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The Handling of NaCl Load in Rats During DOCA-Salt and Goldblatt 2 Kidney-1 Clip Hypertension Development

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The handling of an intraperitoneal NaCl load (2 % body weigth, 0.9 % NaCl) administered twice a week during DOCA-salt and Goldblatt 2K-1C hypertension development has been evaluated. An exaggerated natriuresis was observed in DS-hypertensive rats since blood pressure became higher with respect to normal (C), Doca (D) and uninephrectomized-salt (NS) rats that served as controls. However, this phenomenon was not observed in Goldblatt 2K-1C hypertensive rats (2K-1C) when compared to the response obtained in sham-operated (SO) rats. These results suggest that: 1) An increased blood pressure, *per se*, is not a determinating factor for exaggerated natriuresis. 2) Rise in blood pressure and exaggerated natriuresis may be related through a common mechanism in Doca-salt hypertension.

Key words: Sodium chloride load, Experimental hypertension.

FARNSWORTH and BARKER (6) were the first to recognize that patients with essential hypertension have a high rate of salt and water excretion following the administration of a salt load. This phenomenon, called the exaggerated natriuresis of hypertension, has been reported to occur in a variety of models of experimental hypertension (9, 14, 20). As high perfusion pressure is the obvious common factor in increased blood pressure, it may be essential to the phenomenon of exaggerated natriuresis. However, the presence of this phenomenon in DOCA-salt normotensive rats (15) and in normotensive subjects treated with aldosterone (17) in the escape phase, and the absence of an exaggerated natriuretic response in some strains of SHR (1, 13) would not support this possibility. The present experiments were designed to study the time-course relation in two models of arterial hypertension, between arterial pressure increase and renal function with regard to the kidney's ability to excrete an acute saline load.

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Materials and Methods

Animals. — Male Wistar rats initially weighing 125-150 g were used. All the rats were fed the same laboratory diet containing 73 mEq/kg of Na^+ and 185 mEq/kg of K^+. These rats were distributed at random in the different experimental groups: Control rats (C): These rats were kept under normal laboratory conditions and given tap water and rat chow *ad libitum* without any treatment (n = 6). DOCA-rats (D): There were treated with 12.5 mg/week of deoxycorticosterone enantate, s. c. (CORTIRON Depot, Schering) (n = 6). Uninephrectomized-salt rats (NS): The left kidney was removed under ether anesthesia and the rats received 1 % NaCl as drinking water (n = 6). DOCA-salt rats (DS): There were uninephrectomized, 1 % NaCl was given as drinking water and the rats were injected with 12.5 mg/week, s.c. of deoxycorticosterone enantate (n = 6). Goldblatt 2 kidney-1 clip rats (2K-1C): The left kidney was exposed under ether anesthesia and a silver clip with an inner diameter of 0.2 mm was placed in the left artery (n = 6). Sham-operated rats (SO): The left kidney was exposed under ether anesthesia without clipping (n = 6).

Experimental protocol. — C, D, NS and DS groups were used for DOCA-salt hypertension study; SO and 2K-1C groups were used for 2 kidney-1 clip hypertension study. Systolic blood pressure was measured twice a week by the tail cuff method in unanesthetized artificially warmed animals. Repetitive measurements were made until three consecutive values agree to within 10 mmHg. These were averaged to obtain the value recorded. To adapt the animals to the procedure, pressure was measured on several occasions prior to beginning the study.

During the course of the experiment, all the rats were placed into individual metabolic cages. Twice a week the rats were submitted to an isotonic saline loading test. Between 9 and 10 a.m. each rat was required to breathe ether to stimulate reflex emptying of the bladder. This urine was discarded. The rats were then injected with isotonic saline solution (2 ml/100 g body wt.), replaced in the metabolic cage and deprived of access to food and drinking fluid for 8 hours. Urine was collected in graded tubes containing olive oil. Urine samples were taken, 2 and 8 hours after injection, for diuresis (U_v), urinary sodium ($U_{Na}V$) and urinary potassium ($U_K V$) determinations. Na^+ and K^+ were measured by flame photometry (CORNING 435).

Statistical methods. — One-way Anova tests were made in order to compare the different groups of the different determinations. If the ANOVA was significant, the Newmann-Keuls technique was used to make all paired comparisons among groups, keeping the overall error small according to Bonferroni's rule. Data are expressed as mean ± SE.

Results

DOCA-salt hypertension. — Blood pressure increased from 124 ± 7.7 to 190 ± 6.3 mmHg in DOCA-salt rats, significant differences appearing from the 6th day of evolution. In rats from the NS and D groups, the changes in blood pressure were not significant, being the same as in control rats (C) (fig. 1).

