High-Fat Diet Elevates Blood Pressure and Cerebrovascular Muscle Ca²⁺ Current

Dixon W. Wilde, Kenneth D. Massey, Glenn K. Walker, Alan Vollmer, Roger J. Grekin

Abstract—Dietary fat contributes to the elevation of blood pressure and increases the risk of stroke and coronary artery disease. Previous observations have shown that voltage-gated Ca²⁺ current density is significantly increased in hypertension and can be affected by free fatty acids (FAs). We hypothesized that a diet of elevated fat level would lead to an increase in blood pressure, an elevation of L-type Ca²⁺ current, and an increase in saturated FA content in vascular smooth muscle cell membranes. Male Osborne-Mendel rats were fed normal rat chow or a high-fat diet (Ob/HT group) for 8 weeks. Blood pressures in the Ob/HT group increased moderately from 122.5±0.7 to 134.4±0.8 mm Hg (P<0.05, n=26). Voltage-clamp examination of cerebral arterial cells revealed significantly elevated L-type Ca²⁺ current density in the Ob/HT group. Voltage-dependent inactivation of the Ob/HT L-type channels was significantly delayed. Total serum FA contents were significantly elevated in the Ob/HT group, and HPLC analyses of fractional pools of FAs from segments of abdominal aorta revealed that arachidonic acid levels were elevated in the phospholipid fraction in Ob/HT. No differences in vascular membrane cholesterol contents were noted. Plasma cholesterol was significantly elevated in portal venous and cardiac blood samples from Ob/HT rats. These findings suggest that an elevation of plasma FAs may contribute to the development of hypertension via a process involving the elevation of Ca²⁺ current density and an alteration of channel kinetics in the vascular smooth muscle membrane. (Hypertension. 2000;35:832-837.)

Key Words: hypertension, obesity ■ calcium ■ ions ■ hyperlipidemia ■ obesity ■ chromatography

The ontogeny of hypertension in the spontaneously hypertensive stroke-prone rat involves significant alteration of the physiology of vascular smooth muscle cells (VSMCs), including alteration of the composition of the phospholipid bilayer.¹ The change in membrane composition is accompanied by increased numbers of L-type Ca²⁺ channels² and increases in membrane concentrations of long-chain fatty acids (FAs).³ This compositional change may contribute to increased membrane microviscosity and a reduced sensitivity to Ca²⁺-dependent membrane stabilization.⁴ Shifts in vascular smooth muscle membrane lipid (and protein) composition lead to increased ⁴⁴Ca²⁺ uptake.⁴ Calcium homeostasis in rabbit aortic smooth muscle cells is sensitive to intracellular cholesterol enrichment,⁵ in which enrichment with cholesterol-laden liposomes has been shown to increase L-type channel current⁶ and norepinephrine-gated Ca²⁺ influx.⁴

Recent reports describe a reduction in the risk of ischemic stroke with increased dietary fat.⁷ Increased dietary intake of ω3-FAs has been associated with a reduced risk of sudden cardiac death.⁸ In addition, the content of dietary fat may play a direct role in the genesis of hypertension and atherosclerosis.⁹ Thus, the quantity and type of dietary fat can have important effects on the development of cardiac disease, hypertension, and stroke. Transmembrane Ca²⁺ influx seems to be an important signal transduction mechanism affected by these membrane FA modifications. Diet-induced increases in membrane concentrations of linoleic acids have been shown to influence transmembrane Ca²⁺ flux in leukocytes,¹⁰ In hypertension, bilayer modification and increased density of voltage-gated Ca²⁺ channels influence vascular excitability, leading to increased vascular tone. Other studies have demonstrated that long-chain free FAs (FFAs) (eg, oleic, linoleic, arachidonic) increase L-channel current in ventricular myocytes and modulate dihydropyridine binding in cardiac myocytes, possibly through modification of the physicochemical properties of the lipid/protein interface.¹¹

Data from our laboratory and others have demonstrated increased levels of L-type Ca²⁺ channel current and increases in the ratios of L-type to T-type current in various VSMCs from hypertensive rats.¹²,¹³ However, there has been no indication of a change in the kinetics of the L-channel population with the hypertensive state, nor have any relationships been established among increased membrane microviscosity, hypertension, and Ca²⁺ channel activity. We hypothesized that dietary hyperlipidemia-induced hypertension involves increases in VSMC membrane levels of long-chain

