G-Protein Function Is Reduced in Hypertension

Ross D. Feldman, Jozef Chorazyczewski

Abstract A functional impairment in vasodilator tone may be important in the pathogenesis and/or maintenance of elevated peripheral vascular resistance in hypertension. Previous studies of hypertensive subjects have demonstrated impaired β-adrenergic-mediated vasodilation paralleling a reduction in lymphocyte β-adrenergic-stimulated adenyl cyclase activity. We have suggested that this impairment is related to a defect in G-protein function. To determine whether this defect alters the coupling between the G-protein complex and adenyl cyclase, we performed [3H]forskolin binding studies in lymphocytes from hypertensive subjects, older normotensive subjects, and younger normotensive control subjects. Maximal specific [3H]forskolin binding was used as an index of adenyl cyclase binding sites. Gpp(NH)p-, NaF/AlCl3, and isoproterenol-stimulated binding were used as indices of G-protein/adenyl cyclase coupling. In the absence of other stimulators, maximal [3H]forskolin binding was not significantly different among groups. However, both Gpp(NH)p- and isoproterenol-stimulated [3H]forskolin binding were significantly decreased in lymphocytes from hypertensive subjects. Overall, Gpp(NH)p- and isoproterenol-stimulated [3H]forskolin binding were significantly inversely correlated with blood pressure. No differences in NaF/AlCl3-stimulated [3H]forskolin binding were detected between groups. These studies indicate that G-protein/adenyl cyclase coupling is impaired in lymphocytes from younger hypertensive subjects and may contribute to the blood pressure-related defect in β-adrenergic-stimulated adenyl cyclase activity (Hypertension. 1997; 29[part 2]:422-427.)

Key Words • G-proteins • adenyl cyclase • adrenoceptors

Impaired vasodilator function may be important in the elevation of peripheral resistance and hence in the pathogenesis and/or maintenance of the hypertensive state. Previous studies from our laboratory and those of others have shown impaired β-adrenergic-mediated vasodilation in younger predominantly white hypertensive subjects. Impaired β-adrenergic-mediated vasodilation has also been reported with aging but not by all investigators.

To explain the defect in β-adrenergic-mediated function with hypertension, our laboratory (and those of others) has focused on the β-adrenceptor/adenyl cyclase complex. Activation of this system is dependent predominantly on the interaction of three protein components (1) the receptor that contains the recognition site for hormone binding, (2) the enzyme adenyl cyclase that catalyzes synthesis of cAMP, and (3) the guanine nucleotide regulatory protein complex (G-protein), a heterotrimer that couples receptor occupancy with stimulation of catalytic activity. In the study of alterations in adrenergic responses in hypertension, the lymphocyte has been used as a model for the human vascular β-adrenceptor/G-protein/adenyl cyclase complex. The validity of this approach is based on several lines of evidence. First, previous studies have reported that lymphocyte isoproterenol-stimulated adenyl cyclase activity is correlated with isoproterenol-stimulated vasodilation. Second, indices of β-adrenceptor/G-protein coupling in lymphocytes (viz, β-adrenceptor-mediated adenyl cyclase activity and the ability to form the high affinity state of the receptor) are regulated in parallel with arterial and venous β-adrenergic responsiveness. Using this model, we and others have shown that lymphocyte β-adrenergic responsiveness is reduced in hypertension (as reviewed in References 14 through 16). This defect in lymphocyte β-adrenergic responsiveness may be predominantly characteristic of younger white hypertensive subjects. Additionally, with aging, impaired leukocyte β-adrenergic responsiveness has been reported by some but not all investigators.

In hypertension, the impairment in β-adrenergic-mediated response in lymphocytes has been related to a defect at the level of the receptor/G-protein interaction. Our most recent studies have suggested an impairment in stimulatory G-protein (Gs) function (but not expression). These studies also suggested a divergent pattern of alterations in G-protein function between hypertensive and older subjects.

