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Effect of Endopeptidase 24.11 Inhibition in Conscious Cardiomyopathic Hamsters

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ABSTRACT

An elevated plasma concentration of atrial natriuretic peptide (ANP) is characteristic of congestive heart failure (CHF) in both humans and animals. One strategy for facilitating the biological actions of this circulating hormone is to inhibit its metabolism. Neutral endopeptidase 24.11, an enzyme known to degrade ANP, has been implicated in the metabolic clearance of this hormone. Inhibition of endopeptidase 24.11 may produce increases in the local concentrations of ANP in organs rich in the enzyme, such as the kidney, thereby enhancing local actions, *e.g.* natriuresis. Exogenous administration of ANP to CHF patients produces limited natriuresis, perhaps because of the associated fall in arterial pressure. This approach of blocking endopeptidase 24.11 activity in CHF could offset the general effects of exogenous administration of ANP and result in enhanced natriuresis. We therefore undertook studies to determine if thiorphan, an endopeptidase 24.11 inhibitor, would increase circulating ANP levels and enhance natriuresis in conscious cardiomyopathic hamsters with CHF. Cardiomyopathic hamsters had significantly lower ($P < .05$) basal mean arterial pressures in comparison to normal hamsters (90 ± 2 *vs.* 135 ± 3 mm Hg). Treatment with thiorphan (10 mg/kg i.v. bolus followed by 10 mg/kg/hr i.v. infusion) did not change arterial pressure in either group and produced a 3-fold increase in urinary sodium excretion in the cardiomyopathic group but not in the normal hamsters. This natriuretic response in the cardiomyopathic hamsters was associated with a doubling of the plasma concentration of ANP. These results indicate that inhibition of endopeptidase 24.11 increases natriuresis and circulating ANP concentrations in this model of CHF. Thus, altering the metabolism of endogenous ANP may provide a new therapeutic approach for the management of CHF.

ANP possesses hemodynamic, renal and endocrine actions which could alleviate the symptoms of CHF. Several studies have evaluated ANP as a therapeutic agent for treating CHF (Cody *et al.*, 1986; Crozier *et al.*, 1986) and have demonstrated a markedly depressed natriuretic response to exogenous ANP in CHF patients. The mechanisms which are responsible for this attenuation of ANP-induced natriuresis have not been identified; however, the depressor activity of ANP may contribute to underperfusion of the kidneys (Crozier *et al.*, 1986).

Rather than administering exogenous ANP, an alternative approach to utilizing ANP as a therapeutic agent is to decrease the metabolism of the endogenous hormone. Evidence indicates that ANP is metabolized by endopeptidase 24.11 and inhibitors of this enzyme prevent the degradation of ANP (Olins *et al.*, 1987, 1989; Sonnenberg *et al.*, 1988; Stephenson and Kenny, 1987). Local concentrations of ANP could be expected to increase greatly during endopeptidase inhibition in tissues such as the kidney which contain large amounts of the enzyme (Gee *et al.*, 1985). Thus, an inhibitor of endopeptidase 24.11 might prove to be more effective than exogenous ANP in promoting natriuresis because exogenous ANP broadly affects cardiovascular functions, resulting in decreased arterial pressure which can counteract ANP-induced natriuresis. In contrast, by selectively raising ANP concentrations within the kidney, one could expect enhanced natriuresis because renal perfusion pressure would be maintained. Moreover, one might predict that the natriuretic response to endopeptidase 24.11 inhibition would be amplified in conditions of elevated circulating levels of ANP such as CHF (Burnett *et al.*, 1986; Cody *et al.*, 1986; Laragh, 1986). This study was undertaken to determine whether inhibition of endopeptidase 24.11 would elicit an enhanced natriuresis and increase the circulating levels of endogenous ANP in conscious hamsters with CHF.

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Methods

Instrumentation and protocol. Male CM and NM hamsters, weighing 120 to 160 g, were obtained from Canadian Hybrid Farms (Nova Scotia, Canada). While anesthetized with methohexital sodium (60 mg/kg i.p.), a short-acting barbiturate, femoral arterial and venous cannulas were inserted to monitor arterial pressure and to administer saline or thiorphan. The flared end of a length of PE-190 tubing (Clay-Adams, Parsippany, NJ) was inserted into the dome of the urinary

ABBREVIATIONS: ANP, atrial natriuretic peptide; CHF, congestive heart failure; CM, cardiomyopathic; NM, normal; MAP, mean arterial pressure; UV, urine flow; r-ANP, rabbit ANP; IgG, immunoglobulin G; UNaV, urinary sodium excretion.

bladder *via* a midline abdominal incision. Before the hamsters awakened, they were placed into Lucite holders which protected the catheters.

