REVISTA ESPAÑOLA DE FISIOLOGIA, 41, 107-116. 1985

Effects of Changes in Circulating Volume and in Arterial Pressure on Plasma Renin Activity in the Intact Rat

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(Received on May 7, 1984)

A. SOLER, C. GARCIA DEL RIO, L. GARCIA-TORRES, F. VARGAS, A. MARTIN-ANDRES and C. OSORIO. *Effects of Changes in Circulating Volume and in Arterial Pressure on Plasma Renin Activity in the Intact Rat.* Rev. esp. Fisiol., 41, 107-116. 1985.

The effects of changes in arterial pressure and in circulating volume on Plasma Renin Activity (PRA) in the intact rat were compared by two experimental procedures. Gradual volume depletion was induced by intraperitoneal injection of a hyperoncotic polyethylenglycol solution (PEG) in absence of acute changes in Systolic Arterial Pressure (SAP). SAP was measured in the conscious state by the tail cuff technique. Plasma Protein Concentration (PPC) and Hematocrit (Hct) increases after PEG injection were compared as the index for measuring the Plasma Volume Reduction (PVR). PRA showed a significant ($p < 0.001$) linear relationship with PPC, suggesting a direct dependence of renin secretion on volume depletion.

Acute changes in the circulating volume were induced by controlled hemorrhages of 5.0, 10.0, 15.0 and 20.0 ml of blood/kg body weight. The increase in PRA showed a significant relationship with the changes in circulating volume, but it did not show any dependence on the changes in Mean Arterial Pressure (MAP). Our results suggest that, in the intact and conscious rat, renin secretion responds to the information from the cardiopulmonary volume receptors rather than to that from the high pressure receptors.

Key words: Arterial pressure, Circulating volume, Renin secretion.

Evidence that carotid, aortic and cardiopulmonary receptors participate in the control of renin release comes from several studies. Most of these studies have investigated the influence of each Receptor System (RS) separately, using different experimental procedures that imply deactivation or blockade of some of the three RS (3-5, 10, 11, 14, 21, 23).

However, the complexity of the reported results have suggested to several authors that the renin release could be mediated by the interaction of the three RS, and the interpretation of the results could be conditioned by the experimental procedure used (20, 22).

Moreover, deafferentation of any RS seems to involve a change in the basal

information that the remaining systems could perceive (4, 14, 16, 17, 23) and, probably, in the renin secretory responsiveness induced by additional changes on basal information (1, 12, 21). Furthermore, other receptors could be implicated in the central blood pressure regulation and the renin secretion, since renal denervation can alter the central mechanism governing sympathetic outflow (12).

Therefore, the available information appears fragmentary and inadequate to provide a satisfactory model about the contribution of the three RS, and its integration, on the renin release in the whole animal.

On this basis we have designed the present work in an attempt to study the interaction of the high pressure receptors and the volume receptors on renin secretion, trying to avoid the alterations produced by the suppression of any of the RS on the remaining ones. The experimental procedures used, gradual volume depletion by intraperitoneal injection of hyperoncotic solutions and controlled hemorrhages, allow the analysis of the renin secretory response to simultaneous changes on arterial pressure and circulating volume in the intact and conscious rat with the three RS in normally working state.

Materials and Methods

Experimental Procedures. Male Wistar rats, weighing between 180 and 300 g, were maintained on ad libitum food and water intake in a constant temperature and humidity controlled room, illuminated between 8:00 a.m. and 8:00 p.m. Rats were handled several times prior experimental testing in order to minimize any stress.

Experiment 1. Fifty-four rats were made hypovolemic by injectig intraperitoneally 20 ml/kg body weight of a 20 % polyethyleneglycol 4000 (Doesder) in 0.9 % NaCl solution (PEG), and divided into five subgroups, that were bled under nembutal anaesthesia (40 mg/kg body weight, Serva) at 30, 60, 90, 120 or 240 min after PEG injection. Control group was injected i.p. with same amounts of isotonic saline vehicle and bled 60 min after the injection. Water intake was restricted after PEG or saline injection.