The diuretic and natriuretic responses (fig. 2), either 2 and 8 hours after volume expansion, were significantly greater in the DS hypertensive rats than in the normotensive rats (groups C, NS and D). Diuretic response became significant on the 9th day and the natriuretic response on the 6th day of evolution. Furthermore, DS-rats excreted most of the total amount (taken to be 8 hours) of sodium

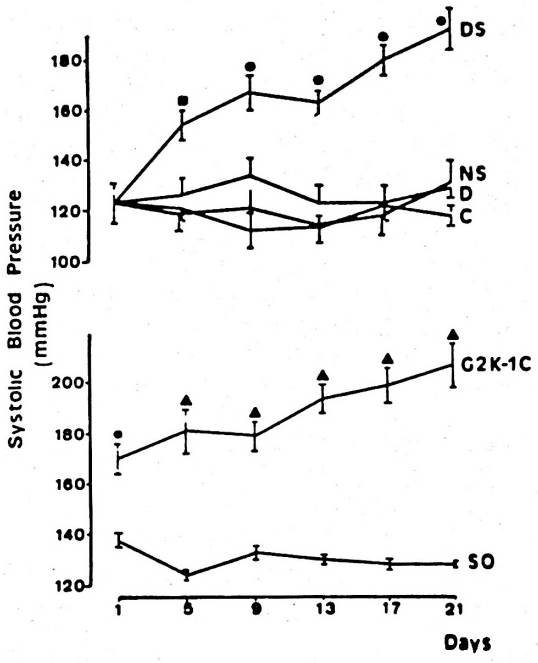

Fig. 1. *Evolution of systolic blood pressure measured by plethysmography during DOCA-salt and Goldblatt 2 kidney-1 clip hypertension development.* C = Control, D = DOCA-treated, NS = uninephrectomized-salt, DS = DOCA-salt, SO = Sham-operated and G2K-1C = Goldblatt 2 kidney-1 clip rats. ■ $p < 0.05$, ▲ $p < 0.01$, * $p < 0.001$, in comparison with their respective controls.

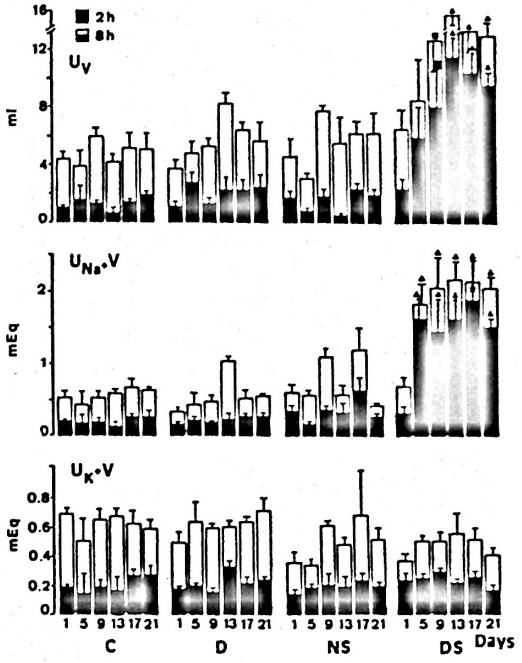

Fig. 2. *Diuretic (U_V) natriuretic ($U_{Na}V$) and kaliuretic ($U_K V$) responses to isotonic saline loading (2 % body weight, NaCl 0.9 %) at 2 h (shaded part of the bar) and 8 h (entire lenght of the bar) during DOCA-salt hypertension development.* Abbreviations as in figure 1. ■ $p < 0.05$, ▲ $p < 0.01$ vs. normotensive groups.

and urine volume during the first two hours. Such responses are not found in normotensive rats (C, NS and D). After 2 and 8 hours of saline expansion, kaliuretic (fig. 2) response had a similar evolution in all experimental groups during the study.

