inhibited following stress or glucocorticoids administration [7]. The results obtained here and in previous work with AChE activity might indicate that both ChAT and AChE activities are affected by stress and glucocorticoids might mediate this effect.

1 This work was supported by a grant from the National Aeronautics and Space Administration (NSG 2183 and NAG 2-411), a grant from the National Institutes of Health (NIH RR 0811) and a grant from the Division of Research Resources (NIH grant RR 03020).
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0014-4754/92/030265-04$1.50 + 0.20/0

Effects of blood sampling, anesthesia and surgery on plasma vasopressin concentration in rats

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Received 31 May 1991; accepted 10 September 1991

Abstract. The influence of blood sampling, anesthesia and surgery on plasma vasopressin concentration was assessed in rats. Mean plasma concentration in conscious, chronically catheterized rats was 1.4 ± 0.1 pg/ml (n = 6). This value remained constant over repeated plasma samplings in the same animals. On the other hand, decapitation increased the plasma vasopressin concentration to 6.0 ± 2.4 (in pg/ml) (n = 6), inactin anesthesia to 2.9 ± 0.6 (n = 6), anesthesia and femoral cannulation to 13.3 ± 5.8 (n = 6) and surgery for renal micropuncture to 81.3 ± 35.0 (n = 6). It is concluded that the level of circulating plasma vasopressin is highly dependent on the sampling technique and is closely related to the extent of surgery.
Key words. Vasopressin; rats; surgery; renal micropuncture; anesthesia.

The plasma concentration of the peptide hormone vasopressin may vary over a wide range, depending on the physiological state of the animal. In the present work, we focused our attention on concentration changes following various experimental procedures, such as anesthesia and the surgery involved in renal physiology studies. It has already been shown that surgical preparation of rats for micropuncture of renal tubules results in a marked change in hematocrit and plasma volume [1]. A sequential study of the different events of such surgery indicated that inactin anesthesia and femoral artery catheterization were not responsible for this increase in hematocrit. However, the stress induced by physically manipulating the animal increases the hematocrit by about 3%. Complete micropuncture surgery involving tracheotomy, cannulation of the jugular vein, a midline abdominal incision

extended laterally to expose the left kidney, and cannulation of both ureters, raised the hematocrit by 6 to 10%. No significant changes in red cell volume or mean plasma systemic arterial blood pressure were observed under these conditions, whereas plasma volume decreased markedly, suggesting an internal redistribution of extracellular fluid [1]. Since these events may influence the plasma vasopressin concentration and interfere with studies on renal function, we decided to investigate the effects of these experimental procedures on the plasma concentration of antidiuretic hormone in rats.

Methods

Four series of experiments were performed on 10-month-old WAG/Rij female rats. In the first, the effect of sampling on plasma vasopressin was assessed in 6 conscious rats. The animals were chronically catheterized under gas anesthesia (1/3 O_2, 2/3 NO_2 and 4% fluothane)[2]. PE10 catheters were inserted in the left femoral vein and artery and pushed until the abdominal blood stream was reached. The free ends of the catheter were passed under the skin, brought out at the back of the neck and held in a small glass pipe ligatured to the skin. After a recovery period of 3 to 4 days, the animals were placed in metabolic cages with free access to food and water and extensions were added to the catheters. When the rat was quiet or sleeping in its cage, a 0.8-ml blood sample was withdrawn from the arterial catheter by gentle suction over a 10–20-min period. The blood was centrifuged to obtain the plasma fraction and the blood cells resuspended in an equal volume of plasma from a donor rat and returned to the animal through the vein catheter. An hour later a second blood sample was collected under the same conditions.

In a second series we tested the influence of decapitation on plasma vasopressin concentration. Rats were chronically catheterized and plasma samples collected as in the first series. An hour after returning the blood cells in an equivalent volume of plasma, the rats were decapitated and blood was collected and centrifuged for plasma vasopressin determination.

In a third series the effect of anesthesia was evaluated in the following way. Plasma was collected in conscious chronically catheterized rats as in the two previous series and the animals were then anesthetized by intraperitoneal injection of 10 mg/100 g body weight inactin. The rats were placed on a heated table to keep the body temperature at 37 °C and blood was sampled an hour later through the chronically implanted arterial catheters.

In a fourth series the influence of the surgery usually performed in studies related to renal physiology was assessed in six anesthetized rats. Each animal was anesthetized by intraperitoneal injection of 10 mg/100 g BW of inactin and placed on a temperature-controlled table. PE10 catheters were introduced in the left femoral artery and vein through a small incision in the skin of the leg. Blood was sampled from the artery and blood cells returned to the animal in the usual way. Sham micropuncture surgery was then performed as previously described [3]. Tracheotomy was carried out and PE10 catheters inserted into the right jugular vein and right and left ureters. The left kidney was exposed through an abdominal incision, superfused with paraffin oil, and its surface illuminated by an optic fiber connected to a xenon arc lamp. The animal was then perfused with a priming dose of 1 ml of a 0.9% NaCl solution, followed by continuous infusion of the same solution at a rate of 20 µl/min. An hour after completion of surgery, corresponding to half way through the micropuncture experiments, a blood sample was removed from the left femoral artery. Blood cells were returned to the animal and an hour later the rat was bled by puncturing the abdominal artery.

