Anomalous Changes in the Plasma Antidiuretic Activity of Hydrated Dogs After the Cessation of Intravenous Infusion of Arginine Vasopressin

In our previous experiments we found that an antidiuretic effect obtained by i.v. infusion of arginine vasopressin, as well as lysine vasopressin, in rats, persisted for some time after the infusion was interrupted[1]. One of the possible reasons for such a protracted action could be a persistence of the substance in the circulatory system or its accumulation in some tissue compartment. The short half life of both vasopressins in blood is well documented in nonhydrated animals[2], but not the changes in their plasma level at longer time intervals. We attempted to follow these changes in hydrated dogs and found a deviation from linear kinetics of vasopressin excretion.

Materials and methods. 6 female dogs (9.7–23.5 kg) were anesthetized with pentobarbital (35 mg/kg), tracheotomized, the ureters were cathetarized and hydration was commenced by the continous i.v. administration of 0.45% NaCl + 2.5% glucose solution at the rate of 5 ml/min. 1 h later infusion of arginine vasopressin ('Pitressin', Parke-Davis) (0.3–2.0 nmol/h/kg[3]) was initiated. In 2 animals, urine flow rate (measured within 5 min intervals) rose during hydration from 0.03–0.06 ml/min to 3.5–5.3 ml/min. In 4 of the animals the hydration did not result in an increased urine flow. Diuresis began, however, immediately after initiation of vasopressin infusion. This may possibly be attributed to a 'diuretic' effect of arginine vasopressin in certain physiological conditions[4,5]. The survival of a biological effect (antidiuretic or diuretic) for about 25 min after the cessation of the infusion was noted particularly in dogs of the first group (antidiuretic, see Figure 1). The filtration rate, measured as the clearance of endogenous creatinine, did not show substantial changes in any period of the experiment.

Antidiuretic activity of plasma was determined several times during and after infusion of vasopressin by withdrawing 1–2.5 ml of blood from the femoral artery and assaying by a previously described method[6]. In the first 3 experiments the antidiuretic activity was determined in native plasma and in the remaining experiments after deproteination of blood samples with trichloroacetic acid and freeze drying. In view of the high infusion rate it is assumed that the exogenous vasopressin is predominant in comparison with other endogenous substances with antidiuretic activity; therefore we shall only speak of the level of arginine vasopressin (AVP).

Results and discussion. After 1.5–2 h of infusion a steady state plasma concentration of AVP was reached (see Figure 2). The concentration at this moment was 0.40–5.75 nmol/l). The infusion was interrupted at this point, and a rapid decrease in plasma AVP-concentration (half life 1.5–5.7 min) was observed within the first 7–10 min. Following this the AVP-concentration rose until a maximum was reached 15–20 min after the cessation of infusion. The peak attained during this interval was 45–100% of the steady state level. The final phase is a steady decrease of the AVP-concentration from a previous peak level.

In general, substances displaying a similar time course of the plasma level (called 'reaktive Stoffe' by DOST[7]) may have a tissue distribution non-linearly dependent on their concentration in blood plasma or other body fluids, exhibit changes in their own tissue distribution as a consequence of their biological effect (e.g. in the case of vasoactive substances) or exhibit an 'active accumulation' in some tissue compartment. Preliminary calculations on an analogue computer[8] show the third hypothesis to most plausible. In this case, the anomaly might be associated with the depot function of the neurohypophysis. It is known that after an i.v. injection of tritiated lysine-vasopressin to the rat, the highest radioactivity per mg of dry tissue is in the neurohypophysis[9]. In vitro experiments show that about 50% of the total tissue radioactivity is accounted for by vasopressin, with split products and inactive forms constituting the residual part[10]. Furthermore, under in vitro conditions some kind of an accumulation of vasopressin in the neurohypophysis also occurs[10]. Dog neurohypophysis contains about 16 antidiuretic units of vasopressin[11]. Of this amount, only

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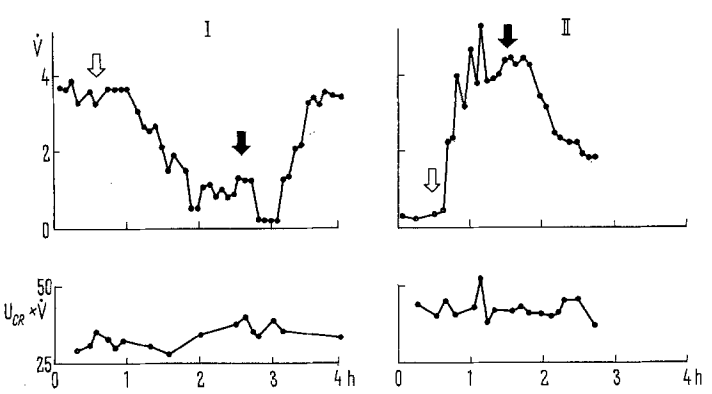

Fig. 1. Antidiuretic (I) and diuretic (II) effect of arginine vasopressin in female dog (1–15.7 kg, infusion 0.36 nmol/h/kg, 2–15.0 kg, infusion 0.74 nmol/h/kg). White arrow, initiation of Pitressin infusion; white arrow, initiation of vasopressin infusion, black arrow, termination of infusion. $\dot{V}$, urine flow rate (ml/min), $U_{cr}\dot{V}$, excretion of 'endogenous' creatinine (mg/min).