Two hours were allowed for the animals to recover. An i.v. infusion of 0.9% NaCl (16 μl/min) was then started in all animals and continued throughout the experiment. After a 50-min equilibration, urine was collected and MAP was recorded during a 30-min control period. The animals were assigned randomly to one of two treatments; either they were maintained on saline alone or given a 10 mg/kg i.v. bolus of thiorphan followed by a 10 mg/kg/hr i.v. infusion. This randomization procedure resulted in four experimental groups; CM-Saline (CMs maintained on saline; $n = 8$), CM-Thiorphan (CMs treated with thiorphan; $n = 8$), NM-Saline (NMs maintained on saline; $n = 9$) and NM-Thiorphan (NMs treated with thiorphan; $n = 8$). Once the thiorphan or saline infusions were underway, urine was collected and MAP was recorded for a 30-min experimental period. At the end of this experimental period, 2 ml of arterial blood was collected for measurement of plasma ANP concentration.

Some hamsters (5 CM and 10 NM, chosen randomly) were anesthetized with sodium pentobarbital at the conclusion of the experiment to examine the gross morphology of the viscera and determine the weights of dissected cardiac atria and ventricles.

Physiological and biochemical analysis. Urine volume was determined gravimetrically and sodium excretion was calculated from urine sodium concentration, as measured by flame photometry (model IL943, Instrumentation Laboratories, Lexington, MA) and urine flow. Arterial pressure was monitored continuously by connecting the arterial cannula to a Statham P23ID (Gould, Oxnard, CA) transducer which was coupled to a Gould Pressure Processor and 2800S polygraph (Gould Inc., Cleveland, OH). MAP was derived electronically. MAP was sampled at 2-min intervals to quantitate the average arterial pressure during each 30-min period of an experiment.

The concentration of ANP in the plasma was determined by a double-antibody method, as described previously (Trapani *et al.*, 1989). All determinations were performed in duplicate using rANP(99–126) as standard, r-[^{125}I]ANP as the labeled ligand, rabbit anti-human ANP serum and goat anti-rabbit IgG serum. Unlabeled ANP at 21 pg/tube produced 50% displacement of bound r-[^{125}I]ANP in this assay. The intra- and interassay coefficients of variation were less than 8%.

Statistics. Data were analyzed by one-way and two-way analysis of variance and Student's *t* test for data with equal variance and Wilcoxon rank-sum test for comparisons with unequal variances using the SAS statistical software package (SAS Institute Inc., Cary, NC). Values were considered to be different when $P < .05$. Data are expressed as mean ± S.E.M.

Drugs. Racemic thiorphan {(±)-N-[1-oxo-2-(mercaptomethyl)-3-phenyl-propyl]-glycine} was synthesized by previously published procedures (Bindra, 1982; Roques *et al.*, 1985). ANP(99–126), rabbit anti-human ANP serum and goat antirabbit IgG serum were obtained from Peninsula Laboratories (Torrance, CA). r-[^{125}I]ANP was purchased from Amersham Corp. (Arlington Heights, IL).

Results

The CM hamster is characterized as having a remarkably enlarged atrial mass. This is shown in table 1, where the atrial weight, normalized per 100 g of body weight, was 3 times greater in the CM hamsters compared to NM hamsters. Although there was no difference in the mass of the ventricles, those from the CMs were visibly dilated. These characteristics have been described previously in animals with severe heart failure (Basjusz *et al.*, 1969; Franch *et al.*, 1988). Several hamsters with marked edema and other signs of advanced disease did not survive the surgery.

Also displayed in table 1, MAP was significantly lower ($P < .05$) in the CM animals. MAP did not change during the course of the experiment in either the CM or NM hamsters, regardless of treatment with thiorphan or saline.

TABLE 1

Basal arterial pressure, UV, UNaV and heart weights in CM and NM hamsters

Values are mean ± S.E.M. Atrial Wt., atrial weight in mg/100 g of body weight; Ventr. Wt., ventricular weight in mg/100 g of body weight.