Therefore, the current evidence to date would suggest that the impairment in lymphocyte β-adrenergic subjects was related to an impairment in G-protein function (at least in part) and differs from the alterations in β-adrenergic responses seen with aging. However, in human hypertensive subjects, the studies addressing this issue have depended almost entirely on assessment of catalytic activity determined from alterations of cAMP concentrations. These studies generally use membrane preparations and are dependent on a number of other modulators, including phosphodiesterases, ATP, and ions (eg, Ca2+) that selectively affect catalytic function. Our recent studies suggesting impaired G-protein-mediated effects were based on reduced cholera toxin-mediated ADP-ribosylation of the α subunit of Gs. However, the relationship between alterations in the extent of cholera toxin-mediated ADP ribosylation and G-protein function is indirect (as discussed in Reference ?). An alternative approach has been developed to assess G-protein/adenyl cyclase interactions in intact and permeabilized cells in-
dependent of the determination of cAMP levels. It utilizes the affinity of the diterpen forskolin to bind selectively to adenyl cyclase. Notably, the effects of forskolin on adenyl cyclase are modulated by G-protein interactions. The extent of forskolin-stimulated activation of adenyl cyclase is enhanced by an interaction with Gs. Furthermore, forskolin binding to adenyl cyclase is enhanced by stabilization of the interaction between the Go and adenyl cyclase. With this technique, G-protein function has been assessed by the extent to which G-protein- and/or receptor-specific ligands enhance specific [3H]forskolin binding. This approach has been used successfully to assess the regulation of G-protein function.

Therefore, to assess alterations in G-protein function in hypertension independent of the functional assessment of cAMP concentrations, we have determined the extent of G-protein–mediated enhancement of [3H]forskolin binding. The data to be presented demonstrate that G-protein–enhanced [3H]forskolin binding is reduced in lymphocytes from hypertensive but not older normotensive subjects. Further, G-protein–enhanced labeling is significantly inversely correlated with blood pressure independent of age, consistent with a blood pressure–related reduction in human lymphocyte G-protein function.

Methods

Subject Protocol

Younger and older normotensive subjects and younger borderline hypertensive subjects were studied. Younger normotensive subjects were between the ages of 20 to 36 years, healthy, and not taking any medications on a regular basis or any medications for at least 1 month before study. Older normotensive subjects were between the ages of 45 and 63 years, healthy, and not taking any medications on a regular basis or any medications for at least 1 month before study. Hypertensive subjects were all white, between the ages of 22 and 36 years, otherwise healthy, and had neither renal nor cardiovascular complications. Hypertensive subjects had not taken any antihypertensive medications (or any other mechanisms) for at least 1 month before study. The criteria for classifying blood pressure status of subjects were as described previously. The borderline or mildly hypertensive subjects had pressures >140/90 mm Hg on at least 20% of daytime automatic ambulatory blood pressure readings (Spacelab model 90207). All subjects were instructed to maintain a high sodium intake for 3 days before study. Compliance with dietary instruction was assessed by urinary sodium determinations based on overnight collections. The protocol was approved by the Human Subjects Review Committee, University of Western Ontario. Informed consent was obtained from each subject before study.

Assessment of [3H]Forskolin Binding in Human Lymphocytes

[3H]Forskolin binding was assessed in mononuclear leukocyte preparations. Mononuclear leukocytes were separated from whole blood by the method of Boyum and as described previously. Mononuclear cells were harvested via separation on Ficoll-Hypaque gradients and resuspended in Hanks’ balanced salt solution (HBSS, pH 7.4 at 4°C) with 33 mM HEPES, 0.5 mM EDTA, and 1 mM magnesium sulfate (buffer A), followed by centrifugation for 10 minutes at 400g and resuspension in buffer A with digitonin (10 μg/mL) for 15 minutes at 4°C. Cells were washed twice at 400g for 10 minutes at 4°C in buffer A without digitonin and resuspended in HBSS (pH 7.4 at 4°C) with 33 mM HEPES, 1.25 mM EDTA, and 5 mM magnesium sulfate. The cells were incubated at a concentration of 8 X 10⁶ cells/mL for 3 hours at 4°C. Initial studies showed [3H]forskolin binding achieved steady state before 3 hours at 4°C at the concentrations of radiolabeled used. Incubations were terminated by vacuum filtration with rapid washing (less than 5 seconds) with a 1/10 dilution of buffer A at 4°C. Radioactivity on filters was measured by liquid scintillation spectroscopy (Beckman LS 6000). Nonspecific binding was determined as the extent of [3H]forskolin binding in the presence of excess unlabeled forskolin (10 μM/L). At the [3H]forskolin concentrations used, nonspecific binding accounted for less than 25% of maximal G-protein–enhanced binding (i.e., in the presence of NaF/AlCl₃, see below). Binding site density and Kd for specific [3H]forskolin binding was determined by computerized nonlinear curve fitting (Inplot4, GraphPad Software).

