Table 3. Uterine decidualization in androgenized female rats given multiple s.c. injections of physiological saline (Groups 11–12) or 400 ng LHRH (Groups 13–14) at 14:00 h every 4 days, starting before (Day 25) or after (Day 37) vaginal opening, until 53 days of age.

Group	Beginning of LHRH treatment	No. of rats	Uterine weight (mg)* Traumatized horn	Control horn
11	Day 25	5	279 ± 84^a	225 ± 21^a
12	Day 37	5	250 ± 20^a	243 ± 17^a
13	Day 25	7	1340 ± 90^c	203 ± 17^a
14	Day 37	7	675 ± 50^b	240 ± 26^a
15#	–	6	1350 ± 106^c	210 ± 20^a

* Values are mean ± SEM. Values with different alphabetical superscripts in any one column differ at a level of $p < 0.05$ (Duncan's new multiple range test). # Normal pseudopregnant rats: pseudopregnancy was induced by transplantation of a pituitary under the renal capsule.

nized female rats, vaginal cervical stimulation does not induce functional corpora lutea,[14] but reserpine treatment or isotransplants of pituitaries beneath the kidney capsule do[8,14]. Barraclough and Fajer[5] have reported that progesterone secretion by the corpora lutea of androgenized rats decreases and that administration of prolactin results in increased secretion of progesterone by these corpora lutea. When a pituitary gland was transplanted from a normal rat into the kidney capsule of an androgenized rat to maintain functional corpora lutea, implantation occurred in some of the rats. These findings suggest that failure of embryos to implant may result from a deficit of progesterone from the induced corpora lutea.

The vaginal opening in androgenized female rats occurs at approximately 32 days of age. The uterine sensitivity to the decidual reaction, i.e. endometrial scratching, elicited a better response ($p < 0.05$) in the rats that received multiple LHRH injections before vaginal opening. In fact, the response was the normal decidual response such as is observed in pseudopregnant rats. These results indicate that the cyclic LH surges before vaginal opening may be necessary for the induction of pregnancy in androgenized rats.

Acknowledgment. I thank Dr H. Horikoshi, Sankyo Pharmaceutical Co., Tokyo for the gift of LHRH and Dr T. Etoh for help with parts of these experiments.

1 Barraclough, C. A., Recent prog. Horm. Res. *22* (1966) 503.
2 Wagner, J. W., Erwin, W., and Critchlow, V., J. Endocr. *79* (1966) 1135.
3 Borvendeg, J., Hermann, H., and Bajusz, S., J. Endocr. *55* (1972) 207.
4 Hahn, D. W., and McGuire, J. L., Endocrinology *102* (1978) 1741.
5 Barraclough, C. A., and Fajer, A. B., Proc. soc. exp. Biol. Med. *128* (1968) 781.
6 Kramen, M. A., and Johnson, D. C., Fert. Steril. *22* (1971) 745.
7 Mennin, S. P., Kubo, K., and Gorski, R. A., Endocrinology *95* (1974) 412.
8 Sawada, T., Yasuda, T., and Ichikawa, S., Jap. J. Anim. Reprod. *14* (1968) 65.
9 Matton, P., and Maurasse, C., Biol. Reprod. *32* (1985) 1095.
10 Sawada, T., J. Reprod. Fer. *80* (1987) 595.
11 Sawada, T., J. Endocr. *110* (1986) 507.
12 Sawada, T., Endocr. jap. *34* (1987) 273.
13 De Feo, V. J., Endocrinology *72* (1963) 305.
14 Zeilmaker, G. H., Acta endocr. *46* (1964) 571.

0014-4754/88/060511-03$1.50 + 0.20/0

Antagonism of V_2-receptor effect of antidiuretic hormone by atrial natriuretic peptide in man

J. Brown*, M. L. Forsling[a], G. Valdes, J. D. H. Slater[b] and C. T. Dollery

Royal Postgraduate Medical School, Du Cane Road, London W12 (England), [a] Department of Physiology, Middlesex Hospital Medical School, Cleveland Street, London W1 (England), and [b] Cobbold Laboratories, Middlesex Hospital, Mortimer Street, London W1 (England)
Received 2 December 1987; accepted 2 March 1988

Summary. Human α-atrial natriuretic peptide (h-αANP) makes the urine of dehydrated volunteers hypotonic to plasma despite high circulating concentrations of antidiuretic hormone. Urinary dilution with h-αANP also occurs in subjects receiving indomethacin. Therefore, h-αANP antagonises effects of antidiuretic hormone on distal tubular V_2-receptors in man, probably without involving prostaglandins.
Key words. Atrial natriuretic peptide; antidiuretic hormone; hydraulic conductivity; prostaglandins.

