REVISTA ESPAÑOLA DE FISIOLOGIA, 44 (3), 265-272, 1988

Effect of Atrial Natriuretic Peptide on Arterial Pressure and Renal Function in Cirrhotic Rats with Ascites

C. López, W. Jiménez*, V. Arroyo, J. Gaya, F. Rivera and J. Rodés

Laboratorio Hormonal y Unidad de Hepatología
Hospital Clínico Provincial
Universidad de Barcelona
08036 Barcelona (Spain)

(Received on November 17, 1987)

C. LOPEZ, W. JIMENEZ, V. ARROYO, J. GAYA, F. RIVERA and J. RODES.— *Effect of Atrial Natriuretic Peptide on Arterial Pressure and Renal Function in Cirrhotic Rats with Ascites.* Rev. Esp. Fisiol., 44 (3), 265-272, 1988.

Glomerular filtration rate, urine volume, sodium excretion and mean arterial pressure were measured in 10 rats with Cl_4C induced cirrhosis presenting sodium retention and ascites, and in 10 control rats before and during the iv administration of the 28 aminoacid rat α-Atrial Natriuretic Peptide (α-ANP) (a bolus of 1 μg followed by a constant infusion of 33 ng/min). α-ANP induced a similar increase in glomerular filtration rate and filtered sodium load in both groups of rats. In contrast, the increase in urine volume and sodium excretion produced by α-ANP was significantly lower in cirrhotic rats (from 13.8±1.9 to 37.9±9.1 μl/min., and from 0.5±0.1 to 3.3±1.0 μEq/min) than in control animals (from 14.6±1.3 to 102.5±17.7 μl/min., $p<0.005$; and from 1.0±0.3 to 14.1±3.2 μEq/min., $p<0.001$). The results indicate that in rats with experimental cirrhosis and ascites there are blunted diuretic and natriuretic responses to α-ANP, probably as a consequence of the exaggerated tubular sodium reabsorption present in these animals.

Key words: Experimental cirrhosis, Ascites, Atrial natriuretic factor, Sodium excretion.

Atrial natriuretic factor (ANP), is a peptide or a group of peptides synthesized by the atrial myocytes of mammals with powerful effects on systemic hemodynamics and renal sodium and water metabolism. During the last few years, the development of specific and sensitive techniques to measure the plasma level of ANP and the availability of synthetic atrial natriuretic peptides have allowed the investigation of the role of ANP in the homeostasis of extracellular fluid volume and arterial pressure and in the pathogenesis of arterial hypertension and sodium retention in humans and experimental animals. At present, several studies on the plasma concentration of ANP in cirrhosis have

* To whom all correspondence should be addressed.

been published and they showed conflicting results. Whereas some studies found increased plasma levels of ANP in these patients (1, 12, 13, 22), others showed normal or even reduced plasma concentrations of this compound (5, 7, 14). However, at present, there are very few data on the effect of ANP in systemic hemodynamics and renal function in cirrhosis (17).

In the current study, the effect of α-ANP on arterial pressure, glomerular filtration rate (GFR), urine volume and sodium excretion was investigated in rats with carbon tetrachloride induced cirrhosis and ascites and in control animals. The aim of the study was to investigate if the renal response to ANP is impaired in rats with cirrhosis and ascites.