Systolic Arterial Pressure (SAP) was measured in the conscious rats before PEG or saline injection, and at 30 min intervals after the injection until the end of the experiment, by the tail-cuff technique with a sphygmomanometer pneumatic sensor unit (Technical Instruments Inc.) switched on a two channel recorder (Devices MX2). Individual SAP values were determined by averaging five consecutive readings. Each rat underwent at least two preliminary series of SAP determinations to minimize any stress.

Experiment 2. Twenty-six rats were anesthetized with nembutal (40 mg/kg) and a polyethylene catheter (PE 1), containing 1000 units/ml of a heparine isotonic NaCl solution, was inserted in the right carotid artery, placed subcutaneously and exteriorized on the dorsum of the neck. The rats were then allowed to recover for 48 hours before the experiment. The catheters were washed with isotonic saline and refilled with heparine solution 24 hours after implantation. Rats were restrained several times in a small Lucite cage (21 × 8 × 8 cm) before Mean Arterial Pressure (MAP) determination. The arterial catheter was attached to a pressure transducer (Bell and Howell 4-442) switched to the recorder. MAP was continuously monitored during 30 min until stabilization. Then, rats were divided into five subgroups, one of each underwent a controlled hemorrhage of 5, 10, 15 or 20 ml of blood per kg of body weight. The hemorrhage period was always shorter

than 90 seconds. Arterial pressure was again continuously monitored after the hemorrhage during 30 min, after which the last blood extraction was rapidly performed and the rats were finally killed with nembutal.

Blood samples and determinations. Blood samples were drained in experiment 1 by abdominal aortic puncture with siliconed syringes and needles. In experiment 2 initial and final samples were drained out throughout the arterial catheter. In all cases, blood samples were collected into two series of plastic chilled tubes containing 0.2 ml of 6 % Na_2 EDTA solution or 0.2 ml of 5,000 u.i./ml heparine solution. The tubes were gently shaked and small aliquots of heparinized blood were drawn immediately into WINTROBE tubes. All tubes were centrifuged at 400 *g* and 4 °C for 30 min and plasmas were carefully removed. Plasma containing Na_2 EDTA were kept frozen at — 18 °C until later PRA determination.

Post-centrifugation WINTROBE values were multiplied by the plasma trapped factor (0.96) for Hematocrit (Hct) determination. Plasma Na^+ and K^+ concentrations and osmolarity were on the day measured by flame photometry (Corning Instruments Ltd.) and freezing point depression (Automatic Osmette, Precision Systems Inc.) respectively. Plasma Protein Concentration (PPC) was measured by the biuret technique. Plasma Volume Reduction (PVR) was calculated by the following equation:

$$PVR = 100 \times (1 - P_o/P_i),$$

using PPC values from PEG injected rats (P_i) and from control saline injected rats (P_o), and expressed as percentage (18).

Plasma Renin Activity (PRA) was measured using a radioimmunoassay for Angiotensin I (AI, Cea-Sorin), according to the method of HABER *et al.* (8). PRA was calculated as the amount of AI generated from the endogenous renin substrate per ml of plasma during the incubation of the samples for 60 min at 36 °C and pH 6. Plasma AI blanks were deducted from the total generated amount of AI. Within-assay variation in duplicated samples averaged 7.1 %.

Statistics. Analysis of variance or polynomic regression analysis were used to assess statistical significance.

Analysis of variance (Anova). In experiment 1 plasma Na^+ concentration was tested by one-way Anova and the Newman and Keuls method of comparison, and plasma K^+ concentration and plasma osmolarity were tested by one-way analysis for unequal variances. In experiment 2 plasma Na^+ and K^+ concentrations and plasma osmolarity were tested by one-way Anova, and MAP values were tested by two-way analysis (blocks) and Dunnet test.

Regression analysis. PPC/time and PRA/PPC in experiment 1, and ΔPRA/ΔMAP in experiment 2 were tested by polynomic regression analysis. In experiment 1 Hct/time was tested by polynomic regression with repeated observations on y at each x, PRA/time was tested by the linearity test with repeated observations on y at each x, and SAP/time was tested by regression of the two-way classification (blocks) with observations in the cells. In experiment 2 ΔPRA/Δvolume was tested by weighed polynomic regression, and Δvolume/Δpressure/ΔPRA was tested by the backward elimination procedure for selection of the best regression equation (6).