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FAs, which may contribute to increased membrane Ca\(^{2+}\) influx through the augmentation of L-type Ca\(^{2+}\) channel current. Using a rat model of central obesity, we discovered that a high-fat diet induces modest increases in blood pressure accompanied by large increases in inward Ca\(^{2+}\) channel current (I\(_{Ca}\)) and a rightward shift in the voltage-dependent inactivation of the channel population. To our knowledge, this is the first observation of a significant shift in channel population kinetics in a hypertension model and is one that increases the window current for Ca\(^{2+}\) in these cells. We simultaneously studied the FA composition of the VSMC membrane to determine whether a change in the abundance of any nonesterified FAs (NEFAs) in the membrane parallels the altered biophysical properties of the cells leading to these delayed kinetics.

### Methods

#### Animal and Cell Models

Adult, male Osborne-Mendel rats were fed ad libitum either a control rat chow (Purina 5001; L/NT group) or a high-fat diet (Teklad TD95407; Harlan; Ob/HT group) supplemented with AIN-76 mineral mix (Harlan) and Teklad 40060 vitamin mix (Harlan) for a period of 8 weeks. A comparison of these diets is shown in Table 1. The Osborne-Mendel strain was chosen for its tendency to become obese when on a high-fat diet. Water was supplied ad libitum. The animals were maintained on a 12-hour day/night cycle. Blood pressures were monitored with tail-cuff plethysmography. Animals were anesthetized with 3% halothane (volume percent in 95% O\(_2\)/5% CO\(_2\)) in an anesthesia chamber and decapitated. Segments of abdominal aorta were harvested, cleaned of adventitia, weighed, and frozen in liquid N\(_2\) for lipid extraction and analysis. Brains were removed and placed into chilled 0.1 mmol/L Ca\(^{2+}\) Hanks’ buffered salt solution containing (in mmol/L) NaCl 140, KCl 5.4, KH\(_2\)PO\(_4\) 0.44, NaH\(_2\)PO\(_4\) 0.42, NaHCO\(_3\) 4.17, CaCl\(_2\) 0.1, HEPES 5, and glucose 5.55 at pH 7.3. Single relaxed VSMCs were isolated from major brain arteries and subjected to voltage clamp as described previously.\(^{12}\)

#### Isolation and Characterization of VSMC Membrane and Blood Lipids

Plasma NEFAs were assayed in fasted plasma samples assayed for total FFA through spectrophotometric analysis (NEFA kit; Wako).

#### Results

### Characteristics of the Ob/HT Rat Model

Systolic blood pressures, as measured with tail-cuff plethysmography after 8 weeks, averaged 122.5 ± 0.7 mm Hg for the L/NT rats and 134.4 ± 0.8 mm Hg for the high-fatOb/HT rats (P < 0.05, n = 26; Figure 1). L/NT rats exhibited an average body weight of 477.8 ± 17.6 g compared with 545.2 ± 26.6 g for the Ob/HT rats (P < 0.05). Total FFA content in plasma from L/NT and Ob/HT rats was 0.374 ± 0.032 mEq/L for L/NT rats and 0.812 ± 0.086 mEq/L (P < 0.05) for Ob/HT rats (Figure 1).

Hematocrits for each group were not significantly different, with a value of 50.4 ± 1.6% for L/NT rats and 52.1 ± 3.4% for Ob/HT rats (n = 10). An increase in red blood cell hemolysis was noted in blood samples from Ob/HT animals. To measure the observed hemolysis, absorbance measurements of plasma were made at 414 nm with a spectrophotometer (Beckman Instruments). Plasma from L/NT rats had an average absorbance value of 0.281 ± 0.018, whereas plasma from Ob/HT animals showed significantly higher levels of free hemoglobin, with an absorbance value of 0.837 ± 0.247 (P < 0.05, n = 10 per group).

### Passive Properties of L/NT and Ob/HT Cerebral VSMCs

Total membrane capacitance for cerebral VSMCs isolated from L/NT rats had a mean value of 16.16 ± 0.98 pF. In
VSMCs from Ob/HT rats, total cell capacitance averaged 16.04±0.94 pF (no significant difference from L/NT, n=34 cells). These values were taken from the null circuitry of the patch-clamp amplifier and were not significantly different from membrane capacitance values derived from the integrated area of the capacitative transient with the null circuitry turned off. These values may be considered to represent a rough measure of cell surface area and to indicate no differences in mean cell size after 8 weeks of a high fat or lean diet.

