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Verapamil Impairs Secretion of Stimulated Atrial Natriuretic Factor in Humans

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The adaptation of the secretory rate of atrial natriuretic factor to repeated adequate stimuli and the influence of the calcium antagonist verapamil on the release of atrial natriuretic factor were investigated in 16 patients. In eight patients (Group 1) right atrial pressure was abruptly increased by rapid right ventricular pacing for 4 min (stimulation I). After a 15 min interval, the identical stimulation was repeated (stimulation II). Eight patients (Group 2) underwent the same protocol but received 5 mg of verapamil intravenously after stimulation I.

Pacing increased right atrial pressure in both groups identically by 76%. In Group 1, release of atrial natriuretic factor caused by the second stimulation (median 290 pg/ml over basal) was significantly (2.5-fold) larger than atrial natriuretic factor release induced by the first stimulation (median 116 pg/ml over basal). In the verapamil-treated patients (Group 2), the effect of right atrial pressure increase on release of atrial natriuretic factor was abolished after stimulation II. In both groups, changes in plasma concentrations of cyclic guanosine monophosphate corresponded to changes in atrial natriuretic factor concentrations.

Thus, the myoendocrine cells are apparently capable of a fast upward regulation of their response to repeated secretory stimuli. Verapamil appears to block the stimulatory effect of a sudden increase in right atrial pressure upon release of atrial natriuretic factor.

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It has been demonstrated in various human and animal models that sudden increases in right atrial pressure induce the release of atrial natriuretic factor (1–8). Rapid ventricular pacing in patients with sinus rhythm proved to be a reliable and effective method to provoke short-term increment in right atrial pressure as well as in atrial natriuretic factor (9). Little is known about the cellular mechanisms involved in the mediation of the release of atrial natriuretic factor. In vitro experiments (10–12) suggest that secretion of atrial natriuretic factor is a calcium-dependent process because Bay K8644, a voltage-sensitive calcium channel activator, induced a sustained increase in the secretory rate of atrial natriuretic factor. This effect was abolished by simultaneous administration of nifedipine, a competitive calcium channel blocker (10).

These observations prompted us to examine whether the release of atrial natriuretic factor in patients could be suppressed by administration of a calcium antagonist. In a recently published study (13) it was demonstrated that verapamil did not alter basal concentrations of atrial natriuretic factor in rats, whereas in two patients with essential hypertension who had elevated levels of atrial natriuretic factor, a decline of the secretion rate of atrial natriuretic factor and blood pressure was observed after 2 weeks of therapy with nifedipine (14). A differentiation between a direct influence of nifedipine on the secretion of atrial natriuretic factor or an indirect effect due to the drop in systemic arterial blood pressure was not possible. The present study was designed to investigate the influence of the calcium antagonist verapamil on the secretion of atrial natriuretic factor induced by a sudden increment in right atrial pressure.

Methods

Study patients. Sixteen patients (5 female, 11 male, aged 38 to 66 years) with moderate coronary artery disease were studied during diagnostic right and left heart catheterization before left ventricular and coronary angiography. All patients had normal ventricular and atrial pressures, a left

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ventricular ejection fraction >40% and stable sinus rhythm at rest. Administration of nitrates, beta-receptor blocking agents and calcium channel antagonists was terminated ≥48 h before the examination. None of the patients was receiving digitalis. All patients had given informed consent.

Protocol. As we have shown in a previous study (9), right ventricular pacing in patients with sinus rhythm results in asynchronous contraction of the right ventricle and right atrium and leads to a sudden increment in right atrial pressure and subsequently to an increase in concentrations of plasma atrial natriuretic factor.

The patients were classified into two groups: Group 1 (n = 8) underwent right ventricular pacing at 150 beats/min for a period of 4 min (stimulation I). After a 15 min interval, the identical stimulation was repeated (stimulation II). Patients of Group 2 (n = 8) were subjected to the same protocol except that they received verapamil, 5 mg intravenously, at the beginning of the 15 min interval after stimulation I. In all patients, right atrial pressure and systemic blood pressure were continuously measured through catheters placed in the right atrium and the femoral artery, respectively. A bipolar lead placed in the apex of the right ventricle and a programmable external pacemaker were used for stimulation. The electrocardiogram (ECG) was monitored throughout the study.

Plasma radioimmunoassay. Arterial blood samples were taken in ice water-cooled ethylenediaminetetraacetate tubes before and after stimulation I and II and immediately separated by centrifugation at 2°C and 2,800 rpm and stored at −70°C until assayed. Plasma concentration of atrial natriuretic factor was measured by radioimmunoassay after extraction through ODS-silica cartridges according to the procedure described for rat and human plasma samples (15). Plasma levels of cyclic guanosine monophosphate (GMP) were measured by radioimmunoassay of ethanol-extracted samples (5).

Statistical analysis. The Wilcoxon matched pairs test was used for statistical intraindividual evaluation. The Mann-Whitney *U* test was used for interindividual comparison. Probability values <0.05 were considered significant.