Results

Experiment 1. The average weight at the time of injection (PEG or isotonic saline) was not significantly different for any group. There was no significant

change in SAP after isotonic saline injection (control group), but a significant linear decrease of — 0.0265 mmHg for each minute was observed in the experimental groups. Extrapolation of this slope means a maximal average decrease of 6.38 mmHg at 240 min after PEG injection, whereas the mean PVR was 29.7 % at this time (table I).

Regression analysis shows that PPC increased linearly with time following the PEG injection (fig. 1). In contrast, hematocrit showed a marked rise during the first 90 min, followed by a stable increase phase (fig. 1). Comparison between PPC and hematocrit showed a greater curvilinear hematocrit increase than the plasma volume reduction could explain. PRA increased linearly during the first 90 min after PEG treatment ($y = 0.5898\ t + 7.936$; $r = 0.8834$); but a transient fall at 120 min and a new increase toward the end of the experiment were observed (table I). Fig. 2 shows a significant relationship between PRA and PPC. Since PVR is estimated as the inverse function of the PPC (see methods), the relationship between renin secretion and plasma volume reduction, subsequent to PEG injection, could be established as a hyperbolic function.

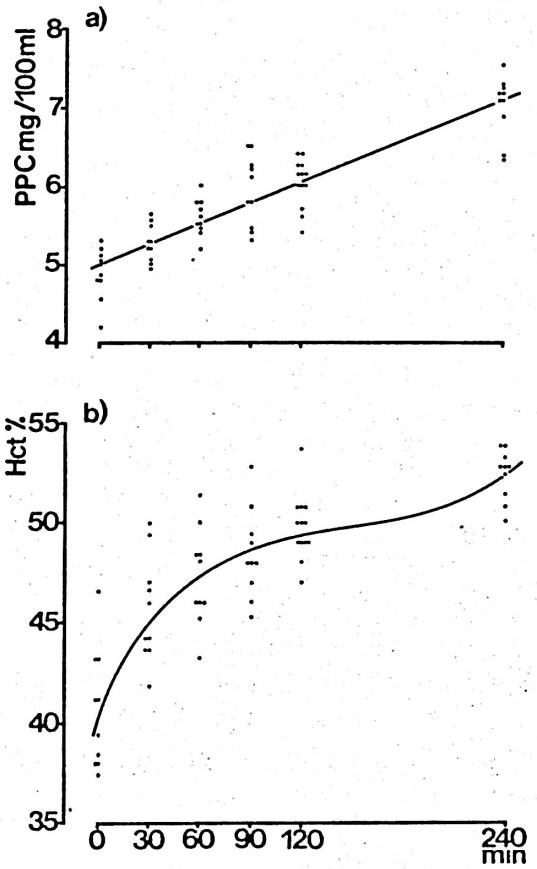

Fig. 1. *Plasma Protein Concentration (PPC) and Hematocrit (Hct) after the intraperitoneal injection of the hyperoncotic PEG solution.* Time 0 expresses the initial value of the control group injected with isotonic saline. PPC increase is given by the equation $y = 0.0084\ x + 5.028$; $r = 0.8925$; $p < 0.001$. Hct increase is given by $y = 40.89 + 0.1711\ x - 1.164\ 10^{-3}\ x^2 + 2.711\ 10^{-6}\ x^3$; r (global) = 0.8517; $p < 0.001$.

Experiment 2. No significant differences between the four experimental groups were observed in weight, initial blood parameters and MAP values before hemorrhages (table II).

Changes in MAP after initial hemorrhages are given in fig. 3. The extraction of 5 ml/kg body weight of arterial blood did not induce any significant change in MAP from the pre-hemorrhage values. Extractions of both, 10 ml/kg and 15 ml/kg, induced immediately falls of MAP (— 12.4 ± 6.1 mmHg, and — 43.8 ± 6.3 mmHg, respectively) after which the initial pressures were reached again within 6 min for the 5 ml/kg hemorrhage and within 27 min for the latter. The fall of MAP was higher after the extraction of 20 ml/kg (— 57.5 ± 5.3 mmHg), but the initial pressure was not reached again during the 30 min experiment period.

