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Both Nifedipine and Bay K 8644 Potentiate the Release of Atrial Natriuretic Peptide in Response to Volume Expansion

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= ABSTRACT=

The effects of a calcium channel blocker and an activator on the release of atrial natriuretic peptide (ANP) were investigated in rats. They were volume-expanded (VE) up to 5% of the body weight over 30 min, by being infused with iso-oncotic saline. Following VE, plasma ANP concentration markedly increased in association with increases in the right atrial pressure. Addition of either nifedipine (0.4 μg/min) or Bay K 8644 (0.4 μg/min) in the infusate potentiated the VE-induced release, although neither of them affected the right atrial pressure. The nifedipine-added group showed a lower mean arterial pressure than the Bay K-added group throughout the infusion period. VE decreased plasma renin concentration, the magnitude of which was attenuated by nifedipine but not by Bay K. It may be hypothesized that a decrease in cytoplasmic calcium is primary stimulus for the ANP release, and an increase plays a role in secondary liberation of the ANP accumulated in the interstitium into the lumen of the atria through myocardial contraction. Further studies will be needed to confirm the hypothesis.

Key Words: Atrial natriuretic peptide, Volume expansion, Calcium, Nifedipine, Bay K 8644, Renin.

INTRODUCTION

An accumulating body of evidence indicates that the main physiological stimulus for the secretion of atrial natriuretic peptide (ANP) is a direct stretch or distension of the atrial wall (Lang et al, 1985; Ledsome et al, 1985). However, the mechanism of signal transduction involved in the stimulus-secretion coupling has not been settled. Although it is tempting to postulate that a rise in intracellular calcium is the stimulus for the ANP release as in most secretory systems (Beridge & Irvine, 1984), the evidence is conflicting.

Since the heart has basically a contractile function which needs an alternate increase and decrease of cytoplasmic calcium, the role for calcium as a mediator of the excitation-secretion coupling may be inevitably complicated.

Ruskoaho et al (1985) observed that an increase in cytosolic calcium induced by treatment with calcium ionophores stimulated the ANP release from the isolated Langendorff preparation. Sonnenberg et al (1984) also have shown that the release of ANP occurs through

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stimulation of the phosphoinositol system which leads to release of calcium from the intracellular stores.

On the other hand, Leung et al (1986) failed to find a correlation between phosphoinositol metabolism in the atria and ANP release. Furthermore, DeBold and DeBold (1989) have suggested that a reduction of cytosolic calcium stimulates the basal ANP release.

While the reason for the contradiction is not clear, most studies were done in vitro. The present study was undertaken to evaluate the role of calcium in mediating the ANP release in vivo. Since the dihydropyridine calcium antagonist (nifedipine) and agonist (Bay K 8644) may provide means by which intracellular calcium can be decreased and increased, respectively, these drugs were superadded when the release was induced by volume expansion (VE).

MATERIALS AND METHODS

Male Sprague-Dawley rats (250-320 g) were allowed free access to water and food until the day of the experiment. Anesthetic was given intraperitoneally as a bolus (pentobarbital sodium, 50 mg/kg). The right femoral artery was cannulated to measure mean arterial pressure (MAP) and the vein to serve as an infusion route. The left jugular vein was also cannulated to reach the right atrium and to measure intraatrial pressure (RAP). A 30 to 60-min equilibration period was allowed to elapse before the infusion started. The following four groups were provided.

1. [Control] group was without any intravenous infusion.

2. [VE] group received an intravenous infusion of iso-oncotic saline (0.9% NaCl with 7 g bovine serum albumin/100 mL) over 30 min. The total volume infused amounted up to 5% of the body weight.

3. [VE+Nife] group used the same protocol as the [VE] group, except that nifedipine (0.4 μg/min) was added to the infusate.

4. [VE+Bay K] group was also the same as the [VE] group, except that Bay K 8644 (0.4 μg /min) was added to the infusate.