All plasma obtained from the various experimental series was stored at − 80 °C for subsequent vasopressin determination. Plasma vasopressin was extracted by Skowsky's bentonite technique [4] and its concentration measured by radioimmunoassay [5] using an antibody kindly donated by Dr L. C. Keil (NASA/ARC, Moffett Field, USA). Statistical analysis was performed using Student's paired t-test.

Results

The mean plasma vasopressin concentration from conscious, 10-month-old female rats was 1.4 ± 0.1 picog/ml. Repeated plasma samplings and vasopressin measurements in the same rats did not significantly change this mean value (table). Decapitation, on the other hand, increased the plasma concentration of vasopressin. The mean values were 1.4 ± 0.3 (n = 6) in conscious rats and 6.0 ± 2.4 pg/ml (n = 6) in sacrificed animals, with a considerable degree of variation: in some rats, the plasma vasopressin concentration doubled after decapitation,

Effect of blood sampling, anesthesia and surgery on plasma vasopressin concentration (pg/ml) in 10-month-old female Wistar rats. In group 1 two successive samples of blood were withdrawn from conscious chronically catheterized rats. In group 2, the blood was first sampled in conscious animals before decapitation. In group 3, serial sampling was performed in conscious and inactin-anesthetized rats. In group 4, the animals were anesthetized and blood sampled from the femoral artery. n is the number of animals. Data are expressed ± SEM. Statistical analysis was made using Student's paired t-test in each group.

Group 1 (n = 6)	Conscious rats 1.4 ± 0.1	NS	Conscious rats 1.3 ± 0.1
Group 2 (n = 6)	Conscious rats 1.4 ± 0.3	$p < 0.05$	Decapitated rats 6.0 ± 2.4
Group 3 (n = 6)	Conscious rats 1.0 ± 0.1	$p < 0.05$	Anesthetized rats 2.9 ± 0.6
Group 4 (n = 6)	Anesthetized rats + mild surgery 13.3 ± 5.9	$p < 0.05$	Anesthetized rats + micropuncture surgery 81.3 ± 35.0

whereas in others it was up to 5 to 8 times higher than the control value. The effect of inactin anesthesia is also shown in the table. Paired t-test analysis revealed a significant increase in plasma vasopressin concentration after anesthesia, although the amplitude of this increase was less than after decapitation. Anesthesia plus mild surgery had a greater effect than anesthesia alone: the mean plasma vasopressin concentration was 10 times that of conscious rats. Complete micropuncture surgery with exposed left kidney further increased the vasopressin level to 81.3 ± 35.0 pg/ml. Finally, bleeding the animals by abdominal aortic puncture at the end of the sham micropuncture experiments caused a tremendous rise in the plasma vasopressin concentration, to 1175 ± 197 pg/ml (n = 6).

Discussion

This study shows how sampling technique, anesthesia and surgery all influence the plasma vasopressin concentration in the rat. Gentle withdrawal of blood samples in conscious unrestrained rats provided reproducible values for vasopressin, close to 1.4 pg/ml in this series. A similar value was obtained when a second blood sample was collected from the same animal, provided that blood cells and the relevant volume of plasma were returned to the animal between the two collections. Such reproducibility of plasma vasopressin values makes it possible to compare hormone concentrations in blood samples collected successively from the same animal under control and experimental conditions.

Decapitation, which is used in many studies for rapid collection of a large volume of blood, resulted in a raised level of plasma vasopressin. The values were 4 times higher than in conscious rats and were in the range of those found by Cowley [5] and Brunner et al. [6] in decapitated Sprague-Dawley rats. It is noticeable that the individual data in such conditions were more variable in the sacrificed than in the conscious animals. This is probably related to the different degree of manual restraint, a stress which is known to increase plasma vasopressin [7].

The effect of anesthesia per se was investigated in the absence of surgical trauma in chronically catheterized rats. Administration of inactin had a minor effect on plasma vasopressin concentration, raising it to 2 to 3 times the control value. This agrees with the effects of the anesthetic agents previously tested [8]. A marked effect was observed after surgery. Skin incision and insertion of a femoral catheter increased plasma vasopressin concentration from 2.9 pg/ml in anesthetized rats to 13.3 pg/ml. Moreover, the rise in vasopressin release was proportional to the extent of surgery. Tracheotomy, laparotomy and renal exposure, the surgery necessary for renal micropuncture, further increased circulating vasopressin to 60 times the resting value. Thus most micropuncture data related to water and solute transport along the nephron corresponds to conditions of vasopressin stimulation.

Finally, drawing blood from animals by aortic puncture has a dramatic influence on vasopressin release. This sampling technique would thus appear to be inappropriate for producing valid determinations of circulating antidiuretic hormone.

In conclusion, repeated removal of limited amounts of blood from conscious chronically catheterized rats seems to be the most reliable sampling method for the measurement of plasma vasopressin concentration. It can be safely used for studies of the changes induced by various experimental conditions such as dehydration and salt depletion. This approach is preferable to decapitation or arterial sampling under anesthesia and surgery. In fact, plasma vasopressin concentration appears to be closely related to the extent of surgery.

Acknowledgments. The authors wish to thank Mrs A. Zimmerman, Mrs C. Meunier-Simi and N. Callo-Bona for technical assistance and Mrs C. Juillard for skillful secretarial aid. This work was supported by the Institut National de la Santé et de la Recherche Médicale, Grant 887005.

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