After the hemorrhage, PRA had significantly risen in all the four groups (table II). Fig. 2 shows a high significant

Table I. *Changes in Sistolic Pressure and Blood Parameters after PEG injection (Mean ± SEM).*

Rat groups	Control group n = 10	30 min PEG n = 10	60 min PEG n = 10	90 min PEG n = 14	120 min PEG n = 10	240 min PEG n = 10
Body weight (g)	202.90±5.20	203.20±4.80	203.80±3.50	202.30±4.30	207.30±3.60	204.30±5.80
Systolic Pressure (mmHg)	125.90±3.10	124.30±3.30	125.10±3.70	121.70±3.40	119.40±5.30	118.70±3.80
Plasma Protein Conc. * (mg/100 ml)	4.89±0.10	5.27±0.07	5.60±0.07	5.94±0.14	6.04±0.08	6.98±0.12
Plasma Volume Reduction % *		—6.89±1.31	—12.52±1.13	—17.11±1.98	—18.78±1.17	—29.75±1.29
Hematocrit % (× 0.96) *	40.65±0.95	45.65±0.83	47.28±0.76	48.72±0.71	49.58±0.46	52.37±0.40
PRA (ng/ml/h) *	9.40±2.20	23.50±3.30	43.20±4.00	61.80±3.70	52.30±2.50	96.30±9.10
Plasma Na^+ Conc. (meq/l)	146.10±0.50	147.80±0.20 **	147.30±0.30	147.90±0.50 **	144.90±0.40	142.00±0.30 **
Plasma K^+ Conc. (meq/l)	3.71±0.03	3.73±0.07	3.66±0.04	3.78±0.09	5.25±0.16 ***	5.62±0.08 ***
Osmolarity (mosm/kg)	285.40±0.70	282.00±2.80	286.60±1.80	283.10±1.90	287.80±1.00	284.40±1.70

* $p < 0.001$ (Regression analysis). ** $p < 0.05$ vs control group; *** $p < 0.001$ vs control group (Student test).

Table II. *Acute volume depletion. Blood parameters before (A) and after (B) controlled hemorrhages (Mean ± SEM).*

	5 ml/kg (n=5)		10 ml/kg (n=8)		15 ml/kg (n=7)		20 ml/kg (n=6)	
Hemorrhage	A	B	A	B	A	B	A	B
Body weight	203.00±5.40		202.80±4.80		209.00±8.90		202.20±7.50	
Hematocrit (%)	46.08±0.97	43.00±1.26**	44.63±0.67	40.53±0.69***	45.19±0.62	39.85±0.58***	45.76±0.70	37.92±0.55***
PRA (ng/ml/h)	3.80±0.60	8.20±0.90***	7.70±1.70	16.30±2.20**	4.60±0.80	16.50±2.00**	5.80±0.60	37.20±3.10***
Plasma Na^+ conc. (meq/l)	143.00±1.10	142.00±1.00	144.10±1.40	141.90±0.90	142.00±0.80	141.90±1.00	142.00±1.30	141.20±1.00
Plasma K^+ conc. (meq/l)	4.34±0.17	4.40±0.20	4.06±0.11	4.33±0.21	4.11±0.17	4.43±0.18	3.98±0.12	4.48±0.18
Osmolarity (mosm/kg)	280.60±2.60	280.60±2.40	280.60±1.80	283.10±1.30	283.60±1.30	281.70±1.80	283.50±1.00	284.20±1.50

