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An endogenous 'hypertensive factor' enhances the voltage-dependent calcium current

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The effects of an immunoaffinity-purified putative endogenous hypertensive factor (HF) on voltage-dependent calcium current in frog cardiac myocytes were assessed. In 9 out of 10 cells, HF reversibly increased the peak amplitude of the calcium current. HF increased peak calcium current density at −5 mV from a control level of 1.8 ± 1.3 pA/pF (mean ± SD) to 4.4 ± 2.0 pA/pF. HF shifted the peak of the calcium current-voltage relationship in the hyperpolarizing direction. HF shifted the voltage dependence of the inactivation of the calcium current to more negative potentials with prepulses from −80 to 0 mV, but the inactivation was not affected with prepulses more positive than 0 mV. Modulation of the voltage-dependent calcium current by HF may be the mechanism underlying its pressor effects.

Ca^{2+} current; Ca^{2+} channel; Hypertension

1. INTRODUCTION

A factor has been isolated from the blood of spontaneously hypertensive rats which elevates blood pressure in normotensive animals and increases lanthanum-resistant calcium uptake by aortic rings [1]. Recent contractility studies have demonstrated that preincubation of aortic rings with this hypertensive factor (HF) had no effect on resting tension, but enhanced potassium- or norepinephrine-induced contractions [2]. This observation suggests that depolarization is required for HF to have an effect on contractility. A hypothesis consistent with these data is that HF is acting on voltage-dependent calcium channels to enhance calcium influx.

HF has been tentatively identified as a low molecular mass (700–800 Da) peptide [3,4]. This compound has now been further purified by monoclonal antibody affinity chromatography and we have examined its effects on the voltage-dependent calcium current (I_{Ca}).

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Abbreviations: HF, hypertensive factor; I_{Ca}, calcium current

2. MATERIALS AND METHODS

2.1. *Electrophysiological recordings*

I_{Ca} was recorded from ventricular myocytes isolated by enzymatic dissociation from the heart of adult *Rana catesbiana* [5,6]. These cells have been shown to contain only one type of Ca channel [7], the well-characterized dihydropyridine-sensitive (L-type) Ca channel. Suction electrodes were used for whole cell recordings. Interference from other ionic currents was minimized by including Cs in pipette and bath solutions to block K currents and tetrodotoxin in the bath to block the fast Na current. Control Ringer solution consisted of (in mM): 103 NaCl, 20 $CsCl_2$, 1.8 $CaCl_2$, 1.8 $MgCl_2$, 5 Na pyruvate, 5 glucose, 10 Hepes, 0.0003 tetrodotoxin; pH 7.4 with NaOH. The recording pipette contained (in mM): 103 $CsCl_2$, 4 $MgCl_2$, 5 EGTA, 5 creatine phosphate, 10 Hepes, 3 ATP, 0.4 GTP; pH 7.1 with KOH.

2.2. *HF purification*

HF was initially purified from rat erythrocytes by molecular sieve and ion exchange chromatography as in [3] and has the following composition: Asp/Asn (1.41), Ser (1.02), Glu/Gln (1.00), and Gly (2.00). Further purification was achieved by affinity chromatography using a mouse anti-HF antibody. Brief-

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ly, BALB/c mice were immunized with a HF-ovalbumin conjugate. Hybridomas producing monoclonal antibodies directed against HF were identified by means of an ELISA assay [8]. Subsequently, the antibody was produced by growing hybridoma cells as ascites tumors in BALB/c mice. The monoclonal antibody present in the ascites fluid was purified by affinity chromatography using protein A AvidGel F (BioProbe International) and an affinity column for purification of HF was prepared by covalently linking the anti-HF monoclonal antibody to FMP AvidGel F. The peptide-containing fraction was dissolved in distilled water and recirculated through the column a miminum of three times. The column was washed with 5 bed volumes of distilled water, followed by 5 bed volumes of 0.01 M sodium acetate buffer, pH 4.5, containing 0.5 M NaCl, and again washed with distilled water. Material specifically bound to the column was eluted with 0.1 M acetic acid and the eluant lyophilized. Bioassay (enhancement of potassium-induced contraction of rat aortic rings) of the lyophilized fractions indicated that activity was present only in the eluant obtained from the acetic acid wash. Following acid hydrolysis this fraction gave a positive ninhydrin reaction. A control, prepared by treatment of the column as described above except that the peptide-containing fraction was not circulated, indicated no biological activity nor the presence of ninhydrin-positive material in the acetic acid eluant.