Materials and Methods

The study was made in 10 adult male Sprague-Dawley rats with cirrhosis and ascites, and in 10 control rats. Both groups were fed *ad libitum* with a normal sodium chow and distilled water as drinking fluid. Cirrhosis was induced with carbon tetrachloride and phenobarbital following a previously described method (15). Cirrhotic rats were studied after they had developed marked ascites. Two weeks before the infusion of α-ANP, these animals and control rats were placed in metabolic cages. After allowing one week for adaptation, measurements of the 24 h sodium intake and urine sodium and aldosterone-18-glucuronide excretion were made. These measurements were performed on three consecutive days and the mean value was considered. Fecal sodium excretion was not measured. Sodium balance was estimated by subtracting the daily sodium excretion from the daily sodium intake. Infusion of α-ANP was performed in all rats the week after completion of the balance studies. Animals were allowed free access to food and water prior to anesthesia (ketamine-HCl 100 mg/kg body weight i.m.). Catheters were inserted in the right femoral artery, left femoral vein and right jugular vein. The bladder was exposed through a suprapubic incision and catheterized. Once surgical procedure was completed, a priming dose of ^{14}C-inulin (3 μCi) was given through the femoral vein catheter followed by a constant infusion (1 ml/h) throughout the study of a Ringer solution of this substance (3 μCi/ml). Mean arterial pressure was continuously recorded throughout the femoral artery catheter using a high sensitivity transducer and a multichannel recorder (MX4P and MT4, Lectromed Lt, Jersey Channels Islands). After allowing 1 h for equilibration, the urine was collected in three 10-min periods before and during the i.v. administration (jugular catheter) of the 28 aminoacid rat α-ANP (Bachem Inc, Torrance, California). The bladder was voided just before completing each clearance period. In the middle of each period a blood sample was taken to measure serum electrolytes and ^{14}C-radioactivity. α-ANP was given as a bolus (1 μg) followed by a constant infusion (6.6 μl/min) of a Ringer solution of the peptide (5 μg/ml). Ten normal rats received vehicle alone to test the stability of the measurements throughout the course of the clearance periods. Since body weight in cirrhotic rats (328±19 g) and control animals (339±12 g) was similar, the dose o α-ANP per kg of body administered in both groups was comparable.

In cirrhotic rats the volume of ascites was measured using ^{125}I-human serum albumin 1 h following α-ANP infusion. One μCi of labeled albumin (0.2 ml) was injected into the peritoneal cavity and, 15 min later, an ascitic sample was obtained. To ensure accurate dilution of labeled albumin into ascitic fluid, all animals were submitted to an external abdominal massage. The abdominal cavity was then entered and portal pressure measured directly in the portal vein using a 21-gauge

butterfly needle (Material Clínico, Barcelona, Spain) connected to a strain-gauge transducer. A small liver specimen was taken from the middle lobe in each animal, fixed in 10 % buffered formalin and stained using hematoxylin, eosin, and Masson's trichome.

Analysis and Calculations.— Urine was collected in preweighed tubes, and the volume was determined gravimetrically. Electrolytes were measured by flame photometry (IL 943, Instrumentation Laboratory, Lexington MA). GFR was estimated by the clearance of ^{14}C-inulin and the ^{14}C-radioactivity of plasma and urine was measured in a liquid scintillation spectrometer (SL 4000 Intertechnique, Paris) using Supersolve X (Kochlight) as scintillating fluid. The urinary concentration of aldosterone-18-glucoronide was measured in urine samples (0.5 ml) adjusted to a pH 1.0 with 1 ml of 0.2 N HCl and kept during 20 h at 30 °C. The resulting (24) aldosterone concentration was then measured with a highly specific radioimmunoassay (CIS Sorin Biomedica, Saluggia, Italy). The coefficient of variation was 10 % for intraassay and 14 % for interassay determinations.

Statistics.— The paired and unpaired Student's *t* test and the nonparametric test of Mann-Whitney were used for the statistical analysis of the results. Values of $p<0.05$ or less were considered significant. Results are presented as mean ± SEM.

Results

Cirrhotic rats showed a significantly lower urinary sodium excretion and a significantly more positive sodium balance than control rats, indicating that they were actively retaining sodium at the time of the study. Urinary excretion of aldosterone-18-glucuronide was significantly increased in cirrhotic rats as compared to control animals. Ascites volume in cirrhotic rats ranged from 5.9 to 32 ml. Portal pressure was increased in all these animals (table I).

Rats receiving vehicle alone did not show significant changes in all the parameters studied throughout the clearance periods (table II).

Table III shows the effect of αANP on mean arterial pressure and renal function in cirrhotic and control rats. Before infusion of α-ANP, there were no significant differences between both groups of animals with respect to urine volume (13.8±1.9 vs 14.6±1.3 µl/min) potassium excretion (2.2±0.4 vs 2.4±0.2 µEq/min) and inulin clearance (2.6±0.4 vs 2.4±0.3 ml/min.). Sodium excretion and arterial pressure, however, were significantly reduced in cirrhotic rats as compared to control animals (sodium excretion: 0.5±0.1 vs 1.0+0.3 µEq/min $p<0.001$; mean arterial pressure: 92±3 vs 119±3 mmHg, $p<0.001$).