** $p < 0.01$ B vs A; *** $p < 0.001$ A vs B.

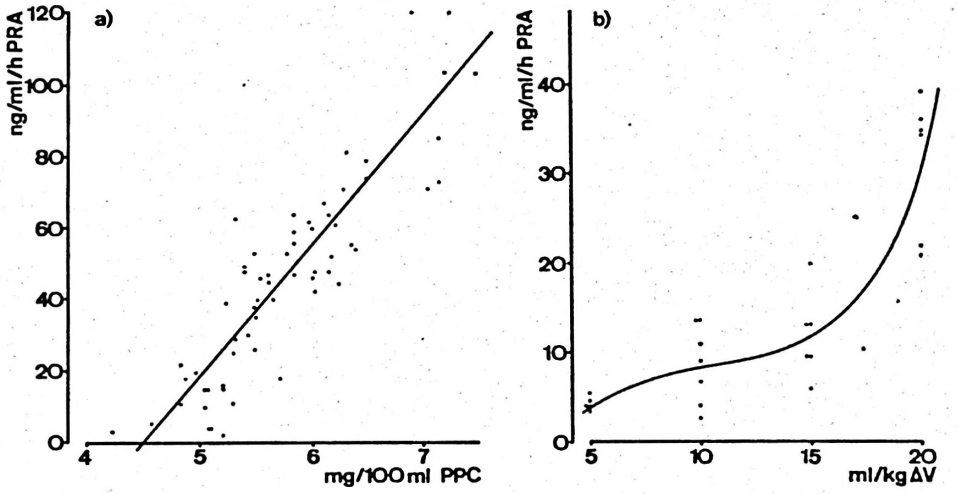

Fig. 2. *Changes in PRA after gradual (a) and acute (b) volume-depletion.*
a) The relationship between PRA and PPC after PEG injection is given by $y = 36.36\ x - 163.68$; $r = 0.8693$; $p < 0.001$. b) PRA increase between pre- and post-hemorrhage values after extraction of 5, 10, 15 and 20 ml/kg body weight; r (global) = 0.9119; $p < 0.001$.

curvilinear relationship between the PRA increase, expressed as the difference among final and initial levels (ΔPRA), and the blood volume reduction, expressed as ml/kg body weight of extracted blood (Δv). This relationship is given by the following polynome:

$$\Delta PRA = -6.96 + 2.72\ \Delta v - 0.0266\ \Delta v^2 - 0.0177\ \Delta v^3 + 8.61\ 10^{-4}\ \Delta v^4;\ r = 0.9119.$$

To assess the dependence of PRA from both the blood volume reduction (Δv) and the maximal decrease in MAP (Δp), a multiple regression adjustment was performed by the backward elimination procedure of ΔPRA with Δv and Δp variables, their powers (Δv^2, Δp^2, Δp^3) and products ($\Delta v \times \Delta p$). The analysis showed that all variables which include the Δp factor must be suppressed from the regression (in this order: $\Delta v \times \Delta p$, Δp^3, Δp and Δp^2), since all the information on ΔPRA is found in the Δv and Δv^2 variables. Moreover, Δv and Δv^2 variables contain all the information given by Δp and the other variables that include the Δp factor.

Discussion

Experiment 1. Extravascular administration of PEG hyperoncotic colloidal solutions, which induce protein-free fluid withdrawal from the intravascular to the interstitial space, has been a classical procedure for depleting the circulating volume in the absence of anemia or hypotensive shock (7, 13, 18). Most authors have indirectly measured the intravascular volume deficit as a function of the non diffusible blood components increase, since direct methods, based on the dilution principle, are difficult to perform in the rat and could interfere with radioimmunoassay techniques. We consider that the blood formed elements increase (7) does not seem the best procedure since, as our results show (fig. 1), the hematocrit increased non-linearly after the PEG administration. This increase is