Voltage-Clamp Analyses of Ca^{2+} Channel Current
Inward voltage-gated Ca^{2+} channel current was significantly elevated in VSMCs from Ob/HT rats compared with L/NT control animals. In the lean VSMCs, \( I_{\text{Ca}} \) activated at \(-35 \text{ mV} \) and reached a maximum inward amplitude of \(-50.6\pm6.2 \text{ pA} \) at \(+20 \text{ mV} \) with an \(-80 \text{ mV} \) holding potential (\( V_h \)). In contrast, \( I_{\text{Ca}} \) in Ob/HT VSMCs activated at the same potential but achieved a maximum of \(-80.8\pm6.5 \text{ pA} \) at \(+20 \text{ mV} \) (\( P<0.05, \text{n}=27 \text{ cells} \)). Normalization of inward current to cell capacitance yielded values of \(-4.0\pm0.8 \text{ pA/pF} \) at \(+20 \text{ mV} \) for L/NT cells and \(-6.1\pm0.8 \text{ pA/pF} \) for Ob/HT cells (Figure 2), which represents a significant increase in current density in Ob/HT cells (\( P<0.05, \text{n}=23 \)). This strongly suggests an increase in Ca^{2+} channel conductance, an increase in Ca^{2+} channel number, or a change in channel kinetics. No evidence was obtained for the presence of T-type \( I_{\text{Ca}} \) in cells from either animal model based on digital subtraction of current records obtained at \( V_h \) of \(-80 \) and \(-40 \text{ mV} \). Current records obtained at \( V_h \) of \(-80 \) or \(-40 \text{ mV} \) (Figure 2) were robust and exhibited the typical slow decay of Ba^{2+} current.

We observed a significant rightward shift in the inactivation curve for OB/HT VSMCs compared with L/NT VSMCs (Figure 3A). In the L/NT VSMCs, Boltzmann fits to plots of relative conductance versus conditioning voltage yielded a sigmoid plot showing a half-maximal inactivation (\( V_{1/2} \)) of \(-14.0\pm1.9 \text{ mV} \). In contrast, \( V_{1/2} \) for Ob/HT VSMCs was \(-8.8\pm0.6 \text{ mV} \) (\( P<0.05 \) compared with L/NT). Slope factors...
VSMCs. For inactivation, activation (A) and activation (B) in L/NT and Ob/HT cerebral arterial decay of inward Ca$^{2+}$ not differ between 2 groups. C, Comparison of time constants for individual values as determined with ANOVA. Current activation did,

\[ \text{Activation in the voltage range of } E \text{ and } \text{Ob/HT cells} \]

\[ \text{exhibit significantly greater degrees of residual current acti-} \]

\[ \text{fication occurred at } V_c \text{ is significantly righthward shifted} \]

\[ (P<0.05, \text{unpaired } t \text{ test}) \text{ in Ob/HT cells.} \]

Significant differences for individual values as determined with ANOVA. Current activation did not differ between 2 groups. C, Comparison of time constants for decay of inward Ca$^{2+}$ channel current decay in L/NT (A) and Ob/HT (C) against step voltage. \( ^* P<0.05 \) with ANOVA.

for the curves were not significantly different. Comparisons of residual nonactivated current reveal that Ob/HT cells exhibit significantly greater degrees of residual current activation in the voltage range of \(-30 \text{ to } +10 \text{ mV.} \) At 0 mV, for example, the residual current in Ob/HT is 197.5% of the L/NT value. In contrast to the inactivation data, no differences were observed in the voltage-dependent activation curves for \( I_{ca} \) in the 2 cell types (Figure 3B). Half-maximal activation occurred at \(-5.0 \pm 2.0 \text{ mV for L/NT cells and at} \]

\[ \text{Ca in both cell types,} \]

\[ \text{Ob/HT cells} \]

\[ (P>0.05). \]

\[ \text{Slope factors for the Boltzmann fit curves were not different. These data indicate} \]

\[ \text{that the window current for} \]