2.3. *HF activity*

HF activity was determined by assessing the ability of the purified fraction to enhance potassium- and norepinephrine-induced contractions of rat thoracic aorta. We have used a quantity of HF sufficient to enhance these contractions by 40%. When the molar content of peptide was estimated by amino acid analysis [2], a similar enhancement of contraction was observed with a peptide concentration of 10^{-8} M.

3. RESULTS

Our first objective was to determine if HF affected the peak amplitude of I_{Ca}. I_{Ca} was activated by a voltage step from -80 to -5 mV for 200 ms every 8 s and the peak amplitude was measured as the difference between the peak inward current and the current at the end of the pulse. Addition of HF resulted in an enhancement of I_{Ca} (fig.1A). HF increased the peak amplitude of I_{Ca} in 9 out of 10 cells tested. The percentage increase of peak I_{Ca} varied from 55 to 750% (mean = 340%, $n = 9$). Under control conditions, the peak calcium current density averaged 1.8 ± 1.3 pA/pF. This was increased to 4.4 ± 2.0 pA/pF in the cells which responded to HF ($n = 9$). The increase in peak I_{Ca} (fig.1B), upon addition of HF, showed a rapid onset and was sustained throughout exposure to HF. Upon return to control Ringer solution, I_{Ca} rapidly came back to basal levels.

A current-voltage relation for the peak I_{Ca} was

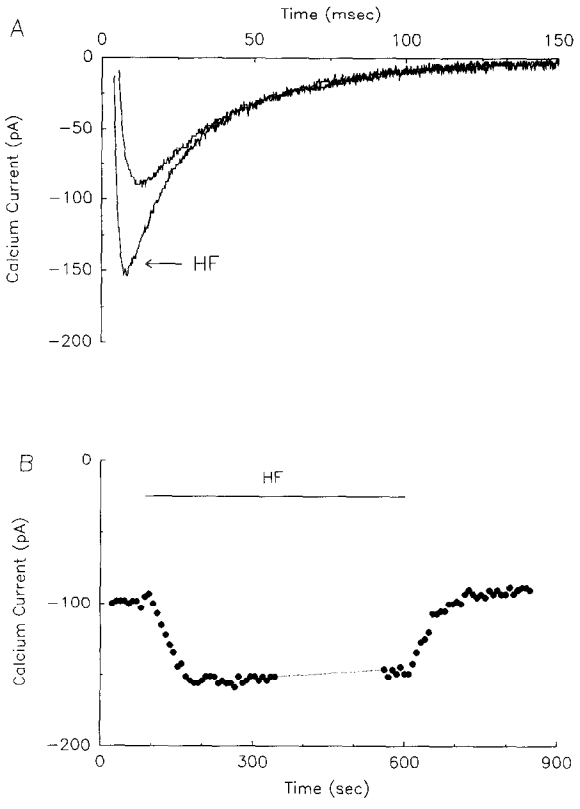

Fig.1. Effect of HF on the voltage-dependent calcium current. I_{Ca} was activated by 200 ms voltage steps from -80 to -5 mV every 8 s. Test solutions were applied by single cell superfusion. (A) Superimposed tracings of I_{Ca} before and during application of HF. (B) Peak calcium current, measured as the difference between the maximum inward current and the current at the end of the pulse, plotted against time. The cell was initially superfused with control Ringer solution and then was exposed to HF-containing Ringer for the period indicated by the bar. Current-voltage, inactivation, and reactivation protocols for I_{Ca} were run during the period indicated by the thin line.

obtained in 3 cells with and without HF (fig.2). HF resulted in an increase in the peak amplitude of I_{Ca} at test potentials in the range of -30 to 20 mV. HF also shifted the peak of the *I-V* curve towards the hyperpolarizing direction. There was no change in the I_{Ca} recorded at 27 mV with HF.

Fig.3 illustrates the voltage dependence of the inactivation of I_{Ca} with and without HF. Inactivation was assessed by measuring I_{Ca} at 0 mV following a 400 ms prepulse to potentials from -80 to 60 mV. This 400 ms prepulse was sufficiently long to ensure that the calcium current was in steady-state inactivation. The inactivation curve exhibited a

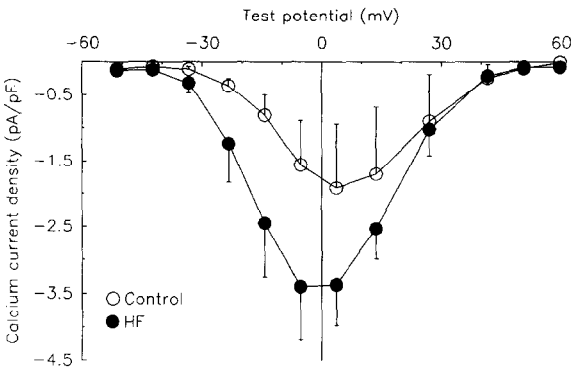

Fig.2. Effect of HF on calcium current-voltage relationship. Peak I_{Ca} in the presence (●) and absence (○) of HF was measured following 200 ms voltage steps from −80 mV and expressed as a fraction of whole cell capacitance. Points are mean of three cells. Error bars represent standard deviations.