Atrial natriuretic peptides may be important hormones for the control of the extracellular fluid volume, and they have many intrarenal actions that could increase the excretion of sodium and water. They increase the rate of glomerular filtration, but they may also reduce sodium and water reabsorption from proximal and medullary tubules[1,2]. Moreover, atrial peptides can antagonise the stimulation of water flow caused by arginine vasopressin (AVP) across the toad bladder[3], and, recently, it has been shown that the synthetic rat atrial natriuretic peptide atriopeptin III can also inhibit the increase of hydraulic conductivity caused by AVP in isolated perfused rabbit collecting ducts[4]. These observations add another possible explanation for the diuretic effect of atrial peptides in the intact animal[4]. So far, however, the complexity of the intact animal and the number of potential intrarenal mechanisms available has not allowed specific mechanisms to be assigned unambiguously to either the natriuretic or water-diuretic effects of atrial peptides in vivo. Despite this, we report prima facie evidence that h-αANP actually does antagonise the effect of endogenous antidiuretic hormone on the hydraulic conductivity of the distal tubular epithelium of normal volunteers and that this mechanism

contributes significantly to the diuretic effect of h-αANP in man.

A first group of 7 normal male volunteers was studied. After overnight dehydration for 12 h, each volunteer passed 3 successive collections of urine, a basal 1-h collection and then two collections, each of 30 min. During the last two collections, subjects received i.v. in one arm 15 pmol kg^{-1} min^{-1} of synthetic h-αANP (Bissendorff Peptide GmbH, FRG). Venous blood was sampled from the opposite arm 30 min before, and 27 and 57 min into this infusion. A second group of 8 normal volunteers was studied in their normal state of hydration after a light lunch. They provided initial samples of urine and blood and then drank 1 l of water. 45 min after drinking, they voided their bladders and a 30-min urinary collection was made. A blood sample was taken at the midpoint of this collection. A third group of 6 normal male volunteers was studied similarly to the first group, except that they were studied on two days. On one day, they received an i.v. loading dose of 24 mg indomethacin (Merck, Sharpe & Dohme, USA) at the beginning of the basal hour and then 15 mg h^{-1} which continued throughout the infusion of h-αANP over the next hour. On the other day, they received only the carrier of the indomethacin (40 ml loading, then 20 ml h^{-1} of 0.9% NaCl) together with the infusion of h-αANP. Plasma AVP was measured by a radioimmunoassay described previously[5]. Plasma renin activity was measured by radioimmunoassay (CIS Ltd, UK). Plasma h-αANP was measured after extraction on a Sep-pak C18 cartridge (Waters Associates) and elution with acetonitrile: trifluoroacetic acid (60% v/v:0.05% v/v). h-αANP in the eluate was assayed by radioimmunoassay (Amersham, UK).

In the first group of volunteers, the mean plasma concentration of h-αANP increased from 10 ± 4 pmol l^{-1} before the peptide was infused to 187 ± 45 pmol l^{-1} at the end of the peptide infusion. This infusion of h-αANP increased the rate of sodium excretion from a basal value of 173 ± 25 μmol min^{-1} (mean ± SEM) to a maximum of 359 ± 55 μmol min^{-1} during the second half of the infusion ($0.02 > p > 0.01$, $t = 3.32$, $d.f = 6$). Water excretion also increased during the infusion of h-αANP so that the mean ratio of urinary to plasma osmolality fell sharply in each subject (fig. 1, lower panel). The corresponding mean urinary osmolalities were 812 ± 49 mosmol kg^{-1} basally and 170 ± 17 mosmol kg^{-1} for the last 30 min of peptide infusion. Despite the consistent production of a hypotonic urine during the infusion of h-αANP, the circulating concentrations of AVP remained high (fig. 1, upper panel). The mean plasma concentration of AVP before infusion of h-αANP was 2.22 ± 0.78 pmol l^{-1} and this was not significantly different from its mean value of 1.38 ± 0.25 pmol l^{-1} at the end of the infusion of h-αANP. One volunteer started with a substantially elevated level of plasma AVP compared to the others (fig. 1). Although this volunteer reported no discomfort, it is known that emotion and pain, as could occur with venepuncture, may sometimes raise AVP levels sharply[6]. This might explain the high AVP levels in this volunteer, particularly as the level fell late in the study, when the subject would have been most accustomed to the protocol. In the second group of volunteers, using the same assay procedures, drinking 1 l of water caused urinary osmolality to fall from 680 ± 90 to 155 ± 30 mosmol kg^{-1} between the basal and final urinary collections. At the same time, the corresponding plasma concentrations of AVP fell from 0.98 ± 0.30 to 0.27 ± 0.10 pmol l^{-1} (fig. 2).