\[ \text{in L/NT and Ob/HT cerebral arterial} \]

\[ \text{of inward Ca$^{2+}$ entry, which could result in an} \]

\[ \text{Elevated Vascular Muscle Ca}^{2+} \text{ Current} \]

\[ \text{Serum and Vascular Smooth Muscle Lipid Profiles in L/NT and Ob/HT} \]

\[ \text{Total plasma FFA content (Figure 1) was significantly} \]

\[ \text{increased in the Ob/HT animals compared with the L/NT} \]

\[ \text{control animals. HPLC results revealed that only arachidonic} \]

\[ \text{acid levels in the phospholipid component were significantly} \]

\[ \text{elevated in the Ob/HT animals (n=28) (Table 2). No other} \]

\[ \text{fractions exhibited signficant changes in the Ob/HT animals.} \]

\[ \text{In L/NT animals, membrane cholesterol level was}\]

\[ 31.3\pm9.8 \text{ mg/mg tissue wet wt, but the Ob/HT animals} \]

\[ \text{exhibited cholesterol values of } 25.4\pm2.7 \text{ mg/mg tissue} \]

\[ \text{wt } (P>0.05 \text{ compared with L/NT, } n=6). \]

\[ \text{Portal venous serum cholesterol concentration was significantly} \]

\[ \text{elevated in the Ob/HT group (to 105.06\pm5.90 \text{ mg/dL}) compared with} \]

\[ 65.96\pm7.5 \text{ mg/dL} \text{ in the L/NT control animals} \]

\[ (P<0.05, n=8). \]

\[ \text{Concentrations of serum cholesterol, which was taken} \]

\[ \text{from the heart via direct cardiac puncture, were} \]

\[ 60.63\pm7.59 \text{ mg/dL} \text{ in the L/NT rats and 95.74\pm5.00 \text{ mg/dL} in the Ob/HT} \]

\[ (P<0.05, n=8). \]

\[ \text{Data are separated by fraction and are presented as mean±SEM in nmol/mg wet wt tissue, with the numbers in parentheses indicating number of samples, each from one vessel. In some cases, samples from a particular fraction were below detectable limits. Data were analyzed with ANOVA } (* P<0.05). \]

%Data are separated by fraction and are presented as mean±SEM in nmol/mg wet wt tissue, with the numbers in parentheses indicating number of samples, each from one vessel. In some cases, samples from a particular fraction were below detectable limits. Data were analyzed with ANOVA (* P<0.05). Arachidonic acid levels were elevated only in the phospholipid fraction. Linoleic acid levels in the nonesterified FA fraction approached significance (P=0.1).\n
\[ \text{TABLE 2. NEFA Contents From Aortas as Determined Through HPLC Separation and Analysis} \]

\[ \text{FA Species} \]

\[ \text{Phospholipids} \]

\[ \text{Triglycerides} \]

\[ \text{NEFAs} \]

\[ \text{L/NT} \]

\[ \text{OB/HT} \]

\[ \text{L/NT} \]

\[ \text{OB/HT} \]

\| \text{L/NT} \|

\| \text{OB/HT} \|

\| \text{L/NT} \|

\| \text{OB/HT} \|

\| \text{L/NT} \|

\| \text{OB/HT} \|

\| \text{L/NT} \|

\| \text{OB/HT} \|

\| \text{L/NT} \|

\| \text{OB/HT} \|
Discussion

Ingestion of the high-fat diet elevated serum FA and cholesterol concentrations without inducing significant alterations in vascular smooth muscle membrane FA and cholesterol compositions. Despite a lack of effect on vascular membrane composition, the high-fat diet caused a modest elevation of systolic blood pressure and a significant increase in inward $\text{Ca}^{2+}$ current density in the Osborne-Mendel rat. Most significant was the rightward shift in the L-type $\text{Ca}^{2+}$ channel voltage-dependent inactivation curve in animals fed the high-fat diet. This suggests that a short period of high-fat diet intake may increase $\text{Ca}^{2+}$ channel numbers or alter channel regulation, leading to increased transmembrane $\text{Ca}^{2+}$ flux. In the cerebral arterial vessels used for these experiments, this process may have a significant impact on vascular reactivity. Although vascular reactivity was not examined in the cerebral vessels used in the present experiments, aortic responsive-ness to the $\text{Ca}^{2+}$ channel opener Bay K 8644 is elevated in animals fed the high-fat diet (J. Ritchey, personal communica-tion, same animals as used in the present study, 1998).