Strain	Atrial Wt.	Ventr. Wt.	MAP	UNaV	UV
			mm Hg	*μEq/min*	*μl/min*
NM	30 ± 1 ($n = 10$)	360 ± 4 ($n = 10$)	135 ± 3 ($n = 17$)	0.10 ± 0.01 ($n = 17$)	2.6 ± 0.5 ($n = 17$)
CM	100 ± 10* ($n = 5$)	362 ± 20 ($n = 5$)	90 ± 2 ($n = 16$)	0.26 ± 0.05 ($n = 16$)	2.9 ± 0.5 ($n = 16$)

* $P < .05$ *vs.* NM hamsters.

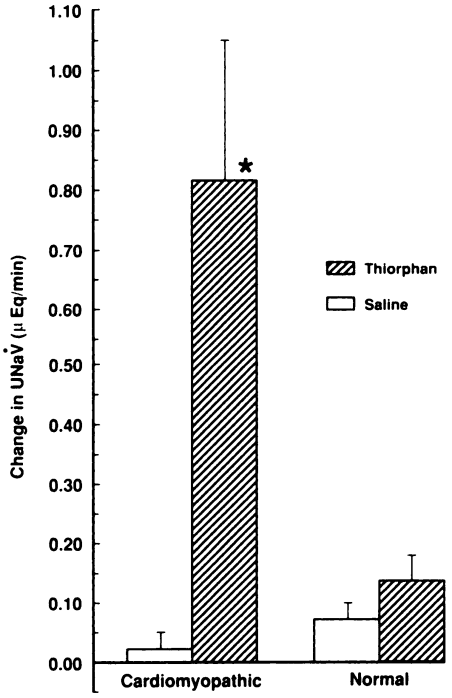

Fig. 1. Absolute change in UNaV for the 30-min period during thiorphan (10 mg/kg i.v. bolus and 10 mg/kg/hr i.v. infusion) or saline infusion in CM and NM hamsters. Values are mean ± S.E.M. for CM-Saline ($n = 8$), CM-Thiorphan ($n = 8$), NM-Saline ($n = 9$) and NM-Thiorphan ($n = 8$) groups. The absolute change in sodium excretion was calculated as the difference between the value measured for the 30-min experimental period and that determined for the 30-min control period. *$P < .05$ *vs.* CM-Saline.

The CM hamsters excreted slightly more sodium ($P < .05$) than NM hamsters during the control period whereas UV was comparable between strains (table 1). Figure 1 shows the absolute increases in UNaV produced by the administration of thiorphan or saline during the experimental period. Sodium excretion did not change in NM or CM hamsters which received saline throughout the experiment. NM hamsters treated with thiorphan did not exhibit a significant increase in sodium excretion when compared to NM hamsters infused with saline. In contrast CM hamsters responded to thiorphan treatment with a 3-fold increase in UNaV. This natriuresis was significant but its magnitude varied considerably between individual animals within the group. Changes in UV paralleled those of sodium excretion (data not shown).

The plasma concentration of ANP was 6-fold higher in the

CM hamsters infused with saline than in the NM hamsters treated similarly (fig. 2). Thiorphan administration increased plasma ANP concentrations 130% in the CM animals but did not alter the levels in the NM animals.

Discussion

ANP is one of many humoral systems which regulate fluid and electrolyte balance. In the present study, we determined whether the natriuretic activity of endogenous ANP could be potentiated by inhibiting endopeptidase 24.11, an enzyme which may participate in its clearance from the circulation. Our results indicate that thiorphan, an inhibitor of endopeptidase 24.11, increased sodium excretion by 310% in hamsters with CHF while doubling their plasma levels of ANP.

When ANP was infused i.v. in patients with CHF, the natriuretic response was blunted in comparison to healthy individuals (Cody *et al.*, 1986; Crozier *et al.*, 1986). In one study (Crozier *et al.*, 1986), the attenuated natriuretic response was associated with a significant fall in arterial pressure. Our data show a substantially different profile. Treatment with the endopeptidase inhibitor produced no effect on MAP and the hamsters with CHF responded with enhanced natriuresis. These data suggest that inhibition of endopeptidase 24.11 may increase sodium excretion by mechanisms other than elevating plasma ANP concentration, possibly by protecting ANP from degradation within the kidney. By inhibiting endopeptidase at the glomerulus (Shima *et al.*, 1988), thiorphan may have increased the local concentration of ANP at this critical renal site, thereby unmasking enhanced natriuresis. Furthermore, if ANP exerts a natriuretic action from the tubular side of the nephron, it is possible that blocking brush-border endopeptidase in the early proximal tubule (Shima *et al.*, 1988) would increase sodium excretion by allowing more intact ANP to reach sites in both the proximal and distal portions of the nephron. Therefore, these local effects, coupled with increased delivery of ANP to the kidney *via* an elevation in plasma levels of the peptide, could greatly increase the concentration of ANP at natriuretic sites along the nephron. Also, because arterial pressure did not fall, the antinatriuretic influence of reduced renal perfusion pressure would not be a factor.