In a previous study using our radioimmunoassay[7], circulating concentrations of AVP in the range 0.95 ± 0.10–1.2 ± 0.4 pmol l^{-1} were associated with urinary osmolalities in the range 695 ± 110–860 ± 50 mosmol kg^{-1} in dehydrated subjects who were given water to drink. Consequently, the urinary osmolality of 170 ± 17 mosmol kg^{-1} found at the end of the infusion of h-αANP in the present study is signif-

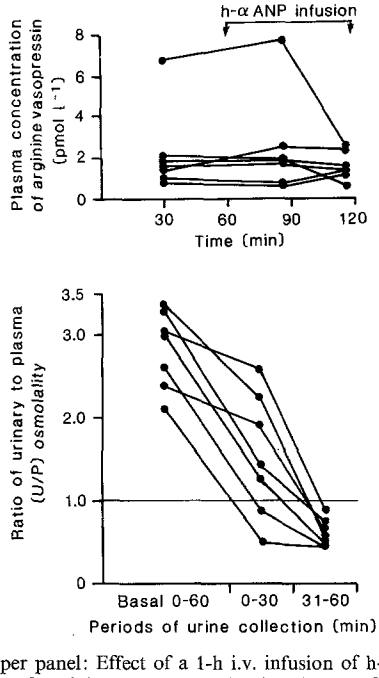

Figure 1. Upper panel: Effect of a 1-h i.v. infusion of h-αANP on the concentration of arginine vasopressin in the plasma of 7 dehydrated volunteers. Corresponding changes of urinary to plasma osmolality ratio are shown in panel below. h-αANP was infused at 15 pmol kg^{-1} min^{-1} in 40 ml of 0.9% NaCl plus 4 ml of 20% human serum albumin at 44 ml h^{-1}. The volunteers were studied with their informed consent and with approval of the Hammersmith Hospital Ethical Committee. Lower panel: Effect of a 1-h i.v. infusion of h-αANP on the ratio of urinary osmolality to plasma osmolality in the same 7 dehydrated volunteers. A 1-h basal collection of urine was followed by two 30-min collections during the peptide infusion.

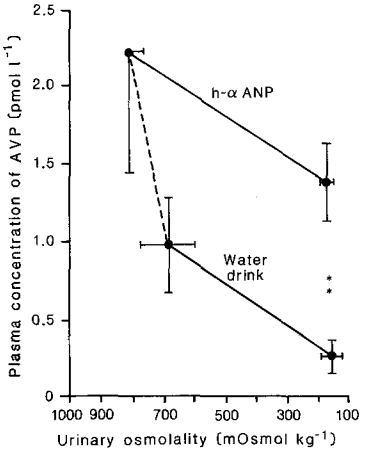

Figure 2. Effect of a 1-h infusion of h-αANP at 15 pmol kg^{-1} min^{-1} as described for figure 1 on urinary osmolality and plasma arginine vasopressin (AVP) in 7 volunteers after 12 h dehydration (upper unbroken line). These results are those of the upper panel of fig. 1, but are expressed here as means ± SEM. They are compared with urinary osmolality and plasma AVP concentration measured by the same assays in 8 normally hydrated volunteers both before and after drinking 1 l of water (lower unbroken line). Both the infusion of h-αANP and the water drink reduced urinary osmolality to similar levels. However, the plasma AVP concentration remained significantly higher after the infusion of h-αANP than after drinking water. ** = $0.01 > p > 0.001$.

icantly lower than would normally correspond to the concurrently measured plasma AVP concentration of 1.38 ± 0.25 pmol l^{-1}. Moreover, this plasma AVP concentration was significantly greater than that of 0.27 ± 0.10 pmol l^{-1} found in our subjects 1 h after drinking 1 l of water ($0.01 > p > 0.001$; $t = 4.1$; d.f. = 13). This was so despite similar osmolalities of 170 ± 17 and 155 ± 30 mosmol kg^{-1} respectively for the corresponding urinary samples (fig. 2).

