THE EFFECT OF NATRIURETIC PEPTIDES ON NA^+ AND CA^{2+} CHANNELS OF MAMMALIAN CARDIAC MYOCYTES

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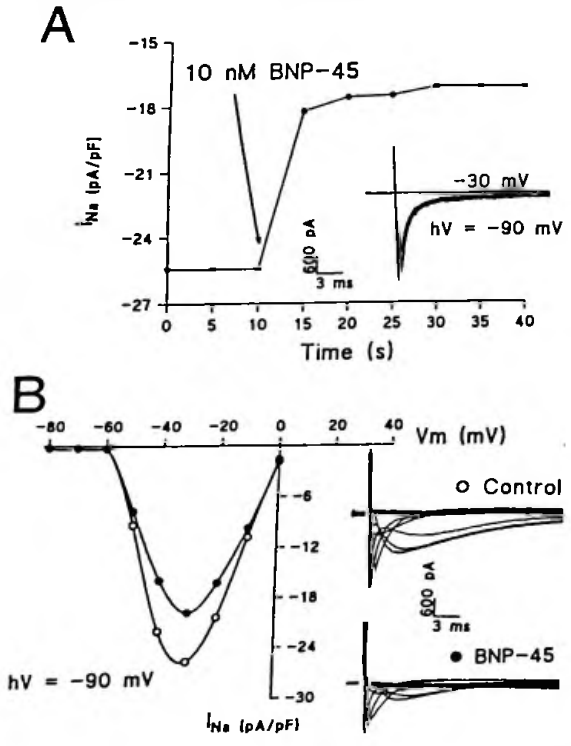
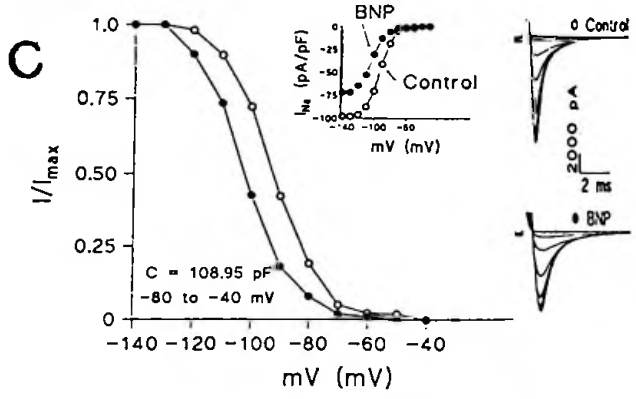
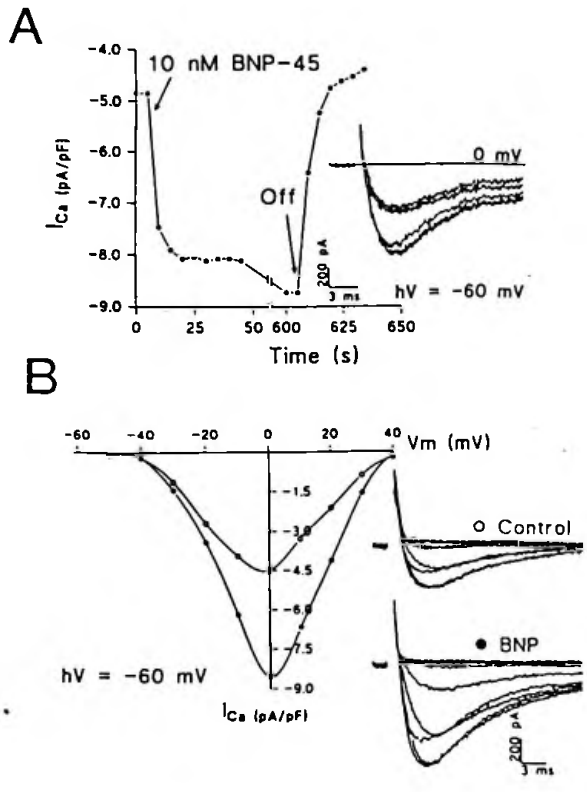
Recently, we reported that ANP (rat, 1-28 a.a.) modulates cardiac Na^+ channels by making them more selective to Ca^{2+} (Sorbera and Morad, Science 247:969, 1990). Two other natriuretic peptides (BNP and CNP) with sequence homology to ANP have been identified and are secreted from the mammalian ventricle (Sudoh et al. Nature 332:78, 1988; Sudoh et al. Biochem. Biophys. Res. Commun. 168:863, 1990). We examined the effect of BNP-45 (rat, 51-95 a.a) and CNP (porcine, 22 a.a.) on I_{Na} and I_{Ca}. Single rat and guinea pig ventricular myocytes were enzymatically isolated (Mitra and Morad, Am. J. Physiol. 249: H1056-H1060, 1985) and whole cell clamped (Hamill et al. Pflügers Arch. 391:85-100, 1981). Ventricular cells with capacitances ranging from 40 to 140 pF were used. The standard external solution contained (in mM): 137 NaCl; 1 $MgCl_2$; 2 $CaCl_2$; 10 HEPES; 10 glucose titrated to pH 7.4 with NaOH. In order to control I_{Na}, $[Na^+]_o$ was reduced to 10 mM and replaced with 127 CsCl. Patch pipettes with resistances of 1 to 3 MΩ were used. The standard internal dialysate contained (in mM): 10 NaCl; 110 CsCl; 5 MgATP; 20 HEPES; 14 EGTA titrated to pH 7.2 with CsOH. In internal solution where GTP (guanosine triphosphate) was added, 1.0 mM $MgCl_2$ in addition to 5 mM MgATP was also included. A rapid (< 50 ms), electronically controlled concentration clamped system was used to externally deliver the hormones to the individual myocyte. I_{Na} was activated by 15 to 30 ms depolarizing pulses applied at 5 s intervals of -30 or -40 mV from holding potentials of -70, -80 or -90 mV. In Figure 1A, I_{Na} was activated by a depolarizing pulse to -30 mV from a holding potential of -90 mV. BNP (10 nM), like ANP, suppressed I_{Na} rapidly (< 50 ms) and reversibly 40.7 ± 2.5 % (n=44) in a dose-dependent manner and at all voltages tested. BNP also shifted the steady state inactivation of I_{Na} by 10-15 mV (Figure 1C), but unlike ANP, BNP did not alter the selectivity of the channel as measured by the change in the reversal potential of I_{Na} (Figure 1B).