Goldblatt 2K-1C hypertension. — In 2K-1C rats, blood pressure was significantly increased with respect to SO rats, from the beginning of the study. In these rats, blood pressure increased progressively (from 170.5 ± 5.3 to 207 ± 8.3 mmHg) while in SO rats, normal levels were maintained (fig. 1). As shown in fig. 3, the diuretic responses of both groups of rats were not significantly different either 2 or 8 hours after saline injection. The natriuretic response in both groups of rats was similar. However, a higher sodium excretion was observed (2 h : $p < 0.05$; 8 h: $p < 0.01$) in SO rats on the 6th day of evolution and 2K-1C hypertensive rats (8 h: $p < 0.01$) on the 21st day. Kaliuretic response was maintained without significant differences until the 17th and 21st days, when total kaliuresis (8 hours) was increased in Goldblatt 2K-1C rats ($p < 0.01$), while no differences were found in potassium excretion between the two groups after

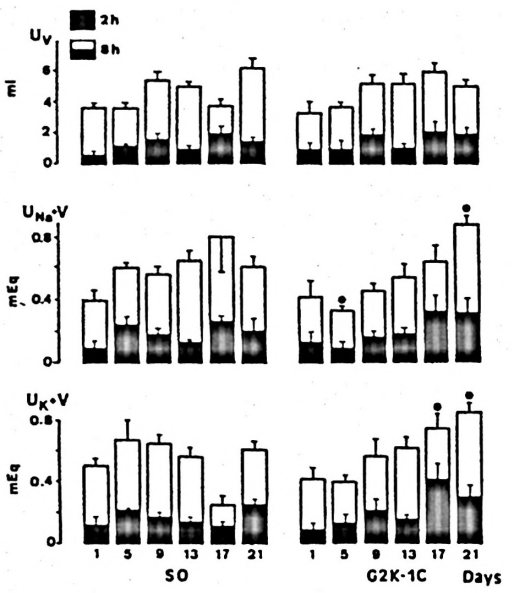

Fig. 3. *Diuretic (U_V) natriuretic ($U_{Na}V$) and kaliuretic ($U_K V$) responses to isotonic saline loading during Goldblatt 2K-1C hypertension development.* Abbreviations as in figure 1 and 2. * $p < 0.01$ vs. SO group.

the first two hours following saline injection.

Discussion

The administration of acute saline loading results in an immediate increase in urinary sodium excretion rate ($U_{Na}V$) which reaches higher levels in a variety of models of hypertension compared to normotensive controls. Our data show that DS-rats exhibit exaggerated diuretic and natriuretic responses during hypertension development. Basically these data agree with the observation of HALL and HUNGERFORD (9), FRIEDMAN *et al.* (7) and BURNETT *et al.* (2) in DOCA-salt treated rats and those obtained by ROVNER *et al.* (17) in patients with primary aldosteronism and in normal subjects treated with aldosterone.

The onset of the diuretic and natriuretic responses were earlier in DS-rats and obviously greater in magnitude than in normotensive animals. These phenomena can only be detected if, in the experimental protocol several measures are made after saline expansion (15, 17, 19). No significant changes to saline loading were observed in the kaliuretic response of DS-rats in comparison with the normotensive controls, as has been reported before by HALL and HUNGERFORD (9) in DOCA-salt rats, WILLIS (20) in SHR and WILLASSEN and OFSTAD (19) in hypertensive patients.

The data show that 2K-1C hypertensive rats exhibit similar diuretic, natriuretic and kaliuretic responses to saline loading to those observed in sham-operated rats during the study. These results do not agree with those obtained in unanesthetized rats after oral isotonic saline loading with this type of hypertension (16) and intravenously isotonic saline infusion in anesthetized rats (14). However, PETERS *et al.* (16) also studied conscious rats with two-kidney Goldblatt hypertension before and after intravenous infusion of an acute load. In response to expansion the unclipped kidney excreted 25-30 % more sodium than the kidney of normotensive rats, but excretion by the clipped kidney was decreased to a similar extent. Thus, bilateral excretion of water and sodium as well as GRF were comparable between normo- and hypertensive rats.

Differences in experimental protocols may explain the disparity between our results in Goldblatt 2K-1C rats and those of PETERS *et al.* (16) and MACKENZIE *et al.* (14) The natriuretic response may be dependent on the route of the saline administration. In this sense, conscious rats made hypertensive by the Grollman technique, exhibit an exaggerated natriuresis in response to an oral load of hypertonic

saline. However, intraperitoneal administration does not elicit this response (4).

Our data show that the rise in blood pressure and the exaggerated natriuretic response to saline loading are time-course related in DS-rats. This exaggerated natriuresis is generally considered as a secondary event following the rise in renal perfusion pressure, since acute normalization of perfusion pressure produced by pharmacological (9) or mechanical (14) procedures, prevents exaggerated natriuresis. However, chronic normalization of blood pressure maintains the exaggerated natriuresis in hypertensive rats (21).

The fact that exaggerated natriuresis does not appear in 2K-1C hypertensive rats (fig. 3), in several models of arterial hypertension (1, 5, 13) or in other circumstances where blood pressure is not high (12, 15, 17), support the notion that the exaggerated natriuretic response to a salt load may be largely independent of high blood pressure.