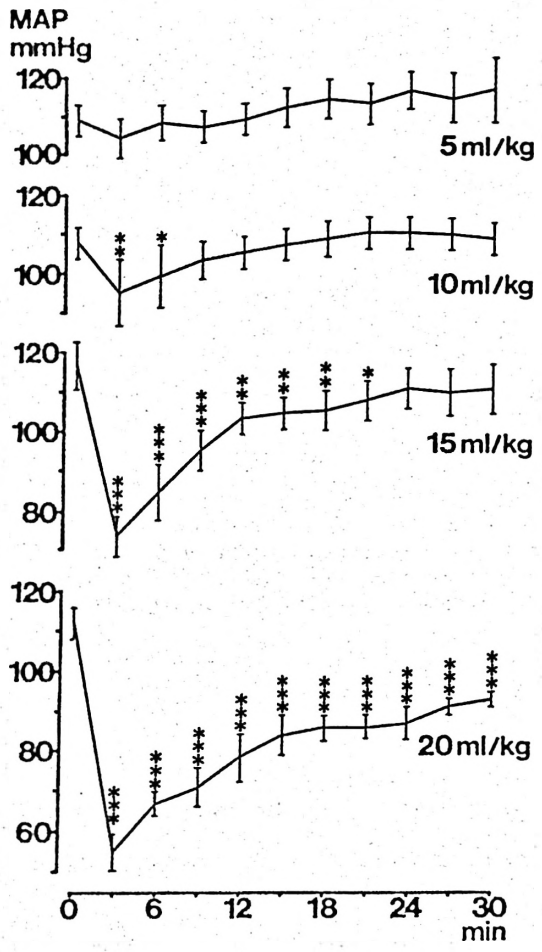

Fig. 3. *Evolution of the Mean Arterial Pressure (MAP) after controlled hemorrhages.* Time 0 expresses the pre-hemorrhage MAP. Values are expressed as the mean ± S.E.M. (* = $p < 0.05$. ** = $p < 0.01$. *** = $p < 0.001$).

higher than the one of the PPC (fig. 1), suggesting that the hematocrit increase is a reflex response to the cardiopulmonary receptors inhibition, since there were no important changes in SAP simultaneously. In this sense, previous works have reported that cervical vagotomy increased MAP, PRA and packed cell volume in dogs maintained at a high sodium diet. Cervical vagotomy also induced additional increases in these three parameters when previously stimulated by sinoaortic denervation (22, 23).

PPC increase seems to be a more adequate method to measure the intravascular volume deficit. Our results show a linear increase in PPC as a function of the time after the PEG injection (fig. 1). Furthermore, we have found a linear relationship between PRA and PPC in absolute values (fig. 2), suggesting that the renin secretion is also a function of the intravascular volume deficit when the arterial pressure is being buffered.

There is a lot of evidence that proves that the cardiopulmonary receptors have a reflex control on the renal activity: Vagal cooling increases renin release in dogs after aortic denervation even when the carotid sinus is perfused at a constant pressure (14, 15). Non-hypotensive hemorrhage of 4 ml/kg in the dog increased renin release before, but not after vagotomy (20). In another report, a 10 % hemorrhage in rabbits with sinoaortic denervation was associated with a significant increase in renal nerve activity (2). Inversely, an increase in left atrial pressure produced by balloon inflation suppresed renin release in dogs maintained on a low-sodium diet, but this suppression was prevented by prior cervical vagotomy or renal denervation (24).

These pieces of evidence could support the conclusion that the linear ratio between PRA and PPC could have originated from the buffering activity in the cardiopulmonary receptors in response to gradual volume depletion.

Experiment 2. Hemorrhage has been another classical procedure for studying the renin secretory response in hypovolemia. Dosage of blood withdrawal according to body weight allows for gradual depletion of the circulatory volume between non-hypotensive hemorrhages and those which induce deep changes in MAP (fig. 3). Statistical treatment of our results could establish significant correla-

tions between the PRA increase and MAP decrease on one hand, and the PRA increase and the volume reduction on the other. However, the study of these three variables as a whole, by the backward elimination procedure for selection of the best regression equation (6), is the most adequate treatment for the analysis of the data, since it allows the removal of any of these variables that do not show dependence on the PRA increase. In this sense, the analytical procedure shows that the PRA increase responds to the information given by volume changes, and all information given by changes in MAP on the PRA increase seems to be superfluous since this latter is completely included in the information given by the volume changes.

These results are in agreement with previous reports by HALL and HODGE (9), who investigated the influence of the extraction rats on the hormonal and pressor responses to the hemorrhage in the dog. Likewise, these are in line with the hypothesis advanced by THAMES (19), who suggests the possibility that cardiopulmonary receptors mediate a specifical neural release of renin that is independent of vasomotor effects, but is effected through sympathetic neurons under the exclusive control of cardiopulmonary receptors. Our findings clearly seem to indicate that, when the rat is in an intact, conscious and hypovolemic state, renin secretion responds more to the information given by the cardiopulmonary volume receptors than the originated in the high pressure receptors.