The i.v. administration of α-ANP was associated with a significant reduction of mean arterial pressure and a significant increase of inulin clearance, urine volume

Table I. *Sodium metabolism, aldosterone excretion and portal pressure in the two groups of rats.*

	Cirrhotic	Control	p value
Sodium excretion (mEq/day)	0.62 ± 0.10	1.81 ± 0.15	<0.001
Sodium balance (mEq/day)	0.76 ± 0.13	0.21 ± 0.16	<0.001
Urinary excretion of aldosterone-18-glucoronide (ng/day)	148.0 ± 17.9	21.4 ± 2.3	<0.001
Ascites volume (ml)	12.8 ± 2.7	—	—
Portal pressure (mmHg)	11.0 ± 1.6	6.0 ± 0.2	<0.001

Table II. *Urine volume, sodium and potassium excretion, inulin clearance and mean arterial pressure in rats receiving vehicle alone.*
Values are mean ± SEM. C1, C2 and C3 represent the three 10-min periods before iv injection of vehicle. C4, C5 and C6 represent the three 10-min periods after iv injection of vehicle

C1	C2	C3	C4	C5	C6
Urine volume (µl/min)					
12.4 ± 1.4	12.1 ± 1.4	12.3 ± 1.7	13.1 ± 1.3	12.4 ± 0.8	12.3 ± 1.3
Sodium excretion (µEq/min)					
0.9 ± 0.09	0.9 ± 0.09	0.9 ± 0.09	1.0 ± 0.1	0.9 ± 0.1	1.0 ± 0.2
Potassium excretion (µEq/min)					
2.4 ± 0.3	2.1 ± 0.1	2.3 ± 0.3	2.3 ± 0.3	2.1 ± 0.2	2.0 ± 0.3
Inulin Clearance (ml/min)					
2.3 ± 0.2	2.1 ± 0.1	2.3 ± 0.1	2.3 ± 0.1	2.1 ± 0.1	2.2 ± 0.1
Mean arterial pressure (mmHg)					
116 ± 2	118 ± 2	120 ± 1	119 ± 1	120 ± 2	119 ± 2

Table III. *Effect of α-ANP on urine volume, sodium and potassium excretion, inulin clearance and mean arterial pressure in control and cirrhotic rats.*
Values are mean ± SEM. C1, C2 and C3 represent the three 10-min periods before iv injection of α-ANP. C4, C5 and C6 represent the three 10-min periods after iv injection.

	C1	C2	C3	C4	C5	C6
Urine volume (µl/min)						
Control	14.8 ± 1.9	15.1 ± 1.7	15.3 ± 2.3	102.5 ± 17.7*	57.1 ± 17.5 *	54.1 ± 7.3 *
Cirrhotic	11.2 ± 2.5	13.2 ± 2.1	13.9 ± 2.2	37.9 ± 9.1 *a	28.9 ± 7.9#c	15.7 ± 4.1 b
Sodium excretion (µEq/min)						
Control	1.2 ± 0.4	0.9 ± 0.1	1.0 ± 0.2	14.1 ± 3.2*	10.3 ± 2.8*	7.0 ± 2.1*
Cirrhotic	0.4 ± 0.1b	0.4 ± 0.1b	0.5 ± 0.1b	3.4 ± 1.0#a	3.4 ± 1.2#b	1.8 ± 0.8c
Potassium excretion (µEq/min)						
Control	2.5 ± 1.1	2.5 ± 0.4	2.4 ± 0.4	6.6 ± 1.2*	5.0 ± 1.0*	3.8 ± 1.1
Cirrhotic	1.9 ± 0.5	2.2 ± 0.5	2.3 ± 0.5	5.2 ± 1.5&	4.5 ± 1.2&	4.3 ± 0.9
Inulin Clearance (ml/min)						
Control	2.3 ± 1.1	2.5 ± 0.4	2.3 ± 0.0	4.6 ± 0.9*	2.9 ± 0.7	2.8 ± 0.6
Cirrhotic	2.3 ± 0.4	2.6 ± 0.4	3.0 ± 0.5	4.6 ± 0.6*	3.3 ± 0.5	3.2 ± 0.5
Mean arterial pressure (mmHg)						
Control	117 ± 3	120 ± 4	122 ± 4	108 ± 5&	102 ± 5#	96 ± 5#
Cirrhotic	93 ± 3a	92 ± 4a	92 ± 4a	76 ± 4#a	69 ± 5#b	78 ± 6#b

& $p < 0.05$, # $p < 0.01$ and * $p < 0.001$ with respect to values before the injection of α-ANP. a $p < 0.001$, b $p < 0.01$ and c $p < 0.05$ with respect to control rats.