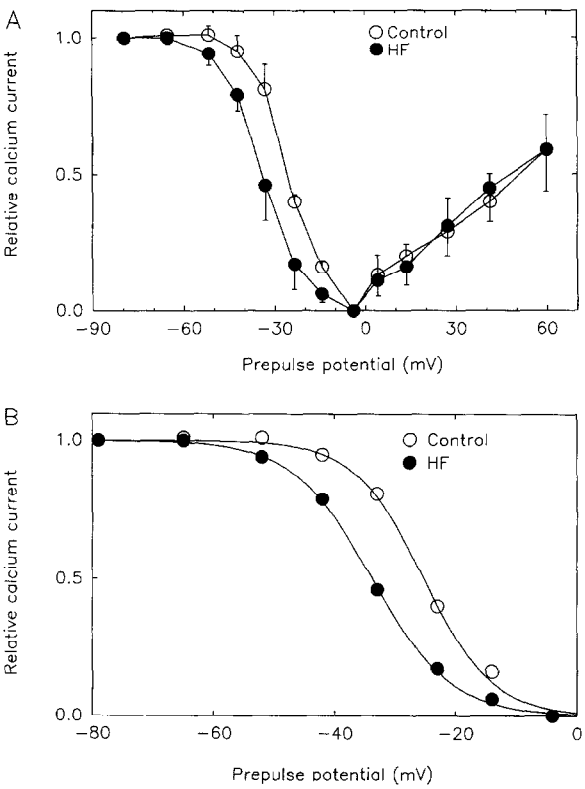

Fig.3. Effect of HF on the voltage dependence of calcium current inactivation. (○) Control, (●) HF. (A) Means and SD of calcium current inactivation measured between −80 and 60 mV. $n = 4$. (B) Fit of data in A between −80 and 0 mV. The points represent the means from A. The lines are the non-linear least-squares fits of the data to $1/(1+\exp\{(E_m - E_{1/2})/s\}$. For control $E_{1/2} = -25$ and $s = 5.6$ and for HF $E_{1/2} = -34$ and $s = 6.4$.

characteristic U-shape (fig.3A) [7]. In the presence of HF, the inactivation of I_{Ca} was greater at more negative potentials. Above 0 mV, however, there was no difference in the degree of inactivation observed with or without HF. To quantify the effects of HF on inactivation, the inactivation curve between −80 and 0 mV was fitted to the relation $1/\{1+\exp(E_m - E_{1/2}/s)\}$ (fig.3B). HF shifted $E_{1/2}$ from −25.2 to −37.8 and increased the slope factor from 5.6 to 6.4. The reactivation, or time course of the recovery from steady-state inactivation, of the calcium current, was measured at 0 mV [7]. HF did not affect reactivation of I_{Ca} ($n = 3$).

4. DISCUSSION

These data clearly demonstrate that HF modulates I_{Ca} by enhancing the amplitude of the current and by shifting the current-voltage and inactivation relationships in the hyperpolarizing direction. These effects of HF are similar to those seen in the presence of dihydropyridine calcium channel agonists [9–12] and less similar to those seen following phosphorylation of the calcium channel [5,7,13]. Inactivation of I_{Ca} is thought to consist of both voltage- and calcium-dependent components [14]. Since HF does not alter inactivation at positive potentials, it is likely that HF affects only the voltage-dependent component of inactivation.

Recently, several peptides have been reported as modulators of calcium channel activity. Angiotensin II increases I_{Ca} recorded from cultured rat cardiac myocytes [15]; however, it does not shift the current-voltage relationship and results in a slight depolarizing shift in the inactivation curve. Thrombin also increases I_{Ca} but does not result in a change in the voltage sensitivity of I_{Ca} [16]. Furthermore, the chemical composition of HF is not similar to these polypeptides [3]. An endogenous peptide purified from rat brain has recently been shown to affect calcium channels. This compound does enhance L-type I_{Ca} in heart and blocks it in neurons. The onset of its action is slower and its amino acid composition is different from HF [17].

The pressor and contractile properties of HF may be explained by its effects on the calcium current. Of course, to relate these effects on I_{Ca} to hypertension, it will be necessary to duplicate these experiments on smooth muscle.

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