The possibility exists in DS-rats that the factor responsible for the rise in blood pressure also mediates the exaggerated natriuretic response, since DS rats show an increase in the digoxin-like factor (3, 11) that possesses natriuretic properties (8) and contributes to the rise in blood pressure (10, 18) in this model of hypertension. On the other hand, it is also possible that the increases in both sodium excretion and blood pressure are independent and are simply associated in some types of hypertension.

In summary, our study show that the natriuretic response to saline loading and blood pressure are time-course related in DS-rats, a phenomenon that is not observed in Goldblatt 2K-1C hypertensive rats.

Resumen

Se estudia la respuesta a una expansión de suero salino isotónico (2 % peso corporal, 0,9 % ClNa) administrada intraperitonealmente dos veces por semana durante el desarrollo de hipertension DOCA-sal y Goldblatt 2 riñones-1 clip. Se observa una natriuresis exagerada en las ratas hipertensas DOCA-salt (DS) desde el inicio de la elevación de la presión arterial, respecto a sus grupos controles: Normales (C), tratadas con DOCA (D) y Uninefrectomizadas-sal (NS). Sin embargo, este fenómeno no es observado durante el desarrollo de la hipertensión Goldblatt 2 riñones-1 clip. Estos resultados sugieren que la elevación de la presión arterial, *per se*, no es un factor determinante de la natriuresis exagerada, y que la elevación de la presión arterial y la natriuresis exagerada, observada en la hipertensión DOCA-sal, pueden estar producidas por un factor patogénico común.

Palabras clave: Expansión salina, Hipertensión experimental.

References

1. Beierwaltes, W. H. and Arendshorst, W. J.: *Circ. Res.*, 45, 721-726, 1978.
2. Burnett, J. C. Jr., Haas, J. A. and Larson, M. S.: *Am. J. Physiol.*, 18, F396-F399, 1985.
3. Casanova, I., Vargas, F., García-Torres, L., Soler, A., Luna, J. D. and García del Río, C.: *VII R. Nal. Liga Española Lucha contra la Hipertensión Arterial*, Pamplona, España. A. 121, 1986.
4. Ezrow, L. and Saperstein, L.: *Am. J. Physiol.*, 194, 436-440, 1958.
5. Farmann, N. and Bonvalet, J. P.: *Pflügers Arch.*, 354, 39-53, 1975.
6. Farnsworth, E. B. and Barker, M. H.: *Proc. Soc. Exp. Biol. Med.*, 52, 74-80, 1943.
7. Friedman, S. M., Hince, J. A. M. and Hardwick, D. F.: *Circ. Res.*, 3, 297-305, 1955.
8. Gruber, K. A., Metzler, C. H., Robinson, T. E. J., Buggy, J., Bullock, B. C. and Lymangrover, J. R.: *Fed. Proc.*, 44, 2795-2799, 1985.
9. Hall, C. E. and Hungerford, S.: *Can. J. Physiol. Pharmacol.*, 62, 436-440, 1983.
10. Kojima, I., Yoshimura, S. and Ogata, E.: *Life Sci.*, 30, 1775-1781, 1982.
11. Kojima, I.: *Biochem. Biophys. Res. Commun.*, 122, 129-136, 1984.
12. Ladd, M.: *J. Appl. Physiol.*, 3, 603-609, 1950.
13. Ledingham, J. and Simpson, O.: *J. Hypert.*, 4, (Supp 3): S245-S247, 1986.
14. Mackenzie, H. S., Norrill, A. L. and Ploth, D. W.: *Kidney Int.*, 27, 731-738, 1985.

15. Pearce, J. W., Sonnemberg, H., Veress, A. T. and Ackerman, U.: *Can. J. Physiol. Pharmacol.*, 47, 377-386, 1968.
16. Peters, G., Brunner, H. and Gross, F.: *Nephron*, 1, 295-309, 1964.
17. Rovner, D. R., Conn, J. W., Knopf, R. F., Cohen, E. L. and Hsueh, M. J-Y.: *J. Clin. Endocrinol.*, 25, 53-64, 1965.
18. Vargas, F., Haro-Martínez, J. M., Jodar, E., Soler-Díaz, A. and García del Río, C.: *J. Pharm. Pharmacol.* (in press).
19. Willassen, Y. and Ofstad, J.: *Hypertension*, 2, 771-779, 1980.
20. Willis, L. R. and Baver, J. H.: *Am. J. Physiol.*, 234, F29-F35, 1978.
21. Willis, L. R.: *Hypertension*, 3, 386-394, 1981.