hypophysectomy was performed through the parapharyngeal approach. Following the operation, a continuous infusion of inulin-^{14}C and lysine-vasopressin (Sandoz) 200 $\mu U\ min^{-1}\ kg^{-1}$ was started in 6 hypophysectomized rats. The other 10 hypophysectomized and 7 normal non-hypophysectomized rats were given only the inulin-^{14}C infusion. After a 1-h equilibration period, a few minutes before starting the first 20-min urine-sampling period, each animal received 200 U heparin i.v. Intravenous infusion of isotonic saline in the amount of 4% of each animal's b.wt was completed in the next 20-min-interval. Another 3 urine samples were taken in the subsequent 60-min-duration of the experiment. Blood samples (0.8 ml) for analyses were withdrawn from the carotid artery in the middle of the first, third and at the end of the fifth period after the cannulas were flushed out by means of an arteriovenous shunt. The volume of blood withdrawn was replaced immediately by the same volume of fresh heparinized rat blood. Arterial blood pressure was recorded continuously by a polygraph. The results were statistically evaluated by means of the Student t-test with correction of t-criterion when the F-values were significant[5,6].

Results and discussion. The results are summarized in the table. The infusion of saline increased significantly sodium and urine excretion in normal rats (group 1). Acute hypophysectomy (group 2) abolished the homeostatically effective increase in renal sodium excretion, and small doses of ADH failed to restore it despite decreasing the free-water excretion (group 3). Another effect of ADH was the maintaining of arterial blood pressure in the acutely hypophysectomized rats at the level found in normal animals (1 vs 3). However, this haemodynamic effect was not of decisive importance to the mechanism of the renal sodium excretion, as the rats in group 1 and 3 excreted different amounts of sodium following the saline infusion inspite of similar arterial pressures. On the other hand, the excretion of sodium was not different in rats in hypophysectomized groups (2 and 3) despite different blood pressures. There was no correlation between GFR and variable amounts of renal sodium excretion.

So, the effect of ADH in the hypophysectomized animals was similar to that found in normal saline loaded rats where also no effect of small doses of ADH on 'volume' natriuresis was found[2]. As in the present experiments, only the urine output was less pronounced in the ADH treated rats due to the increased reabsorption of free water.

It may be concluded from these experiments that the plasma level of ADH which is efficient enough to concentrate urine would not be a prerequisite for the homeostatic mechanism promoting the 'volume' natriuresis.

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Corticosterone secretion after neurogenic stress in intact and hypophysectomized rats

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Summary. The time course of blood and adrenal corticosterone elevation after immobilization stress has been studied in intact and hypophysectomized male rats. The results suggest that the adrenal gland is able to respond to neurogenic stress, increasing the synthesis and release of corticosterone, in the absence of ACTH.

It is generally accepted that the synthesis and release of corticosteroids is mediated by ACTH. Up to now, little attention has been paid to adrenal responsiveness in the absence of this hormone, and the attempts to detect changes in corticosterone levels in hypophysectomized animals subjected to neurogenic stress have proved unsuccessful[2,3]. However, the data presented here might suggest that the adrenal glands of male rats, hypophysectomized 24 h before, are able to synthesize and release corticosteroids when the animals are subjected to immobilization stress.

Male Wistar rats of similar ages were used. The transaural approach was used for hypophysectomy, and 24 h were allowed for recovery before the stress was applied. The immobilization stress was achieved by having the rats held in a prone position, with their 4 limbs fixed to a wooden board, for 5 min. The stress period commenced from the moment the animals were removed from the cage where they had been living. The survival intervals varied between 3 and 45 min. All rats that survived for more than 5 min waited in individual cages. The animals were killed by decapitation, and blood and adrenals were quickly collected. These operations were performed in a room adjacent to where the animals were caged, to minimize uncontrolled stress. Only animals whose hypophysectomy was found to be total by post-mortem inspection were used in this study.

Fluorometric measurements of corticosterone were carried out according to Demoor[4], except that readings were taken 30 min instead of 5 min after adding the sulphuric acid-ethanol reagent. The precision of the method was of 7.3%. Adrenal protein content was measured using the Lowry[5] procedure. The experiments were designed in a fully randomized way. Single (blood samples) or 2-way (left and right adrenals) analysis of variance model I were carried out after logarithmic transformation of the data, in order to avoid the heterogeneity of variances (F-max test). Since the overall analysis of variance was found significant in all the cases, and no difference was detected between left and right glands, an SNK a posteriori test was applied to study the differences among means. The results are given graphically at the bottom of figures 1 and 2. Confidence limits are given, instead of SEM, because of the logarithmic transformation required. Statistical methods were taken from Sokal[6].

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The time courses of blood and adrenal corticosterone elevation following stress in intact animals are shown in figure 1. The blood pattern is in agreement with the results described in current literature[7]. The short-term response of the adrenal gland has not been reported previously. A biphasic curve has been found which looks strikingly similar to that reported for plasma ACTH under ether stress[8], except for a delay of $2^1/_2$ min: this is the latency interval described in vitro between the addition of ACTH and the release of corticosterone[9]. This biphasic response has also been described for corticotropin-releasing factor (CRF) elevation after stress[10]. Figure 1 shows this type of response for adrenal corticosterone as well.

