NH_2- AND -COOH TAILS MEDIATE INDEPENDENTLY THE EFFECTS OF ANP ON Na^+ AND Ca^{2+} CHANNEL OF MAMMALIAN (Rattus norvegicus) CARDIAC MYOCYTES

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Atrial natriuretic peptide (ANP) regulates the cardiac Na^+ channel by enhancing its Ca^{2+} selectivity (Sorbera, L.A. and M. Morad, Science 247:969, 1990; Sorbera, L.A. and M. Morad, Science 252:449,1991). It is evident that this transformation of the channel can only be attributed to the ANP molecule as brain natriuretic peptide (BNP) and cardiac natriuretic peptide (CNP) cannot induce this modification of the Na^+ channel (Sorbera, L.A. and M. Morad, Bulletin 31:124-125, 1992). ANP also suppresses the L-type Ca^{2+} channel in mammalian and frog ventricular myocytes but the kinetics of this effect are relatively slow (~ 20 s). This is consistent with the idea that the ANP-induced suppression of the cardiac Ca^{2+} channel is mediated through binding of ANP to a specific receptor and activating a cGMP generating pathway (Sorbera, L.A. and M. Morad, 1990; Gisbert, M.-P. and R. Fischmeister, Circ. Res. 62, 660-667, 1988). On the other hand, the effect of ANP on the cardiac Na^+ channel is: (i) rapid and reversible (< 50 ms); (ii) independent of the cAMP-dependent phosphorylation of the Na^+ channel; (iii) independent of regulatory G proteins; (iv) not rapidly desensitized in the presence of ANP. These findings suggest that ANP is directly interacting with the channel protein or a novel receptor closely associated with the channel.

The structure of the ANP molecule is known to be essential for its biological function. ANP consists of a central 17 amino acid ring formed by a disulfide bridge between cysteine residues at position 7 and 23 (see Figure 1A). We used several synthetic ANP analogues (Genentech, Inc.) in an effort to determine the structure-function aspects involved in the regulation of the Na^+ and Ca^{2+} current by ANP. In particular, we attempted to identify the minimum molecular size of the ANP peptide necessary to enhance the Ca^{2+} selectivity of the cardiac Na^+ channel.

Figure 1.

In whole cell clamped rat ventricular myocytes dialyzed with 10mM Na^+, ANP analogues were rapidly applied (>50ms) in solutions containing 10mM Na^+ to effectively control I_{Na}. Figure 2A shows a typical time course of rANP1-28 induced enhancement of a Na^+ current activated at -40mV from a holding potential of -90mV. rANP1-28 enhanced I_{Na} by 172.0 ± 9.7 %, increasing Na^+ current density from -22.3 ± 3.5 to -51.8 ± 3.9 pA/pF (SEM, n=13). Enhancement of I_{Na} was rapidly reversible upon removal of rANP1-28 (Figure 2c). rANP1-28 also suppressed the Ca^{2+} current by 32.0 ± 4.7% (SEM, n=13). I_{Ca} was activated by depolarizing pulses to 0mV from a holding potential of -60mV. The ANP-induced suppressive effect was also reversible upon removal of rANP1-28. The effect of various analogues was compared to those of