Resumen

Se estudian los efectos de los cambios en la presión arterial y en el volumen circulante sobre la Actividad Plasmática de Renina (PRA) en la rata intacta, mediante dos procedimientos experimentales: Inducción de una depleción gradual del volumen circulante por inyección intraperitoneal de una solución hiperoncótica de polietilenglicol (PEG), en ausencia de cambios agudos en la Presión Arterial Sistólica (PAS). Esta fue medida en el animal consciente mediante pletismografía en el rabo. Los incrementos en la Concentración de Proteínas Plasmáticas (CPP) y en el hematocrito se comparan como índices de la reducción del volumen plasmático. La APR muestra una relación lineal ($p < 0,001$) con la CPP, mostrando una dependencia directa de la reducción del volumen plasmático sobre la secreción de renina. Inducción de cambios agudos en el volumen circulante realizada mediante hemorragias controladas de 5, 10, 15 y 20 ml/kg peso. El incremento en la APR muestra una relación significativa ($p < 0,001$) con los cambios en el volumen circulante, pero no muestra dependencia de los cambios en la presión arterial media. Los resultados sugieren que, en la rata intacta y consciente, la secreción de renina responde a la información proporcionada a los receptores cardiopulmonares de volumen más que a la proporcionada a los receptores de alta presión.

References

1. ABBOUD, F. M.: *Hypertension*, **4** (Suppl.), 208-225, 1982.
2. CLEMENT, D. L., PELLETIER, C. L. and SHEPHERD, J. T.: *Circ. Res.*, **31**, 824-830, 1972.
3. CUNNINGHAM, S. G., FEIGL, E. O. and SCHER, A. M.: *Am. J. Physiol.*, **234**, H670-H678, 1978.
4. DONALD, D. E.: *Physiologist*, **22**, 39-49, 1979.
5. DONALD, D. E. and SHEPHERD, J. T.: *Ann. Rev. Physiol.*, **42**, 429-439, 1980.
6. DRAPER, N. R. and SMITH, H.: Applied regression analysis. John Wiley and Sons Inc., New York, 1966.
7. DUNN, F. L., BRENNAN, T. J., NELSON, A. E. and ROBERTSON, G. L.: *J. Clin. Invest.* ,**52**, 3212-3219, 1973.
8. HABER, E., KOERNER, T., PAGE, L. B., KLIMAN, B. and PURNODE, A.: *J. Clin. Endocrinol. Metab.*, **29**, 1349-1355, 1969.
9. HALL, R. C. and HODGE, R. L.: *Am. J. Physiol.*, **221**, 1305-1309, 1971.
10. HODGE, R. L., LOWE, R. D. and VANE, J. R.: *J. Physiol. London*, **185**, 613-626, 1969.

11. JARECKI, M., THOREN, P. N. and DONALD, D. E.: *Circ. Res.*, **42**, 614-619, 1978.
12. KLINE, R. L., PATEL, K. P. CIRIELLO, J. and MERCER, P. F.: *Hypertension*, **5**, 468-475, 1983.
13. LEENEN, F. H. and STRICKER, E. M.: *Am. J. Physiol.*, **226**, 1238-1242, 1974.
14. MANCIA, G. ROMERO, J. C. and SHEPHERD, J. T.: *Circ. Res.*, **36**, 529-535, 1975.
15. MANCIA, G., SHEPHERD, J. T. and DONALD, D. E.: *Am. J. Physiol.*, **230**, 19-24, 1976.
16. MUERS, M. F. and SLEIGHT, P.: *J. Physiol. London*, **221**, 283-309, 1972.
17. OEBERG, B. and THOREN, P.: *Acta Physiol. Scand.*, **85**, 164-173, 1972.
18. STRICKER, E. M., VAGNUCCI, A. H., MCDONALD, R. H. and LEENEN, F. H.: *Am. J. Physiol.*, **237**, R45-R51, 1979.
19. THAMES, M. D.: *Fed. Proc.*, **37**, 1209-1213, 1978.
20. THAMES, M. D., JARECKI, M. and DONALD, D. E.: *Circ. Res.*, **28**, 237-245, 1978.
21. WAICKMAN, L. A. and ABBOUD, F. M.: *Clin. Res.*, **28**, 717A-722A, 1980.
22. YUN, J. C.: *Nephron*, **23**, 72-78, 1979.
23. YUN, J. C., DELEA, C. S., BARTTER, F. C. and KELLY, G.: *Am. J. Physiol.*, **230**, 777-783, 1976.
24. ZEHR, J. E., HASBARGEN, J. A. and KURZ, K. D.: *Circ. Res.*, **38**, 232-239, 1976.