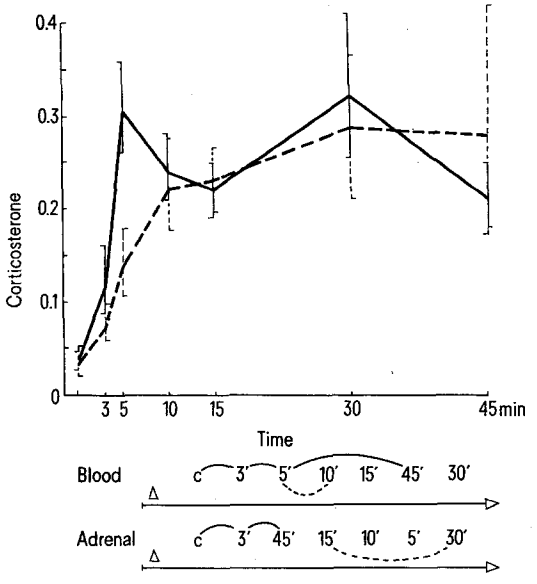

Fig. 1. Time courses for blood and adrenal corticosterone elevation following immobilization stress in intact rats. Mean corticosterone values and 95% confidence limits (n = 9) are plotted against time of survival since the beginning of the stress. ——, adrenal corticosterone (μg/mg Pr); – – – –, blood corticosterone (μg/ml). Statistical analysis is shown graphically at the bottom: The means are arranged in increasing order as shown by the arrow. They are significantly different when a line is drawn between: ⌒ = $p < 0.01$; ⌒ = $p < 0.05$.

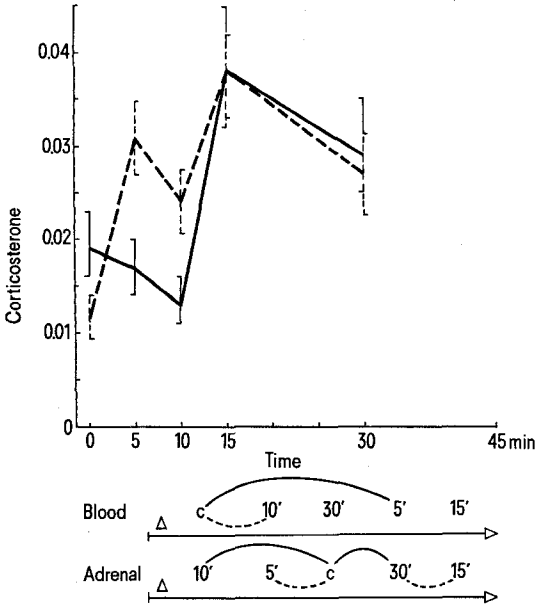

Fig. 2. Time courses for blood and adrenal corticosterone elevation following immobilization stress in hypophysectomized rats. See figure 1 for further details.

The time courses of blood and adrenal corticosterone elevation after stress in hypophysectomized rats are shown in figure 2. We can distinguish 2 phases in the adrenal response: at the beginning, release of corticosterone seems predominant, since decrease in adrenal content parallels increase in blood. The latter increase in adrenal steroid content suggests an active process of corticosterone synthesis.

Other researches have been able to show a certain degree of basal corticosterone synthesis in hypophysectomized animals, by studying the transformation of labelled cholesterol into radioactive corticosterone[11]. In our study, none of the previous ACTH could be present in the animals, since the half-life of this hormone is about 10 min, and they were killed 24 h after the hypophysectomy. As previously stated, hypophysectomies were total. Only a small fragment of pars tuberalis, whose function is still unknown[12], remained around the upper pituitary stalk. The infusion of CRF by way of the internal carotic artery has no steroidogenic effect in such hypophysectomized rats[13]. Therefore, the low rate of synthesis observed could not be attributed to the remaining fragment of pars tuberalis. On the other hand, the interval between the beginning of the stress and the increase in adrenal corticosterone (at least 10 min) makes the possibility unlikely that the latter is mediated by ACTH, whose effects are apparent after 3 min in intact animals.

The fact that previous attempts have failed to show corticosteroid response to neurogenic stress in hypophysectomized animals can be explained, because pentobarbital anaesthetized rats were used and this drug prevents the rise of corticosterone induced by neurogenic stimuli[14]. The existence of an initial period of corticosterone release suggests some form of hormone storage. The lack of organular storage systems (like catecholamine droplets in the adrenal medulla) has been established for cortical adrenal cells. Nevertheless, 2 pools of corticosterone, dialyzable and non-dialyzable, have been reported, as well as changes in the subcellular distribution of these 2 forms under different treatments: ACTH, hypophysectomy, etc.[15,16].

Our data support the results of previous morphological studies carried out by our research group[17], which suggest that adrenal cortical cells are able to respond to neurogenic stimuli in the absence of ACTH. Further evidence is needed in order to explain which mechanisms are involved.

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