After 30 min of infusion in each group, blood samples were taken from the femoral artery and centrifuged at 4°C. The plasma was kept at −20°C until analyzed. Plasma concentrations of ANP and renin (PRC) were determined by radioimmunoassay as described previously (Sessler et al, 1986; Shenker and Grekin, 1986).

The values were compared for their statistical significance using analysis of variance and Student *t*-test, with adjustments made for multiple comparisons by a Bonferroni correction factor where applicable.

RESULTS

Fig. 1 shows the plasma ANP following VE. Compared with [Control], [VE] group showed an 18-fold higher concentration. Both nifedipine and Bay K potentiated the VE-induced release, ie, the plasma values were significantly higher in the [VE+Nife] and [VE+Bay K] groups than in the [VE].

Although PRC was greatly decreased following VE, the value in the [VE+Nife] group was higher than that in the [VE] (Fig. 1).

MAP steadily decreased during VE. The [VE +Nife] group showed lower MAP's than did the [VE+Bay K] group (Fig. 2). While RAP increased during VE, it did not differ among the three VE groups (Fig. 2).

DISCUSSION

The present study showed increases in plasma concentrations of ANP following VE. In addition, both nifedipine and Bay K significantly potentiated the VE-induced increase in plasma ANP.

One may argue that the effects of nifedipine and Bay K on the ANP secretion may have

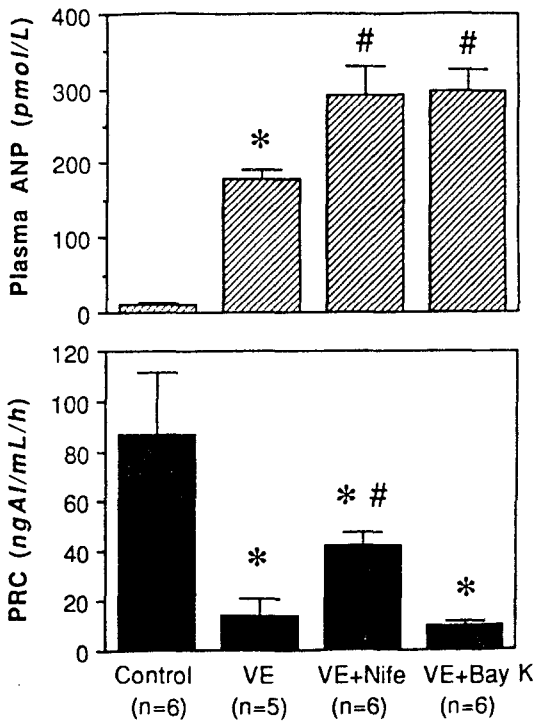

*Fig. 1. Plasma concentrations of atrial natriuretic peptide (ANP) and renin (PRC). *p<0.01, compared with [Control] group. #p<0.01, compared with [VE] group.*