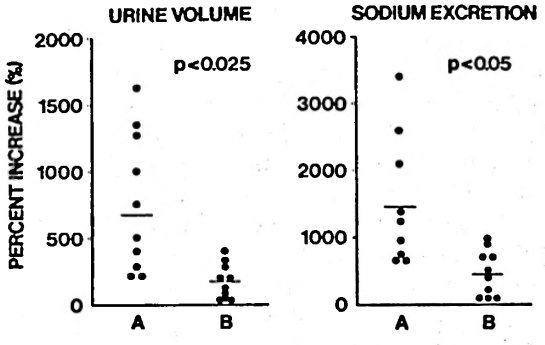

Fig. 1. *Percent increase of urine volume and sodium excretion induced by α-ANP in cirrhotic (A) and control rats (B).*

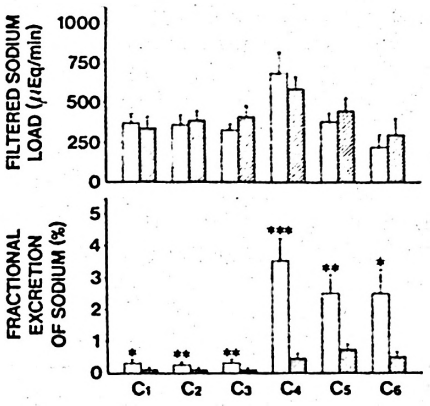

Fig. 2. *Filtered sodium load and fractional excretion of sodium in control (open bars) and cirrhotic (shadowed bars) rats in response to infusion of α-ANP.*

C1, C2 and C3 represent the three 10 min periods before the administration of α-ANP. C4, C5 and C6 represent the three 10 min periods after the i.v. injection of α-ANP. * $p < 0.05$, ** $p < 0.025$, *** $p < 0.001$ with respect to control rats.

and sodium and potassium excretion in the two groups of animals (table III). Cirrhotic and control rats showed a transitory increase in inulin clearance within the first clearance period after starting the infusion of α-ANP. The effect of α-ANP on renal function was more marked within the first 10-min period after its administration in the two groups of rats. However, the maximal effect of α-ANP on arterial pressure occurred within the second period in cirrhotic rats and within the third period in control rats (table III).

Cirrhotic rats remarkably showed a significantly lower diuretic and natriuretic responses to α-ANP than did control animals. Within the first 10-minute period after α-ANP administration, diuresis and natriuresis increased in control rats by 699±176 % and 1408±351 %, respectively. The corresponding values in cirrhotic rats were 183±40 % ($p<0.025$) and 413±134 % ($p<0.05$), respectively (figure 1). Urine volume and sodium excretion in cirrhotic rats were also significantly reduced during the second and third 10-minute period after α-ANP administration, although differences were considerably smaller (table III). In contrast, α-ANP produced similar effect on inulin clearance and mean arterial pressure in the two groups of animals. However, since cirrhotic rats showed lower baseline arterial pressure than control rats, the intensity of the arterial hypotension induced by α-ANP was more pronounced in the former group of animals.

Figure 2 shows the effect of α-ANP on filtered load and fractional excretion of sodium in cirrhotic and control rats. α-ANP induced a similar increase in filtered sodium load in both groups, whereas it produced a significantly lower increase in fractional sodium excretion in cirrhotic rats.

Discussion

During the last few years, numerous studies have shown that carbon tetrachloride induced cirrhosis in rats mimicking human cirrhosis. In addition to a widespread hepatic fibrosis with nodule for-

mation, these animals develop abnormalities of systemic and splanchnic hemodynamics (27), renal function (19, 21) and endogenous vasoactive systems (16) similar to those found in patients with cirrhosis and ascites. On the other hand, although carbon tetrachloride is a well recognized renal toxic agent, there is evidence that tubular damage does not develop in this animal model, since renal histology is normal (16, 20, 21) and the urinary excretion of N-acetyl-β-d-glucosaminidase, a sensitive marker of tubular necrosis, does not increase during the cirrhosis induction program (J. Solá, unpublished observations). Therefore, investigations in this animal model may be relevant to understanding what occurs in human cirrhosis.