10–20% can be readily released, and only after a strong stimulus (severe haemorrhage)[12]. The ultimate capacity of the neurohypophysis is apparently even higher, as documented in the works referred to above, and because of the neurohypophysial accumulation of the endogenous AVP, the percentage of the releasable hormone might be raised and account for an observed peak. If this is true, the question arises as to how a large amount of vasopressin could be released from the neurohypophysis during a very short time period. Two explanations may be considered. First, the release could be stimulated by a sudden drop of the steady and high plasma level of the hormone. Second, the blood flow in pituitary vessels might decrease during the increase of AVP plasma concentration, and rapidly increase when this concentration in the peripheral blood falls; in such a case, outflowing blood would have a high AVP concentration. At present, direct evidence for any particular mechanism of this abnormal AVP elimination is lacking.

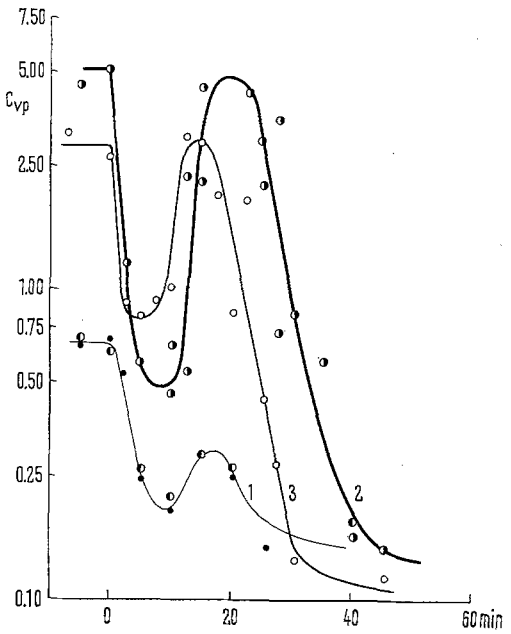

Fig. 2. Changes of plasma concentration of arginine vasopressin (c_{vp}, nmol/l) after cessation of i.v. infusion (zero time). Female dogs: 15.7 kg, AVP-infusion 0.36 nmol/h/kg (●); 9.7 kg, AVP-infusion 0.46 nmol/h/kg (◐); 14.0 kg, AVP-infusion 1.65 nmol/h/kg (○); 15.0 kg, AVP-infusion 0.74 nmol/h/kg (◑).

Zusammenfassung. Die Plasma-Konzentration sinkt beim hydrierten Hunde nach Beendigung einer VP-Infusion in 10 min auf 10–20% der Gleichgewichtskonzentration ab, um dann in ungefähr 20 min auf 40–100% anzusteigen. Nach 2 Stunden war die Konzentration wieder vermindert. Dieser abnorme Zeitverlauf wird mit der Depotfunktion der Neurohypophyse für Vasopressin in Zusammenhang gebracht.

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Cadmium as a Trace Element and Cadmium Binding Components in Human Cells

The rapid expansion of industrial technology has introduced into our environment increasing quantities of cadmium[1]. It is well known that this element is highly toxic. Apart from this, cadmium has teratogenic and tumorigenic properties and it may also play a role in the pathogenesis of hypertensive cardiovascular disease[2–6]. Absorption of Cd^{++} by gastrointestinal tract is poor, nevertheless, an average person absorbs approximately 2 μg Cd/day[7]. For unknown reasons the absorbed Cd is poorly excreted and accumulates in the tissue[6,8,9]. The presence of cadmium binding protein has been demonstrated in equine and human kidneys[10,11]. Similar cadmium binding protein (Cd-BP) appears also in the organs of rats[12,13]. Exposure of rats to $CdCl_2$ by ingestion or s.c. injection induces the synthesis of Cd-BP[13]. Other ions such as Zn^{++}, Hg^{++}, Co^{++}, Cu^{++}, Ni^{++} did not produce this effect. In vivo incorporation of ^{14}C from uniformly labeled cystine-^{14}C into Cd-BP was shown to be increased after exposure of the animals to Cd^{++}. The labeled Cd-BP undergoes a continuous catabolism and resynthesis which is accompanied by only minor losses of intracellular ^{109}Cd[13]. Since in vivo the Cd-BP is confined to intracellular compartment it was of interest to study the interaction of Cd^{++} and Zn^{++} with isolated cells.

Materials and methods. HeLa cells, monkey kidney epithelial cells and human embryonic fibroblasts derived from skin and muscle as well as from lungs were cultured in milk dilution bottles according to Embil et al.[14]. Equimolar mixture of $^{109}CdCl_2$ and $^{65}ZnCl_2$ or carrier free $^{109}CdCl_2$ with radiochemical purity better than 99% were added to culture media and aliquots from the media were drawn periodically for assay of the isotopes. After incubation the cells were washed with Eagles diploid medium and trypsinized[14]. Surviving cells were lysed and the lysate centrifuged at 2000 × *g* for 1 h. The supernatant was separated at +10 °C on Sephadex G-75 column (1.5 × 83 cm) using *Tris*-HCl buffer 0.001 *M*; pH 8.6. Fractions collected from the column were assayed for radioactivity of the isotopes in Nuclear Chicago dual channel gamma scintillation spectrometer.

Results and discussion. Human fetal skin and muscle fibroblasts cultured as monolayer for 8 days in 15 ml of Eagles diploid medium containing ^{109}Cd and ^{65}Zn (1.3 n*M*/ml of each) showed a continuous uptake of ^{109}Cd without detectable change in ^{65}Zn concentration