In the third group of volunteers, the infusion of h-αANP increased sodium excretion from 128 ± 26 to 218 ± 58 µmol min^{-1} on the day with indomethacin and, similarly, from 126 ± 18 to 235 ± 42 µmol min^{-1} on the day without. However, PRA was significantly suppressed by indomethacin before the start of the h-αANP infusion (1.37 ± 0.21 ng ml^{-1} h^{-1} on day with indomethacin vs 1.80 ± 0.29 ng ml^{-1} h^{-1} on day without indomethacin $0.05 > p > 0.01$, $t = 2.9$, d.f. = 5), suggesting the inhibition of at least one renal cyclooxygenase[8]. Despite this, indomethacin did not alter the urinary dilution caused by h-αANP (fig. 3).

Our results confirm the finding of others that infusions of h-αANP do not alter circulating AVP concentrations in dehydrated volunteers[9], but they also allow an important mechanism of the diuretic effect of h-αANP to be identified. h-αANP might increase urinary volume in a number of ways. It does not do so by reducing the plasma concentration of AVP (fig. 1). It might increase urinary volume and reduce urinary osmolality by increasing medullary blood flow and washing out medullary interstitial solute[10]. However, this could not account for a urine which is actually hypotonic to plasma. An increased blood flow would sweep away the extra solute that is actively accumulated in the medullary interstitium. This could reduce the osmolality of the interstitium and of medullary tubular fluid as far as that of the blood entering the medulla from the rest of the body, but no lower. Urinary dilution below isotonicitiy with the rest of the body occurs by the active reabsorption of sodium chloride across the water-impermeable epithelium of the loop of Henle, and it is this hypotonic tubular fluid which ultimately comprises a hypotonic urine[11]. The action of AVP on distal tubular, adenylate cyclase-coupled V_2 receptors renders tubular epithelium beyond the loop of Henle highly permeable to water, so that the hypotonic effluent of the loop of Henle is reconcentrated before it leaves the collecting ducts as urine[11, 12]. In man, unlike some other animals, this process of reconcentration is very efficient, and urine does not become hypotonic in the presence of elevated levels of antidiuretic hormone even at very high rates of tubular flow during profound osmotic diuresis[13]. Consequently, in man, the excretion of a hypotonic urine in the presence of levels of AVP normally associated with antidiuresis is unequivocal evidence that, of all the potential mechanisms, h-αANP acutely increases water excretion to a significant extent by antagonising the increased hydraulic conductivity caused by AVP in the distal tubular epithelium. Changes in medullary blood flow cannot account for the extent of the urinary dilution caused by h-αANP. Prostaglandins also antagonise the increased hydraulic conductivity that AVP causes in isolated perfused collecting ducts[14]. However, normal cyclooxygenase activity does not seem necessary for the antagonism mediated by h-αANP because the peptide causes the same fall in the urinary osmolality of dehydrated volunteers whether or not they were treated with indomethacin (fig. 3). It is unusual to be able to ascribe a change in renal function unambiguously to a particular intrarenal event in the intact animal. However, the evidence given here not only allows such a prima facie argument but does so in normal unstressed man. The evidence therefore complements the previous report that rat atriopeptin III can antagonise the effect of AVP on the hydraulic conductivity of the isolated, perfused collecting duct in vitro[4], and it potentially provides the functional correlate of the receptor-like specific binding of radiolabelled rat atrial natriuretic peptide to rat collecting ducts[15]. As a result, whatever its role in sodium balance, h-αANP may also be involved in man as a hormone of water homeostasis. Levels of immunoreactive h-αANP achieved during the peptide infusions in this study are somewhat higher than those found in healthy individuals[16], and further work is needed to define the role of h-αANP in normal water balance. However, the levels of h-αANP achieved were similar to those found in heart failure[16], a syndrome in which plasma AVP is often increased[17]. Therefore, it is already an important possibility that h-αANP might modulate pathological water retention caused by AVP in heart failure[17]. However, inappropriate secretion of AVP is not the sole cause of increased water retention and hyponatraemia in heart failure. For example, heart failure may increase proximal tubular sodium reabsorption and thus impair both the delivery of sodium to the distal diluting segment and the function of this segment[17]. Consequently, the rôle of h-αANP in counteracting the mechanisms of abnormal water retention in human heart failure merits further investigation.