hANP1-28 and rANP1-28 (Figure 2B). Only those analogues with the amino terminal tail intact, hANF01 and hANF03, produced equivalent effects to the native ANP on the Na^+ channel, i.e. enhanced the Na^+ channel current by 63.6 ± 8.3 % (n=8) and 94.0 ± 1.2 % (n=8) respectively, and shifted the reversal potential by 20-30mV. hANF01, hANF03, rAPI, and rAPII which have deletions on the carboxyl terminal making them less effective guanylate cyclase activators, were ineffective in suppressing the Ca^{2+} channel, an effect reported to be mediated by cGMP (Gisbert & Fischmeister, Circ. Res. 62:660,1988; Sorbera & Morad, Science 247:969,1990). Urodilation of ANP (urodilatin) or oxidation of methionine on the ring of the ANP molecule also blocked the ANP effects on the Na^+ channel. hANP7-28, hANF06, h & rANP3-28, hANP4-28, hANP5-28, and rAPIII with deletions in the amino tail but with an intact carboxyl tail, suppressed only the Ca^{2+} channel by 14.1 ± 3.2 to 29.5 ± 5.8% (SEM, n=54). hANF09 with deleted carboxyl and amino tails but an intact S-S ring structure, failed to alter either the Na^+ or the Ca^{2+} channel. h & rANP8-15 which block ANP-C receptors, failed to alter the Ca^{2+} enhancing properties of ANP1-28 on the Na^+ channels. Finally, to determine if the amino tail alone is in fact the segment of the ANP molecule responsible for the observed alteration of the selectivity of the Na^+ channel, analogue, ANP1-6 (m.w. 704.78) was tested. ANP1-6 (Figure 1B) consists of only the amino tail, S-L-R-R-S-S. This analogue had no significant effect on the Ca^{2+} channel, but enhanced I_{Na} by 145 ± 22% increasing the current from -16.6 ± 1 to -40 ± 3.1 pA/pF (SEM, n=7, Figure 2B). ANP1-6 also shifted the reversal potential for Na^+ 30 to 40mV in a more positive direction. These results definitively assign the amino tail as the portion of the ANP molecule which is interacting with the Na^+ channel resulting in enhancement of the Ca^{2+} selectivity of the Na^+ channel. The carboxyl tail appears, on the other hand, to be required for the I_{Ca} suppressing effect of the hormone. (Supported by NIH grant no. HL16152)

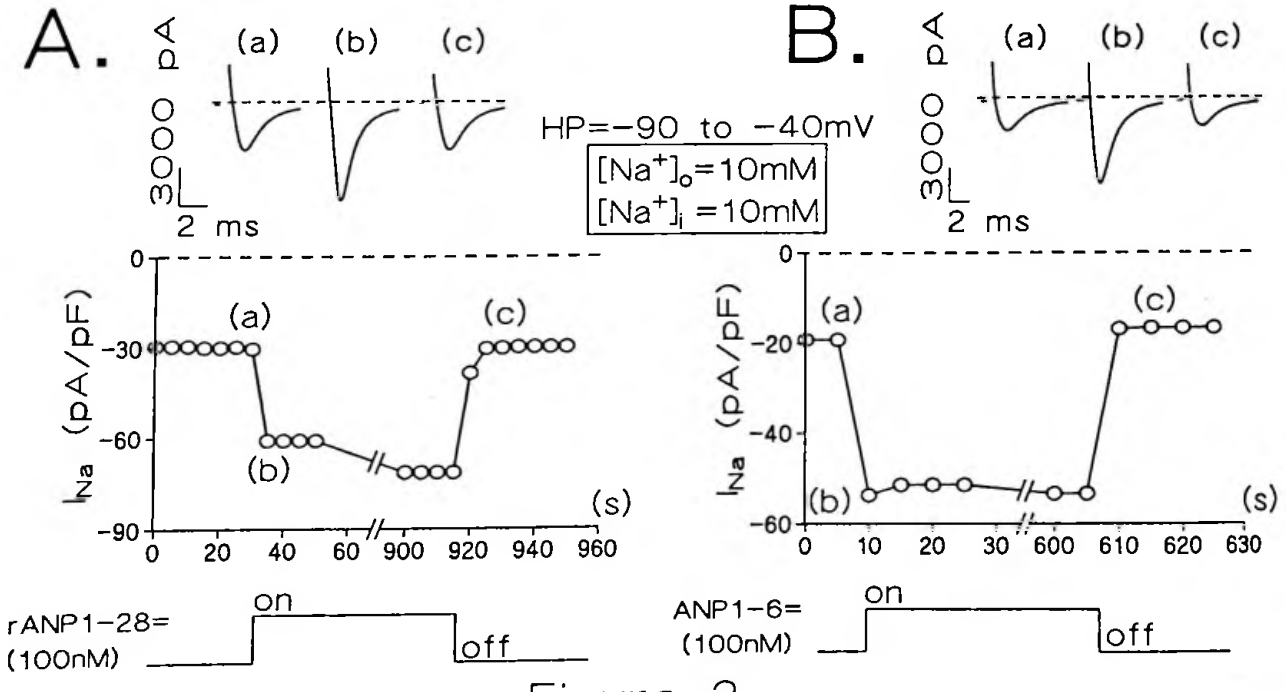

Figure 2.