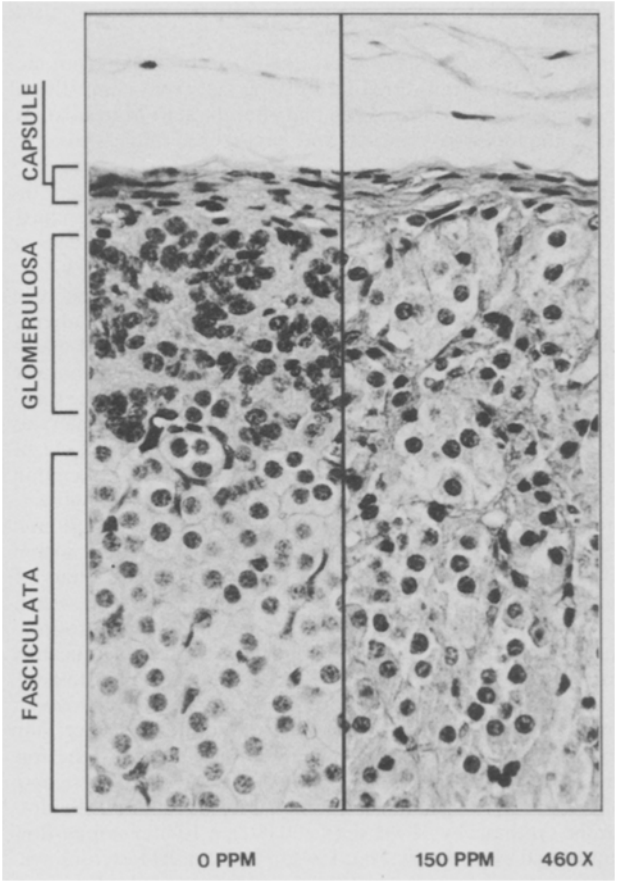

Figure 4. Effect of feeding piglets 150 mg carbadox per kg feed for 5 weeks on the structure of the glomerular zone of the adrenal cortex. The left-hand micrograph represents the control situation, the right-hand micrograph that of the carbadox treatment. Note the complete hydropic appearance of the zona glomerulosa, while the underlying zona fasciculata does not show significant changes.

large decreases in aldosterone levels observed in man with captopril are, however, qualified as a return to normal from elevated levels[5]. Furthermore, extirpation of the kidneys, the major source of renin, does not decrease the aldosterone levels to the extent observed with carbadox[6].

Hence it appears that carbadox interferes directly with the aldosterone-releasing functions of the zona glomerulosa. Giurgea et al.[7] also reported effects of in-feed administration of carbadox on the adrenals of chickens. Although the data given with regard to the amount of carbadox per kg b.wt per day and the in-feed concentration of carbadox seem to be inconsistent, evidence is presented that in chickens carbadox alters the ascorbic acid content of the adrenals. These alterations might reflect effects on the glucosteroid-producing zona fasciculata and zona reticularis, sites of action for which the present results did not give indications. This difference might be attributable to the species difference, but it is possible that also in pigs other parts of the adrenals would be affected with much higher dosages; we interpreted the dosages used in chickens[7] as being at least 10 times higher (mg carbadox $\cdot$ kg^{-1} b.wt $\cdot$ day^{-1}) than in the present study with pigs.

The lack of growth promoting activity of carbadox in dosages up to 50 ppm in this study can be attributed to the 'good health', 'good feed' and 'good housing' conditions of the experimental animals. The toxic activity of carbadox observed, however, indicates a safety-factor smaller than two for the advised dosage of carbadox as a feed additive. With regard to its preventive efficacy against anaerobic pathogens for swine the in vitro MIC-values[8] seem to indicate that this efficacy can be achieved with lower dosages.

An intriguing question is how the majority of the animals treated with 150 ppm carbadox survived and apparently recovered after a 10-week treatment. Probably the residual mineralocorticoidal activity of glucocorticoids, together with that of the renin-angiotensin part of the RAAS provide a mechanism for a minimal survival. Furthermore, the reduced feed consumption by the high dosage-groups seemed to have limited the intoxication. Urine-drinking invariably accompanies dosages of 100 ppm carbadox or higher. This behavior is suggestive of salt-craving, as is known from patients with Addison's disease. In cases of Addison's disease ultimately the whole adrenal cortex becomes dysfuncitonal, whereas carbadox, in the dosages used, selectively affects only the zona glomerulosa of the adrenal cortex. The toxic activity of carbadox as reported above might, however, provide an experimental model for testing aldosterone substitution therapies.