G-protein–stabilized forskolin binding was assessed by the concubination with either the hydrolysise-resistant GTP analogue guanylylimidodiphosphate [Gpp(NH)p, 10 μM/L], isoproterenol (100 μM/L with Gpp(NH)p), or NaF/AlCl₃ (20 μM/L and 20 μM/L, respectively) and [3H]forskolin (50 μM/L, a concentration approximating the Kd for basal, Gpp(NH)p–, and isoproterenol-stimulated [3H]forskolin binding and a saturating concentration for NaF/AlCl₃–stimulated binding; see below). The enhancement of [3H]forskolin binding by the stimulants was expressed as a percentage of basal specific binding (i.e., [3H]forskolin binding in the absence of stimulators subtracted from nonspecific binding). This approach was chosen prospectively based on two major considerations. First, although absolute levels of adenyl cyclase activity may differ between lymphocyte subpopulations, relative stimulation is comparable. Secondly, in preliminary studies we determined that the coefficient of variation is lower when stimulated [3H]forskolin binding is expressed relative to basal specific binding.

Data Analysis

Because measures of potency and relative proportions are normally distributed when logarithmically transformed, these parameters are expressed as a geometric mean ± average SEM. Differences in binding were assessed by ANOVA followed by group comparisons as appropriate. A value of P < 0.05 on a two-tailed test was considered significant.

Results

Assessment of Specific [3H]Forskolin Binding

Forskolin mediated a concentration-dependent and saturable increase in binding. In initial saturation binding studies (n = 3), a Bmax of 5.0 ± 1.4 fmol/million cells (3012

![Graph](attachment:graph.png)

**Fig 1** [3H]forskolin binding saturation binding curves. Nonspecific binding was assessed using excess unlabeled forskolin (10 μM/L). Addition of NaF/AlCl₃ (20 μM/L and 20 μM/L, respectively) enhanced basal [3H]forskolin binding.
Alterations in \(^{3}H\)Forskolin Binding in Older and Borderline Hypertensive Subjects

No significant alterations in overnight sodium excretion were detected between older and hypertensive subjects versus younger normotensive control subjects. No significant difference in blood pressure was apparent between older and younger control subjects. Normotensive and hypertensive subjects were comparably aged. Body mass did not differ among the three groups (Table).

Basal specific \(^{3}H\)Forskolin binding (ie, binding in the absence of G-protein and/or \(\beta\)-adrenergic receptor stimulators) was not significantly altered in older and hypertensive subjects compared with younger normotensive controls (younger normotensives, 4.5±0.3 fmol/million cells [2710 sites/cell]; older normotensives, 5.2±0.3 fmol/million cells [3132 sites/cell]; younger hypertensives, 4.9±0.5 fmol/10\(^6\) cells [2951 sites/cell]). Isoproterenol- and Gpp(NH)p-enhanced \(^{3}H\)Forskolin binding were significantly reduced in lymphocytes from older subjects (Fig 2). In contrast, no alterations in isoproterenol- or Gpp(NH)p-stimulated \(^{3}H\)Forskolin binding were apparent in lymphocytes from older subjects (Fig 2). Further, no alterations in NaF/AlCl\(_3\)-mediated enhancement of \(^{3}H\)Forskolin binding were evident between hypertensive subjects and either older or younger normotensive groups (Fig 2). Overall, isoproterenol- and Gpp(NH)p-mediated enhancement of \(^{3}H\)Forskolin binding were highly correlated (\(r=0.76, P<0.001\), Fig 3). However, neither were correlated with basal \(^{3}H\)Forskolin binding.

Alterations in \(^{3}H\)Forskolin Binding in Hypertensive and Older Subjects

The ability of isoproterenol, Gpp(NH)p, and NaF/AlCl\(_3\) to increase \(^{3}H\)Forskolin binding in lymphocytes from younger normotensive subjects is compared with those from older normotensive and younger hypertensive subjects. Data represent the mean±SE. *P<0.05 vs younger normotensive subjects.