Results

Right atrial pressure (Fig. 1). In control patients (Group 1), mean right atrial pressure was 4.5 ± 2.3 mm Hg during sinus rhythm before the first stimulation and increased significantly to 7.7 ± 2.5 mm Hg during the first stimulation at 150 beats/min. Immediately after the termination of pacing, right atrial pressure decreased to control values. With the second stimulation also at a rate of 150 beats/min, right atrial pressure again increased significantly, to 7.9 ± 2.2 mm Hg. The increment in right atrial pressure was the same for both stimulations. In verapamil-treated patients (Group 2), right atrial pressure increased from 4.6 ± 0.7 to 7.5 ± 1.7 mm Hg during stimulation I and from 4.9 ± 1.2 to 7.3 ± 1.6 mm Hg during stimulation II. Verapamil did not alter the increment in right atrial pressure. Thus, each time pacing was applied, right atrial pressure increased by a constant amount independent of the stimulation period (stimulation I or II) and independent of verapamil administration.

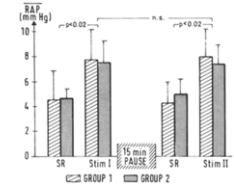
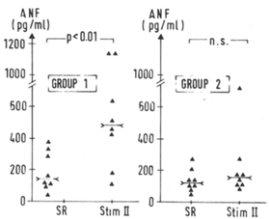


Figure 1. Effect of right ventricular stimulation (Stim) I and II (both for 4 min at 150 beats/min) on mean right atrial pressure (RAP ± SD). Patients of Group 1 were untreated; Group 2 patients received 5 mg verapamil intravenously immediately after stimulation I. ns = not significant, SR = sinus rhythm before both stimulations.

Systemic blood pressure. In both groups, estimates of mean systemic blood pressure were unchanged throughout the protocol. Mean systemic arterial pressure was unchanged by either pacing or verapamil administration.

Atrial natriuretic factor (Fig. 2). In Group 1 and Group 2, median plasma atrial natriuretic factor levels rose from 79 (range 46 to 299) to 258 (range 96 to 1,045) pg/ml ($p < 0.01$) and from 58 (range 21 to 171) to 142 (range 90 to 308) pg/ml ($p < 0.02$), respectively, during the first stimulation, that is, before verapamil treatment in Group 2. For both groups the amount of atrial natriuretic factor increase due to stimulation I showed no statistical difference.

Figure 2. Atrial natriuretic factor (ANF) plasma concentrations during stimulation II (Stim II for 4 min at 150 beats/min) for each patient in Groups 1 and 2. Patients of Group 1 were untreated; Group 2 had received 5 mg of verapamil intravenously. **Horizontal lines** represent median values. Abbreviations as in Figure 1.



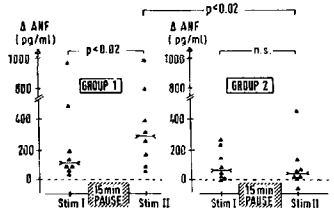


Figure 3. Increases (Δ values) in atrial natriuretic factor (ANF) plasma concentration during stimulations I and II (Stim I and Stim II, both for 4 min at 150 beats/min) for each patient in Groups 1 and 2. Patients of Group 1 were untreated; Group 2 received 5 mg verapamil immediately after stimulation I. **Horizontal lines** represent median values.

Stimulation II increased right atrial pressure identically as compared with stimulation I and provoked different increases in median atrial natriuretic factor levels in the two groups (Fig. 2). Median atrial natriuretic factor concentrations just before stimulation II were 140 pg/ml (Group 1) and 121 pg/ml (Group 2) (p = ns). In the untreated Group 1, median atrial natriuretic factor increased significantly ($p < 0.01$) to 488 pg/ml during stimulation II, whereas in the patients who received verapamil (Group 2), the median value was 157 pg/ml; in this group, stimulation II caused no significant increase in atrial natriuretic factor.

The effect of verapamil to impair pressure-stimulated release of atrial natriuretic factor is illustrated in Figure 3. During stimulation I the amount of atrial natriuretic factor release (expressed as delta values) was comparable in both groups (p = ns). However, in Group 1 (control), the median increase due to stimulation II was 2.5 fold higher than that due to stimulation I ($p < 0.02$). In the verapamil-treated patients (Group 2), no significant difference between release of atrial natriuretic factor due to stimulation I and II was seen. Thus, after administration of verapamil, the stimulatory action of stimulation II was impaired in comparison with the effect of stimulation II in the untreated patients (Group 1) ($p < 0.02$).