The results of the present study clearly demonstrate that diuretic and natriuretic responses to high dosage of ANP was markedly reduced in cirrhotic rats with ascites as compared to control animals. This impaired natriuretic response was related to a higher tubular reabsorption of sodium in cirrhotic rats, since the filtered sodium load increased to a similar degree in both groups of animals. Cirrhotic rats with ascites present marked hyperaldosteronism (16), which increased sodium reabsorption in distal nephron. In addition, as it occurs in human cirrhosis (23), these animals may also present an increased activity of the sympathetic nervous system which stimulates sodium reabsorption in the proximal tubule, loop of Henle and distal and collecting tubules (10). Therefore, the natriuretic effect of ANP in cirrhotic rats with ascites could be reduced because it was counteracted by the tubular action of aldosterone and the sympathetic nervous activity. The lower mean arterial pressure after ANP administration present in cirrhotic rats with ascites, either by activating endogenous vasoactive systems that affect tubular sodium reabsorption or by changing intrarenal hemodynamics, could be an alternative mechanism for the blunted natriuretic response to ANP in these animals.

The results of the current study are in keeping with those of a recent investigation by KOEPKE *et al* (17) in conscious rats with bile obstruction and ascites. The i.v. administration of several dosages of ANP to these animals resulted in a blunted natriuretic effect as compared to control rats. However these authors did not find significant differences in mean arterial pressure between bile ligated and normal rats, thus probably reflecting a different systemic hemodynamic from those present in cirrhosis with ascites (3, 6, 19, 23, 27).

The circulating plasma levels of ANP in man and experimental animals are very low. In healthy man the plasma concentration of ANP reported by most studies ranges from 5 to 130 pg/ml (2, 8, 18, 25, 28). In rats it ranges from 50 to 200 pg/ml (4, 9, 11, 26). In animals included in the present study the plasma concentration of ANP was not measured. However, during the infusion of the peptide it probably would have reached very high values since ANP was administered at a very large dosage (a bolus of 1 μg followed by a constant infusion of 33 ng/min). Despite of this, the natriuretic response was very poor. Several studies in cirrhotic patients with ascites have found increased plasma concentration of ANP, however the peptide levels only were two to three times greater than those in healthy volunteers (1, 12, 13). Therefore it is not surprising that endogenous ANP is unable to reverse the exaggerated tubular sodium reabsorption present in these patients.

To sum up, the results of the current study show that the diuretic and natriuretic responses to a pharmacological dose of α-ANP were markedly reduced in cirrhotic rats with ascites. Since the effect of α-ANP on GFR in these animals was comparable to that in control rats, the blunted diuretic and natriuretic responses in cirrhotic rats were probably related to

the exaggerated tubular sodium and water reabsorption present in these animals.

Acknowledgments

Supported by a grant from «Comisión Asesora de Investigación Científica y Técnica» (CAYCIT 668-84) (Spain). C. López had a grant from «Fondo de Investigaciones Sanitarias de la Seguridad Social» (FISS 86/467) (Spain)

Resumen

Se determinan filtrado glomerular, volumen urinario, excreción urinaria de sodio y presión arterial, antes y durante la adminstración i.v. de Factor Natriurético Atrial de rata de 28 aminoácidos (α-ANP) (un bolus de 1 μg seguido de una infusión constante de 33 ng/min), en ratas control y ratas con cirrosis inducida mediante Cl_4C que presentan retención de sodio y ascitis. El α-ANP produce un incremento similar en el filtrado glomerular y en la cantidad de sodio filtrada en ambos grupos de animales. Sin embargo, el efecto diurético y natriurético del α-ANP es significativamente inferior en las ratas cirróticas con ascitis (de 13,8±1,9 a 37,8±9,1 μl/min, y 0,5± 0,1 a 3,3±1,0 μEq/min) que en las control (de 14,6± 1,3 a 102,5±17,7 μl/min, $p<0,005$; y de 1,0±0,3 a 14,1±3,2 μEq/min, $p<0,001$). Los resultados indican que en las ratas cirróticas con ascitis existe una escasa respuesta diurética y natriurética al α-ANP, probablemente como consecuencia de la hiperabsorción tubular de sodio que presentan estos animales.

Palabras clave: Cirrosis experimental, Ascites, Factor natriurético atrial, Excreción de sodio.