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Inhibition of aldosterone synthesis induced by flow-stop in the Mongolian gerbil adrenal gland superfused in vitro

M. Fenske

Lehrstuhl für Tierphysiologie, Universität Bayreuth, D-8580 Bayreuth (Federal Republic of Germany), 7 March 1986

Summary. Stopping of superfusion flow for short periods resulted in a significant accumulation of aldosterone within the Mongolian gerbil adrenal gland superfused in vitro. Aldosterone amounts in the first 2-min samples after the re-starting of superfusion were positively correlated with the length of flow-stop; however, they were significantly lower than calculated amounts: 5-min stop: 37 ± 1% inhibition, 10-min stop: 51 ± 1% inhibition. In addition, aldosterone secretion was significantly suppressed during prolonged incubation. The results suggest that aldosterone and glucocorticosteroid amounts in adrenal tissue may modulate basal corticosteroidogenesis and that self-suppression forms an important part of the control mechanisms involved in corticosteroidogenesis.

Key words. Adrenals; in vitro superfusion; aldosterone; Mongolian gerbils.

Evidence from studies using either incubated whole tissue, tissue blocks or isolated cells[1-14] suggests a direct inhibitory effect of corticosteroids on their own synthesis at the adrenal level. Physiological interpretation of these results, however, is difficult since 1) intermediates and end-products accumulate in the medium, altering the dynamics of steroidal interconversions[15,16], 2) corticosteroid amounts were added in the microgram range, and 3) exogenous and endogenous steroids do not form a homogeneous pool and are metabolized differently[15,16]. To overcome these limitations, adrenocortical tissue was continuously superfused with medium removing synthesized steroids within 1–2 min from the superfusion system[17], and superfusion flow was repeatedly stopped for 1-20 min resulting in elevated steroid concentrations within adrenocortical cells and extracellular matrix. By using this in vitro technique it was possible to investigate the very fast changes in synthesis and secretion of aldosterone induced by small increments of steroid concentrations within the adrenal gland.

Material and methods. Mongolian gerbils (*Meriones unguiculatus*, 8–10 months of age) from our colony were used. They were kept singly in plastic cages under controlled temperature (22 ± 1 °C), relative humidity (55%) and lighting conditions (light from 02.00 to 14.00). Food (gerbil pellets: Altromin, Lage, FRG) and water were available ad libitum.

Superfusion. Within 30 s after decapitation, adrenals were removed, dissected free of fat and adhering tissue, cut into 8 slices of similar size and placed in the superfusion flask (retention vol. 0.2 ml). Tissue was maintained at 36 °C and superfused with medium (Krebs-Ringer bicarbonate, plus 1.2 mmol/l glucose) at a flow of 0.5 ml/min for 60 min to allow the cells to reach steady-state conditions before any experimental manipulations were started. Then two 2-min samples were collected, and superfusion flow was stopped for 1, 5 or 10 min, or for 5, 10, 15 or 20 min (collection time: 2 min). After each test period, glands were superfused for an additional 10 min with 0.5 ml/min to allow tissues to return to baseline secretion.

Incubation. Adrenal slices were superfused for 60 min with medium and then transferred into plastic vials containing 1.0 ml medium. At 30, 60, 90 and 120 min after the start of incubation, 50-µl aliquots were removed and kept frozen until assayed by radioimmunoassay[18].

Statistics. The Mann-Whitney U-test was used to compare the differences between mean values. The differences were considered to be not significant if the calculated values exceeded the 5% probability values ($\alpha > 0.05$).

Results. Within 60 min after start of superfusion, aldosterone secretion reached a constant baseline (0.33 ± 0.13 ng/adrenal pair/min, $\bar{x} \pm SD$, n = 55). While in vitro release varied markedly between experiments (39%, n = 55), secretion within the same experiment was very constant, as is evident from the small variation in aldosterone amounts measured in 2-min intervals (0.019 ± 0.018 ng/adrenal pair/min, n = 133). The effects of short-lasting stops of superfusion flow on aldosterone secretion from adrenals superfused in vitro are summarized in figure 1. Stops of flow for 1, 5 and 10 min, or for 5, 10, 15 and 20 min resulted in a significant accumulation of aldosterone within adrenocortical tissue and superfusion flask. After re-start of superfusion steroids were removed from the superfusion system within 4 min. Amounts of aldosterone in the first 2-min superfusate samples after re-start of superfusion were significantly correlated with the length of flow-stop (0–10 min: r = 0.97, n = 80, $p < 0.0001$; 0–20 min: r = 0.94, $p < 0.0001$). However, amounts in superfusate samples were significantly lower than concentrations calculated (values were calculated by the formula: calculated values (%) = basal secretion (100%) × length of flow stop (min). While a 5-min stop of flow inhibited aldosterone synthesis by 37 ± 1% ($\alpha < 0.001$), a 10-min stop of flow suppressed synthesis by 51 ± 1% ($\alpha < 0.001$). Aldosterone syn-

