-H⁺ antiport through an alkaline shift in the XI is primarily designed to protect the cell from acidification. In fact, under specific circumstances, the activity of the Na⁺-H⁺ antiport may decrease in concert with an alkaline shift in the XI. Boyarsky et al. (232) recently reported that experimentally induced diabetes in the rat resulted in an alkaline shift of the XI in hepatocytes in association with reduced activity of the Na⁺-H⁺ antiport at acidic pH, levels. The latter perturbation may result from an array of metabolic disturbances in diabetes. However, the alkaline shift in XI would tend to sustain the basal pH.

Because the expression of alterations in the parameters of the Na⁺-H⁺ antiport appears to reflect the underlying perturbations in Ca⁺ and PKC, it is improbable that abnormalities would be observed in the genetic makeup of this transport system in hypertensive patients. The findings of Lifton et al. (233) are in agreement with this concept. Because recent studies suggest the existence of more than one Na⁺-H⁺ antiport isomer (234), it is possible that in specific target tissues (e.g., renal tubules) chronic perturbations in Ca⁺ homeostasis would promote a greater expression of mRNA for one of the Na⁺-H⁺ antiport isomers.

Finally, the postulated Ca⁺-PKC–Na⁺-H⁺ antiport link in essential hypertension and NIDDM supports the polygenic origin of these diseases and the overlap in their clinical expressions. A rise in Ca⁺ can result from genetic variations in the expression of multiple cellular processes that regulate this ion. Likewise, cellular responses through PKC and other systems to attenuate the rise in Ca⁺ may demonstrate a spectrum of expressions dictated by the genetic makeup of the individual and by environmental factors. These considerations should be incorporated into future investigations of insulin resistance and essential hypertension.

CONCLUSION

What is so remarkable about essential hypertension is the diversity of the abnormalities it expresses at the cellular level. These are better understood because of the recognition that essential hypertension and NIDDM share a common characteristic, namely insulin resistance. Such a fact underscores the use of analogy as a powerful tool in biological research. At the cellular level, both Ca⁺ and PKC appear to mediate insulin resistance as well as promote rises in blood pressure and the expression of NIDDM. This concept is replete with implications for the treatment of essential hypertension and the prevention of its complications. In principal, normalization of Ca⁺ in specific target tissues should improve PKC status, enhance local blood perfusion, control the systemic blood pressure, and ablate insulin resistance.

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