References

1. Arendt, R. M., Gerbes, A.L., Ritter, D., Stangl, E., Bach, P. and Zähringer, J.: *J. Hypert.*, 4, (suppl. 2), S131-S135, 1986.
2. Arendt, R. M., Gerbes, A. L., Stangl, E., Glatthor E. *et al*: *Klin. Wochenschr.*, 65, (S 8), 122-126, 1987.
3. Arroyo, V., Bosch, J., Mauri, M., Viver, J., Mas, A., Rivera, F. and Rodes, J.: *Eur. J. Clin. Invest.*, 9, 69-73, 1979.
4. Baranowska, B., Gutkowska, J., Talbot, P., Genest, J. and Cantin, M.: *Neurosci. Lett.*, 76, 119-123, 1987.
5. Bonkowsky, H., Hartle, D., Simon, D., Mallen, B., Kutner, M. and Glambos, J.: *Hepatology* 6, 1213, 1986.
6. Bosch, J., Arroyo, V., Betriu, A., Mas, A., Carrillho, F. Rivera, F., Navarro-López, F. and Rodes, J.: *Gastroenterology*, 78, 92-99, 1980.
7. Bughart, N., Wernze, H. and Diehl, K. L.: *Klin. Wochenschr.*, 64 (S6), 103-107, 1986.
8. Cappuccio, F. P., Markandu, N. D., Buckley, M. G., Sagnella, G. A., Shore, A. C. and Macgregor, G. A.: *Clin. Sci.*, 72, 531-539, 1987.
9. Debinski, W., Gutkowska, J., Kurchel, O. Racz, K., Buu, Nt., Cantin, M. and Genest, J.: *Biochem. Biophys. Res. Comm.*, 134, 279-283, 1986.
10. Di Bona, G. F.: *Rev. Physiol. Biochem. Pharmacol.*, 94, 75-181, 1982.
11. García, R., Lachance, D., Thibault, G., Cantin, M. and Gutkowska, J.: *Biochem. Biophys. Res. Comm.*, 136, 510-520, 1986.
12. Gerbes, A. L., Arendt, R. M., Rutter, D., Jüngst, D., Zahringerand, J. and Paum-Gartner, G.: *N. Engl. J. Med.* 313, 1609-1610, 1985.
13. Gines, P., Jiménez, W., Navasa, M. *et al.*: *J. Hepatol.*, 3 (Suppl. 1), S30, 1986.
14. Henriksen, J. H., Schyten, H. J., Bendtsen, F. and Warberg, J.: *Liver*, 6, 361-368, 1986.
15. Jiménez, W., Martínez-Pardo, A., Arroyo, V. *et al.: Am. J. Physiol.*, 250, F749-752, 1986.
16. Jiménez, W., Martínez-Pardo, A. and Arroyo, V.: *Hepatology*, 5, 245-250, 1985.
17. Koepke, J. P., Jones, S. and Dibona, G. F.: *Am. J. Physiol.*, 252, R1019-R11023, 1987.
18. Kouchi, O., Takayaki, I. and Hidekazu, H. *et al.: Lancet*, i, 106, 1986.
19. López-Novoa, J. M., Rengel, M. A. and Hernando, L.: *Am. J. Physiol.*, 238, F353-F357, 1980.
20. López-Novoa, J. M., Rengel, M. A., Rodicio, J. L. and Hernando, L. A.: *Patologia*, 9, 233-240, 1976.
21. López-Novoa, J. M., Rengel, M. A., Rodicio, J. L. and Hernando, L.: *Am. J. Physiol.*, 232, F315-F318, 1977.
22. Morgan, T., Imada, T. and Inagami, T.: *Hepatology*, 6, 1215, 1986.
23. Ring-Larser, H., Hesse, B., Heriksen, J.H. and Christensen, N. J.: *Hepatology*, 3, 304-310, 1982.
24. Ross, E. J.: Aldosterone and Aldosteronism. Lloyd-Luke, Ltd. (Medical Books), 1975.
25. Sagnella, G. A., Markandu, N. D., Shore, A.

C. and McGregor, G. A.: *Lancet*, i, 179-181, 1986.
26. Saito, H., Inui, K., Matuskawa, Y. *et al.: Biochem. Biophys. Res. Comm.*, 137, 1079-1085, 1986.
27. Vorobioff, J., Bredfeldt, J.E. and Groszman, R.: *Gastroenterology*, 1120-1126, 1984.
28. Weidman, P., Hellmueller, B., Dominick, E. *et al: J. Clin. Endocrinol. Metab.*, 62, 1027-1036, 1986.