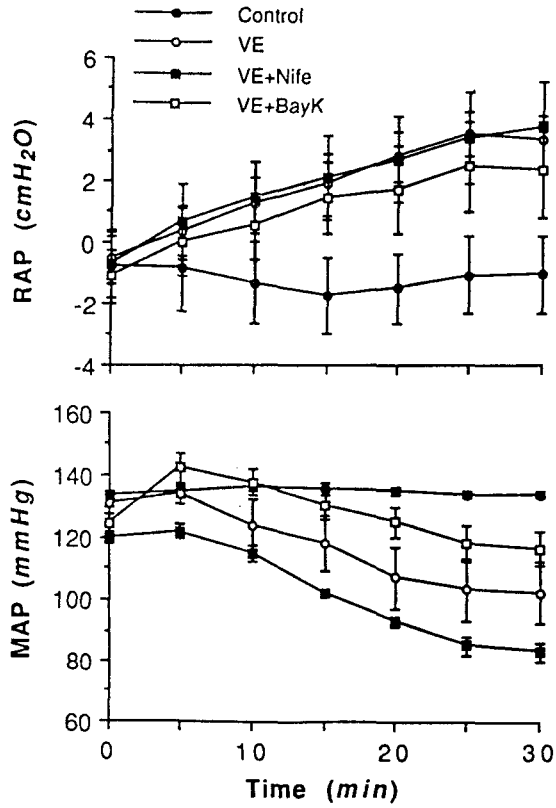

Fig. 2. Right atrial pressure (RAP) and mean arterial pressure (MAP) during volume expansion. The value at 0 min denotes the basal pressure before volume expansion started. MAP in [VE+Nife] group was significantly lower than that in [VE+Bay K] group during the infusion (p<0.01).

resulted from their contribution to systemic hemodynamic factors rather than from their modulatory effects on transmembrane calcium flux in the atrial tissue. Although MAP's in the [VE +Nife] group were lower than those in the [VE +Bay K], however, ANP values were not different between them. Furthermore, the magnitude of increases in RAP during VE did not differ among the three VE groups. These findings suggest that the higher ANP in the [VE +Nife] and [VE+Bay K] groups than in the [VE] can be ascribed to differences neither in MAP nor in RAP. The effects of the drugs directly on the ANP-secreting cells may be responsible for the differences in plasma ANP concentration.

PRC was greatly decreased following VE, which may be attributed to a direct effect of VE and/or the increased ANP. The higher PRC in the [NE+Nife] group than in the [VE] suggests that the nifedipine made the cytoplasmic calcium in renin-secreting cells low enough to elicit the release of renin. It may be extrapolated, therefore, that the amount of nifedipine was also effective to modify the release of ANP from its secretory cells.

Previous studies usually examined effects of either an increase or a decrease in calcium separately and the results were conflicting. What really is the role of calcium in the stimulus-secretion coupling for the ANP? It has been reported

that ANP release induced by osmotic stretch of isolated atrial myocytes or KCl depolarization is potentiated by reducing either extracellular or intracellular calcium (Greenwald et al, 1989). This finding may support the hypothesis that the ANP secretion is rather negatively modulated by raising intracellular calcium, such as were observed for renin (Henrich & Campbell, 1986) and parathyroid hormone (Shoback et al, 1983). The present study, where the ANP release was potentiated by nifedipine, may also substantiate a role for a decreased cytoplasmic calcium in releasing the hormone.

On the contrary, the finding that Bay K potentiated the secretion suggests a positive role for calcium in the stimulus-secretion coupling. It has been found by other investigators that Bay K caused a sustained increase in ANP (Matsubara et al, 1988). Moreover, ANP secretion following α_1-adrenergic or muscarinic cholinergic stimulation in the isolated perfused rat heart suggests that the common intracellular calcium mobilization induced by the phosphoinositol response is involved in the secretory mechanism (Matsubara et al, 1988).

In summary, the present study provides evidence for both an inhibited and a stimulated calcium influx to increase the ANP secretion in vivo. It seems contradictory that the two drugs acting in an opposite fashion similarly potentiate the secretion. Cho et al (1990) have recently suggested a "sequential mechanism" for the release of ANP in vitro, in which the release of ANP from the atrial myocyte into the extracellular space is followed by translocation of ANP into the atrial lumen. In this context, the secondary mechanism alone may not be as effective as the first. This may explain why Bay K alone has no effect on ANP secretion of the atrial or ventricular myocytes in cultures (Uusimaa et al, 1990).

It may be hypothesized, therefore, that ANP release from atrial myocytes is primarily stimulated by a decrease in cytoplasmic calcium, and then the accumulated ANP in the interstitium is squashed into the atrial lumen through myocardial contraction by increased cytoplasmic calcium. As has been suggested by DeBold and DeBold (1989), this may represent an adaptation of cardiocytes to accomplish their dual contractile and secretory functions. Further studies will be necessary before the hypothesis is confirmed.

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