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**Age, Blood Pressure, Body Mass, and Sodium Excretion in Study Groups**

<table>
<thead>
<tr>
<th>Age, y</th>
<th>Younger</th>
<th>Older</th>
<th>Hypertensive</th>
</tr>
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<tbody>
<tr>
<td>27±1</td>
<td>49±2</td>
<td>27±2</td>
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</table>

**Mean arterial pressure (average 24-h), mm Hg**

<table>
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<th>Older</th>
<th>Hypertensive</th>
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<tr>
<td>87±1</td>
<td>89±2</td>
<td>99±2</td>
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**Body mass index, kg/m\(^2\)**

<table>
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<th>Younger</th>
<th>Older</th>
<th>Hypertensive</th>
</tr>
</thead>
<tbody>
<tr>
<td>23.2±0.6</td>
<td>23.9±0.6</td>
<td>23.9±1.2</td>
</tr>
</tbody>
</table>

**Sodium excretion, mEq/24 h**

<table>
<thead>
<tr>
<th>Younger</th>
<th>Older</th>
<th>Hypertensive</th>
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<tbody>
<tr>
<td>155±13</td>
<td>152±29</td>
<td>149±17</td>
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**Fig 3.** Correlation between Gpp(NH)p- and isoproterenol-stimulated \(^{3}H\)Forskolin binding (\(r=0.76, R^2=0.58, P<0.001\)).
binding (data not shown). Both isoproterenol-stimulated and Gpp(NH)p-stimulated [3H]forskolin binding were significantly inversely correlated with blood pressure (isoproterenol-stimulated [3H]forskolin binding versus 24-hour mean arterial pressure: \( r = -0.47, P = 0.02 \); Gpp(NH)p-stimulated [3H]forskolin binding versus 24-hour mean arterial pressure: \( r = -0.71, P = 0.04 \), Fig 4). In contrast, there was no correlation between age and either isoproterenol- or Gpp(NH)p-stimulated [3H]forskolin binding (data not shown).

**Discussion**

Alterations in receptor-stimulated and G-protein-stimulated adenyl cyclase activity have previously been demonstrated in lymphocytes from hypertensive subjects (as discussed above). Furthermore, alterations in G-protein function have been suggested based on reduced cholera toxin-mediated substrate labeling of the \( \alpha \)-subunit of the stimulatory G-protein. The present studies demonstrate that these alterations in lymphocyte G-protein properties parallel a reduction in G-protein-stimulated [3H]forskolin binding. In contrast, no alterations in G-protein-stimulated [3H]forskolin binding were apparent in lymphocytes from older normotensive subjects.

Forskolin has been previously shown to interact with adenyl cyclase with a high affinity, and the use of this reagent was instrumental in the original purification of the enzyme. It is notable that forskolin has cAMP-independent effects, including inhibition of glucose transport, of nicotinic receptor function, and of voltage-dependent potassium channels. However, these non-adenyl cyclase-mediated effects of forskolin are common to 1,9 dideoxyforskolin, a naturally occurring analogue of forskolin that neither inhibits high-affinity binding of [3H]forskolin nor activates adenyl cyclase. The high affinity of [3H]forskolin binding demonstrated in these studies, and lack of effect of 1,9 dideoxyforskolin in inhibiting [3H]forskolin binding, is consistent with an effect of forskolin binding to adenyl cyclase. In the lymphocyte, consistent with other models, both receptor and G-protein specific stimulators significantly enhance [3H]forskolin binding in respect to maximal binding as well as to binding affinity. The estimate of basal binding site concentration of 3012 sites/cell is quantitatively similar to \( \beta \)-adrenergic receptor density (of approximately 10^3 sites/cell-12).