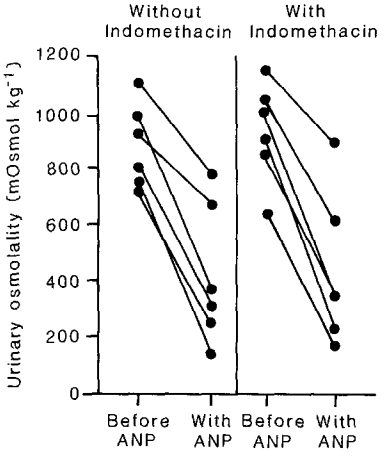

Figure 3. Effect of indomethacin on urinary osmolality during the second half of a 60-min infusion of 15 pmol kg^{-1} min^{-1} of h-αANP as described for figure 1. Individual results are shown for 6 dehydrated subjects, studied on two days. On both days, urine was collected for 1 h before the peptide infusion, and a 30-min collection was made in the last half of the peptide infusion. On one day (right hand panel), subjects were treated with indomethacin. On the other day (left hand panel), they only received the carrier for the indomethacin.

* Present address and address for correspondence: The Physiological Laboratory, Downing Site, Downing Street, Cambridge CB2 3EG.

1 Ballermann, B. J., and Brenner, M. B., J. clin. Invest. *76* (1985) 2041.
2 Harris, P. J., Thomas, D., and Morgan, T. O., Nature *326* (1987) 697.
3 Grosso, A., Spinelli, F., and de Sousa, R. C., Reg. Pept., Suppl. *4* (1985) 104.
4 Dillingham, M. A., and Anderson, R. J., Science *231* (1986) 1572.
5 Forsling, M. L., and Williams, H., J. Physiol., Lond. *346* (1984) 49.
6 Moses, A. M., and Miller, M., in: Handbook of Physiology, section 7, Endocrinology *4* (1974) 233.
7 Phillips, P. A., Rolls, B. J., Ledingham, J. G. G., Forsling, M. L., Morton, J. J., Crow, M. J., and Wollner, L., New Engl. J. Med. *311* (1984) 753.
8 Dunn, M. J., and Zambraski, E. J., Kidney Int. *18* (1980) 609.
9 Gnadinger, M. P., Weidmann, P., Rascher, W., Lang, R. E., Hellmuller, B., and Uehlinger, D. E., J. Hypertension *4* (1986) 623.
10 Sosa, R. E., Volpe, M., Marion, D. M., Atlas, S. A., Laragh, J. H., Vaughan, E. D., and Maack, T., Am. J. Physiol. *250* (1986) F529.
11 Berliner, R. W., and Bennett, C. M., Am. J. Med. *42* (1967) 777.
12 Michell, R. H., Kirk, C. J., and Billah, M. M., Biochem. Soc. Trans. *7* (1979) 861.

13 Goldberg, M., McCurdy, D. K., and Ramirez, M. A., J. clin. Invest. *44* (1965) 182.
14 Handler, J. S., Kidney Int. *19* (1981) 831.
15 Koseki, C., Hayashi, Y., Toprikai, S., Furuya, M., Ohnuma, N., and Imai, M., Am. J. Physiol. *250* (1986) F210.
16 Tikkanen, I., Fyhrquist, F., Metsarinne, K., and Leidenius, R., Lancet *2* (1985) 66.
17 Brown, J., in: New Perspectives in Cardiovascular Medicine, vol. 1, p. 9. Ed. C. Wood. Royal Society of Medicine, 1987.