During continuous exposure to BNP, I_{Na} slowly recovered, suggesting possible desensitization of the response. CNP (1-100 nM) had no consistent effect on I_{Na} in the same set of cells. In GTP (0.5 mM) dialyzed cells, BNP's suppressive effect on I_{Na} was reduced to 10.1 ± 1.8 % (n=15). Dialysis with 1 mM GDPß-S (guanosine 5'-O-(2-thiodiphosphate) did not alter the suppressive effect on I_{Na}.



We also examined the effect of BNP and CNP on I_{Ca}. I_{Ca} was activated at 5 s intervals by 15 to 30 ms depolarizing pulses to 0 mV from holding potentials of -30, -40, -50, or -60 mV. In Figure 2A, I_{Ca} was activated by a depolarizing pulse to 0 from a holding potential of -60 mV. BNP (10 nM) rapidly enhanced I_{Ca} 163.40 ± 7.1 % in some cells (n=37, Figure 2B), but in others suppressed I_{Ca} by 21.44 ± 1.5 % (n=38). In cells dialyzed with 0.5 mM GTP, BNP only suppressed I_{Ca} by 25.27 ± 11.4 % (n=7); and no enhancing effect was ever found. BNP had little or no effect on I_{Ca} in cells dialyzed with GDPß-S. CNP, once again, had no consistent effect on I_{Ca}.



Thus when the Na^+ or Ca^{2+} channels were phosphorylated, the effect of BNP on the Na^+ channel was significantly reduced or completely reversed in the case of the Ca^{2+} channel. Our experiments suggest specific and differential effects of natriuretic peptides ANP, BNP, and CNP on the Na^+ and Ca^{2+} channel. While ANP regulates the Na^+ channel by altering its selectivity, BNP modulates the Na^+ channel by regulating its gating. CNP, on the other hand, appears to have minimal involvement in the regulation of cardiac Ca^{2+} and Na^+ channels.

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