Cyclic guanosine monophosphate. Median plasma cyclic GMP concentrations (Fig. 4) corresponded to the alterations in plasma atrial natriuretic factor concentrations, increasing during stimulation I from 3.1 to 4.9 pmol/ml (Group 1) and from 2.8 to 4.6 pmol/ml (Group 2). During stimulation II, cyclic GMP increased from 5.4 to 8.5 pmol/ml in control patients and from 4.3 to 5.4 pmol/ml in verapamil-treated patients. In both groups, increases in median cyclic GMP were 1.8 pmol/ml after the first stimulation. In Group 1 (control), cyclic GMP increased by 3.1 pmol/ml after the second stimulation. However, in Group 2 (verapamil), median cyclic GMP increased by only 1.1 pmol/ml after stimulation II. There was a linear correlation between atrial natriuretic factor and cyclic GMP levels before stimulation I in both groups that was highly significant ($r = 0.73$, $p < 0.005$).

Discussion

Our results indicate for the first time that the calcium channel antagonist verapamil is effective in nearly totally suppressing the secretion response of atrial natriuretic factor after an abrupt increase in atrial pressure. This has not been shown before in animals or humans. Because right atrial pressure and systemic blood pressure were not affected by verapamil, it appears that calcium-dependent cellular mechanisms are involved in the suppression of atrial natriuretic factor secretion. Verapamil apparently not only inhibits the secretion that is induced by a single immediate increase in right atrial pressure, but also blocks the potentiating effect of repeated stimulations on the release of atrial natriuretic factor.

Effects of single and repeated right atrial pressure increments on release of atrial natriuretic factor. Increments in right atrial pressure induce secretion of atrial natriuretic factor (1–8). As previously shown (9), asynchronous con-

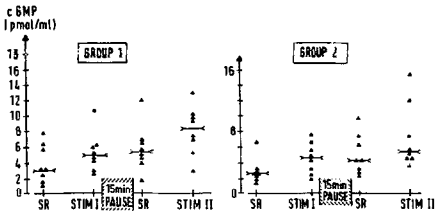


Figure 4. Cyclic guanosine monophosphate (cGMP) plasma concentrations in both groups during right ventricular stimulations (Stim I, Stim II, both for 4 min at 150 beats/min). Patients of Group 1 were untreated; Group 2 received 5 mg verapamil intravenously immediately after stimulation I. **Horizontal lines** represent median values. Abbreviations as in Figure 1.

traction of the ventricles and atria induced by rapid ventricular pacing in patients with sinus rhythm provokes a sudden and reproducible increase in atrial pressure; therefore, pacing is appropriate to investigate the influence of drugs on secretion of atrial natriuretic factor.

Whereas right atrial pressure increased identically during both stimulations, the release of atrial natriuretic factor induced by the second stimulation in Group I was 2.5-fold larger than that induced by the first stimulation. These results indicate that the first secretory stimulus, that is, right atrial pressure increment caused by stimulation I, appears to have a "priming effect" on the release of atrial natriuretic factor caused by the identical second stimulus (16).

Secretion of atrial natriuretic factor as a calcium-dependent process: influence of the calcium channel blocker verapamil. The myoendocrine cells are apparently capable of a fast upward regulation of their response to adequate secretory stimuli. This "priming effect" has not been previously demonstrated for atrial natriuretic factor but it is known for other peptide hormones, such as insulin, for example (17). After administration of verapamil in patients of Group 2, the second stimulation did not induce a significant release of atrial natriuretic factor and the "priming effect" seen in Group I was blunted. Our findings are in agreement with data from other investigators (10–12) who showed in vitro enhanced secretious rates of atrial natriuretic factor after application of calcium agonists (for example, Bay K8644) and demonstrated that this stimulatory action can be blocked by simultaneous administration of the calcium antagonist nifedipine. The agreement of our findings with these results supports the hypothesis that the observed effects were due not to a possible interference of verapamil with the autonomic nervous system but rather to its direct calcium blocking action. Furthermore, it has been shown for other peptide hormones that free intracellular calcium often appears to play a key role in triggering their secretion rate (18–20). Until now, the direct influence of calcium antagonists on the secretion of atrial natriuretic factor in humans has not been elucidated.

In our protocol, the dose of 5 mg of verapamil showed no effect on right atrial and systemic arterial blood pressure, which indicates that the suppression of atrial natriuretic factor is due not to hemodynamic changes but rather to a direct interference with the cells that secrete atrial natriuretic factor.

Concentrations of the second messenger, cyclic guanosine monophosphate. The increases in cyclic GMP, which is known as the second messenger for atrial natriuretic factor (21,22), support the observed changes in atrial natriuretic factor. Between stimulations I and II, cyclic GMP did not return to baseline level; this observation can be explained by the apparent time difference between peaks of atrial natriuretic factor and cyclic GMP and a markedly longer half-time of cyclic GMP of about 30 min, as demonstrated in a previous study (23). The correlation between baseline levels of plasma cyclic GMP and atrial natriuretic factor we found in this study is comparable with recently reported data (24).

Conclusions. Our study supports for the first time in humans the hypothesis that calcium plays a key role in the secretion of atrial natriuretic factor. Repeated stimulations of the secretory system by increase in right atrial pressure result in an upward regulation of the release of atrial natriuretic factor. Voltage-sensitive calcium channel blockers impair stimulated secretion of atrial natriuretic factor.

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