0014-4754/88/060513-04$1.50 + 0.20/0

Effect of an antiglucocorticoid (RU-38486) on hydrocortisone induction of maltase-glucosamylase, sucrase-isomaltase and trehalase in brush border membranes of suckling rats

G. Galand

Laboratoire de Physiologie animale, Faculté des Sciences de Reims, B. P. 347, F-51062 Reims-cedex (France)
Received 26 October 1987; accepted 4 February 1988

Summary. Induction of α-glycosidases by hydrocortisone in suckling rats is inhibited by the daily administration of an antiglucocorticoid (RU-38486). Conversely, RU-38486 injected daily in 15-day-old rats for 7 days does not prevent the spontaneous development of α-glycosidases.
Key words. Antiglucocorticoids; hydrocortisone; maltase-glucoamylase; sucrase-isomaltase; trehalase; brush border; development.

Trehalase, maltase-glucoamylase and sucrase-isomaltase are integral membrane glycoproteins of brush border membranes[1]. Several studies have led to the conclusion that development of neonatal α-glycosidases is not absolutely dependent on glucocorticoids in the intestine[2-4], and not at all in the kidney[5]. Nevertheless, during the first two postnatal weeks, glucocorticoids have unquestionable effects; administration of hydrocortisone to suckling rats[6], rabbits[5] or mice[7] causes their precocious and simultaneous biosynthesis in the intestinal microvillous membrane. But these effects do not prove that glucocorticoids act as the final effectors; glucocorticoids may produce changes in other hormones or substances which in turn may be the true effectors. RU-38486 is a potent antiglucocorticoid, which is capable of fully antagonizing the effects of dexamethasone in vitro as well as in vivo at the level of its receptor[8]. RU-38486 has a strong binding affinity for the glucocorticoid receptor and lacks agonist activity[8]. In this paper, we describe the action of RU-38486 on trehalase, maltase, glucoamylase, sucrase and palatinase activities, prematurely induced by hydrocortisone during the second week of development of the rat. Evidence is given that during the first two postnatal weeks, exogenous glucocorticoids act as a physiological trigger.

Materials and methods. Rats of the Wistar strain, of either sex, were used. All rats were fed ad libitum and had unrestricted access to water. Three sets of littermates were used. The first set was divided into 4 groups; one group was given a s.c. injection of hydrocortisone acetate (Roussel Uclaf,

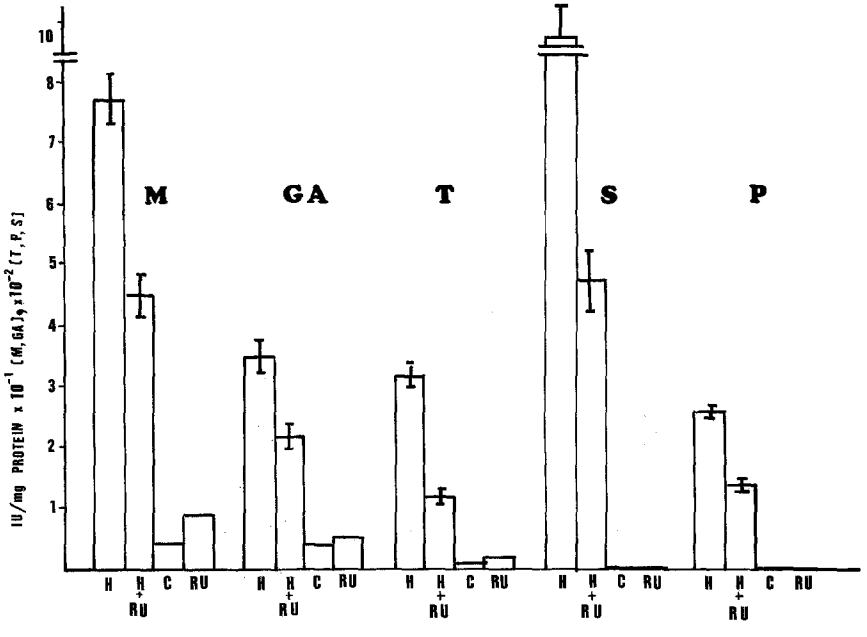

Figure 1. Effects of RU-38486 (25 mg/kg) on α-glycosidases induced by hydrocortisone in the suckling rat. 10-day-old rats were injected with (H) hydrocortisone; (H + RU) hydrocortisone + RU-38486; (RU) RU-38486 as described in the text. (C) control rats; (M) maltase; (GA) maltase-glucoamylase; (T) trehalase; (S) sucrase; (P) palatinase. Results are presented as the mean ± SE of 6 experiments for C and RU and 10 experiments for H and H + RU. Statistical differences between H and H + RU: $p < 0.001$.