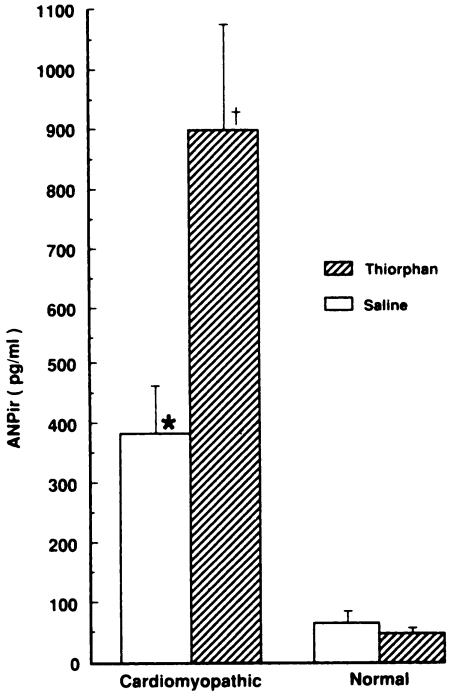

Fig. 2. Plasma concentrations of ANP-immunoreactivity (ANPir) in CM and NM hamsters treated with saline or thiorphan (10 mg/kg i.v. bolus and 10 mg/kg/hr i.v. infusion). Values are mean ± S.E.M. for CM-Saline (n = 8), CM-Thiorphan (n = 8), NM-Saline (n = 9) and NM-Thiorphan (n = 8). *P < .05 *vs.* NM-Saline; †P < .05 *vs.* CM-Saline.

The increase in plasma ANP levels in CM as compared to NM hamsters treated with thiorphan was an interesting aspect of this study. The most probable explanation for this difference is the existence of multiple mechanisms for the clearance of circulating ANP. Several investigators have found more than one binding site for ANP (Leitman *et al.*, 1986; Olins *et al.*, 1988; Takayanagi *et al.*, 1987) and it has been proposed that one of these sites serves a storage/clearance function to regulate plasma levels of the hormone (Maack *et al.*, 1987). Because there is evidence that the number of ANP binding sites decreases under conditions of chronically elevated plasma ANP levels (Morton *et al.*, 1987; Schiffrin *et al.*, 1986), it is reasonable to suggest that CM hamsters may have a reduced number of binding sites and, thus, a reduced capacity to clear circulating ANP *via* receptor-mediated mechanisms. In addition, the elevated ANP levels may nearly saturate the remaining clearance sites. As a result, the regulation of plasma ANP levels in CHF may be more dependent on degradation by endopeptidase 24.11. Under such conditions, endopeptidase inhibition should result in increased circulating levels of ANP in CHF but not in normal animals in which all ANP clearance mechanisms are fully operative.

While increased delivery of ANP to the kidney secondary to elevated plasma levels, increases in ANP concentration at critical renal sites and enhanced delivery of ANP to distal regions of the nephron may fully explain the natriuresis observed in the CM hamsters, other effects of endopeptidase inhibition cannot be excluded at this time. Endopeptidase 24.11 inhibition may elicit natriuresis by interfering with the metabolism of other peptides which have renal activity. There are several known substrates for endopeptidase 24.11 in addition to ANP, including bradykinin (Shimamori *et al.*, 1986; Ura *et al.*, 1987) and substance P (Nau *et al.*, 1986). A recent report (Ura *et al.*, 1987) indicated that endopeptidase 24.11 accounted for 50 to 75% of the degradation of intrarenal kinins. That study also showed that phosphoramidon, an inhibitor of endopeptidase 24.11, caused an increase in urinary sodium and renal kinin excretion. Therefore, some of the renal response exhibited by the CM hamsters may be attributable to the actions of bradykinin or an interaction between bradykinin and intrarenal ANP.

In summary, inhibition of endopeptidase 24.11 by thiorphan produced an increase in urinary sodium excretion and circulating levels of ANP in CM hamsters. These results differ from previous studies which demonstrated that humans with CHF exhibit a depressed renal responsiveness to exogenous ANP. The results of the present study suggest that utilization of inhibitors of endopeptidase 24.11 may provide a useful therapeutic approach for the treatment of CHF.

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