It is unclear what molecular mechanism underlies the increased $I_{\text{Ca}}$ density and shifted inactivation in the Ob/HT animals. Our working hypothesis that the high fat diet would increase VSMC levels of long-chain FAs, leading, perhaps, to a membrane that limited or slowed channel transitions from the open state to the closed state, has not been supported by the HPLC results from aortic muscle samples. The working hypothesis was centered on the notion that an increase in $\text{Ca}^{2+}$ channel activity can contribute to increased tone in the vessels. The data show increased $\text{Ca}^{2+}$ current density, delayed channel inactiva-tion, and elevation of total plasma NEFA and arachidonic acid levels in the VSMC membrane, all in the face of only a modest increase in blood pressure. In other studies that demonstrate elevated $\text{Ca}^{2+}$ current density in hypertension, the elevation in current density is associated with significantly elevated systolic pressures rather than the borderline hypertension observed in this study.

Research by Huang et al.\textsuperscript{11} showed activation of $\text{Ca}^{2+}$ channels in myocardial cells by long-chain NEFAs (including arachidonic, oleic, linoleic, and so on). The activation of $I_{\text{Ca}}$ was independent of the activities of protein kinases A and C, G proteins, eicosanoid production, or nonenzymatic oxida-tion, strongly suggesting a direct effect of the FAs on the L-type channel. The proposed mechanism involved either the alteration of the local lipid domain of the channel or direct interaction of the FA with the channel. Although individual FAs may protect the myocardium in some cases and contrib-ute to its injury in others, their role in vascular smooth muscle ion channel regulation is less clear. Our recent evidence demonstrates that linoleic acid, for example, can act as vascular smooth muscle hyperpolarizing factor by stimulating Na$^+$/K$^+$-ATPase activity.\textsuperscript{16} In rabbit coronary VSMCs, long-chain FAs were more effective than short-chain species in directly increasing maxi-K$^+$ (BK) channel activity.\textsuperscript{17} $\Omega3$-Polyunsaturated FAs (eg, eicosapentaenoic, docosahexaenoic) also inhibit receptor-mediated nonselective cationic currents in cultured A7r5 cells.\textsuperscript{18} Arachidonic and linoleic acids, although still inhibitory, showed much less effect in this system, whereas oleic and stearic acids showed no inhibition. Although evidence suggests an ion channel inhibitory action of individual FAs in vascular smooth muscle cells, the activation of L-type channels in myocardium leaves some room for speculation that these molecules may exert different actions at different sites. We have been unable to correlate changes in specific VSMC membrane FAs and cholesterol with the increased $\text{Ca}^{2+}$ current density and altered inactivation properties in the Ob/HT rats. Thus, it may be that mechanisms secondary to the hyperlipidemia contrib-ute to increases in $\text{Ca}^{2+}$ channel current activity.

There is a rightward shift in the inactivation kinetics for $I_{\text{Ca}}$ in the Ob/HT animals. Because current activation kinetics were not different in the 2 groups, the delayed inactivation in the Ob/HT animals indicates a larger “window current” for $\text{Ca}^{2+}$ entry. This shift in kinetic properties for the L-channel population may be important in that it is evident even at membrane potentials around $-40$ to $-30$ mV and therefore in the range of depolarization that these cells may experience in vivo. What is surprising in this study is that a relatively large increase in inward $\text{Ca}^{2+}$ current density occurs with only a modest increase in systolic pressure. This hints at a lack of synchrony between pressure elevation and increased inward current. Recent data from our group show that $I_{\text{Ca}}$ may be elevated in both Wistar-Kyoto and spontaneously hyperten-sive stroke-prone rats fed the high-fat diet, despite a reduction of blood pressure in both fat-fed groups compared with control animals fed normal chow (D.W.W. and D.F. Bohr, unpublished observations, 1998). The molecular mechanism for the increase in $I_{\text{Ca}}$ density observed in the Ob/HT animals remains undiscovered. Our revised working hypothesis, based on current data, is that elevated serum FA levels have a direct influence on vascular smooth muscle membrane functions. It is possible that these effects are centered in the lipid annulus of the channel protein.

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References


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