The reduction in Gpp(NH)p- and isoproterenol [+Gpp(NH)p]-stimulated [3H]forskolin binding in lymphocytes from hypertensive subjects is consistent with an impairment in G-protein-mediated function. Several lines of evidence would suggest that the locus of this defect is at the level of the G-protein. First, specific basal [3H]forskolin binding was not altered between groups. Second, isoproterenol-mediated enhancement of [3H]forskolin binding was highly correlated with Gpp(NH)p-stimulated binding and comparably reduced in hypertensive subjects, suggesting a common mechanism of regulation. Last, in vitro studies demonstrated that pretreatment of lymphocytes with isoproterenol did not alter isoproterenol-stimulated [3H]forskolin binding under conditions that impair \( \beta \)-adrenergic-mediated adenyl cyclase activity. This would suggest that isoproterenol-stimulated [3H]forskolin binding is insensitive to the changes in receptor/G-protein coupling that occur with acute agonist exposure. These results parallel the finding in S49 lymphoma cells where agonist pretreatment of intact cells did not alter G-protein-stimulated [3H]forskolin binding assessed in permeabilized cells under conditions mediating homologous desensitization of the \( \beta \)-adrenergic receptor pathway.

NaF/AlCl\(_3\)-mediated enhancement of [3H]forskolin binding was not altered in lymphocytes from hypertensive subjects. This is particularly notable because our recent studies have reported impaired NaF-stimulated adenyl cyclase activity in lymphocytes from a similar group of hypertensive subjects. The explanation linking these findings is unclear. However, a previous report suggested that NaF-stimulated enhancement of [3H]forskolin binding is not a sensitive index of heterologous regulation of G-protein-stimulated adenyl cyclase activation. In that study, Gpp(NH)p-stimulated [3H]forskolin binding was a more sensitive index, perhaps related to the lesser potency of Gpp(NH)p in enhancing the [3H]forskolin binding. Our finding that NaF/AlCl\(_3\)-enhanced binding was unaltered in hypertensive subjects (assessed at a [3H]forskolin concentration associated with its "B_max" effect), although Gpp(NH)p- and isoproterenol-enhanced binding were reduced (assessed at a [3H]forskolin concentration approximating their K \( \text{B} \)\( \text{s} \)) is consistent with this result. Overall, these data would suggest that G-protein regulation as assessed by [3H]forskolin parallels...
a shift in the $K_d$ but not the $B_{max}$ for binding. Additionally, NaF is a tyrosine phosphatase inhibitor. Recent studies have suggested that tyrosine kinases may have additional G-protein–independent effects on adenyl cyclase.  

As discussed above, impaired G-protein–stimulated adenyl cyclase activity in lymphocytes from hypertensive subjects has been previously reported. Additionally, we have recently demonstrated a decrease in cholera toxin–mediated ADP ribosylation of Go, in lymphocytes from a comparable group of younger hypertensive subjects studied under identical conditions. In those studies, there were no alterations in Go, expression as assessed by immunodetection. Together with the present studies, a consistent impairment in G-protein function in lymphocytes from younger white hypertensive subjects has now been demonstrated using three distinct approaches.

Finally, these studies provide further evidence that alterations in lymphocyte β-adrenergic responses in hypertension are distinct from the pattern seen with aging. As discussed above, an impairment in lymphocyte β-adrenergic responsiveness has variably been seen with aging. However, these studies have been generally confounded by the age–dependent increase in blood pressure. In contrast, in the present study, blood pressures in older and younger normotensive groups were virtually identical. Our finding that overall G-protein–mediated enhancement of [3H]forskolin binding was inversely correlated with mean arterial pressure over all age groups studied is consistent with the hypothesis that age-related alterations in this transmembrane signaling process may be confounded with the age-related increase in blood pressure.

In summary, these studies have demonstrated the utility of [3H]forskolin binding in lymphocytes as a measure of G-protein function. Furthermore, they demonstrate an impairment in G-protein–mediated function specific to lymphocytes from hypertensive subjects. Overall, the present data, and those from previous studies, have suggested that in these younger white hypertensive subjects, an impairment in G-protein function may underlie the impairment in transmembrane signaling characteristic of the defect in the β-adrenergic receptor/G-protein/adenyl cyclase complex in the hypertensive state.

Acknowledgments

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References


20. Zahniser NR, Parker DC, Bier-Lamng CM, Miller IA, Gerber JG, Nies AS. Comparison between the effects of aging on agonist and agonist interactions with beta-adrenergic receptors on human mononuclear and polymorphonuclear leukocyte membranes J Gerontol 1984; 43:M151–M157


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