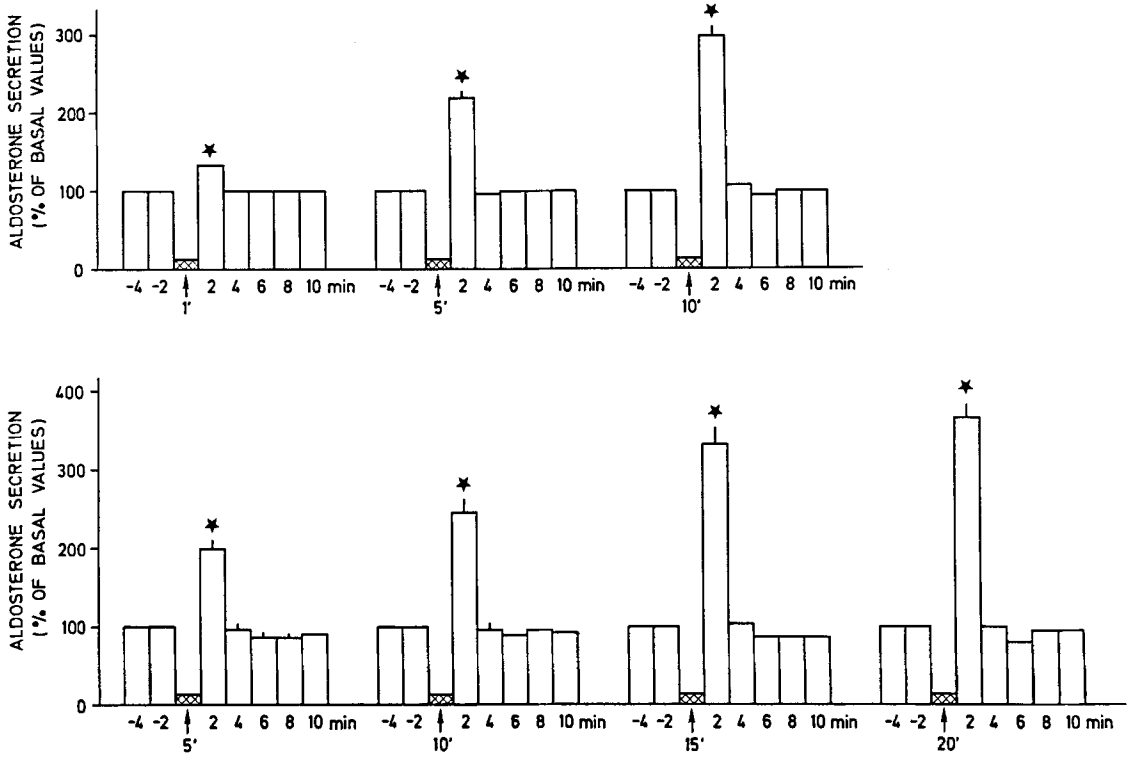

Figure 1. Effects of superfusion flow-stops on aldosterone secretion from Mongolian gerbil adrenal glands superfused in vitro. After 1 h of superfusion, two 2-min samples were collected and superfusion flow was stopped for 1, 5 or 10 min (upper part, n = 20), or for 5, 10, 15 or 20 min (lower part, n = 6). After each test, glands were superfused with medium for 10 min to allow adrenal cells to return to basal secretion. Stops are indicated by arrows. Mean ± SEM, *$\alpha < 0.001$.

thesis was maximally suppressed when superfusion flow was stopped for 20 min (67 ± 1%, fig. 2). In addition, a significant inhibition of aldosterone synthesis occurred during prolonged incubation. The percentage of inhibition was as follows: 30 min: 0%, 60 min: 35 ± 4%, 90 min: 51 ± 3%, and 120 min: 56 ± 4% (all $\alpha < 0.0001$).

Discussion. While steroidogenesis in the adrenal gland superfused in vitro has been the subject of several studies in the rat[19,20] or Mongolian gerbils[17,21-23], no work has been undertaken hitherto to study the effects of exogenous steroids, or of elevations of the endogenous corticosteroid pool induced by cessation of flow, on corticosteroidogenesis in adrenocortical tissue superfused in vitro. The present work shows that by using the stop-flow superfusion technique it was possible to suppress corticosteroidogenesis for short time periods by small, physiological increases of corticosteroids within the adrenal gland, and to measure changes in synthesis and secretion of aldosterone (figs 1–3) and glucocorticosteroids (fig. 3) within 1–4 min after restart of superfusion. Inhibition of aldosterone synthesis was maximal when flow was stopped for 20 min (67 ± 1%, fig. 2). In agreement with recent findings in isolated rat, guinea pig or bovine adrenocortical cells[9,10,13], not all corticosteroidogenesis was suppressible under these experimental conditions. Compared to the results obtained in the superfusion experiment, those provided by the incubation technique were surprising as it took much more time (90 min) to reach a similar inhibition to

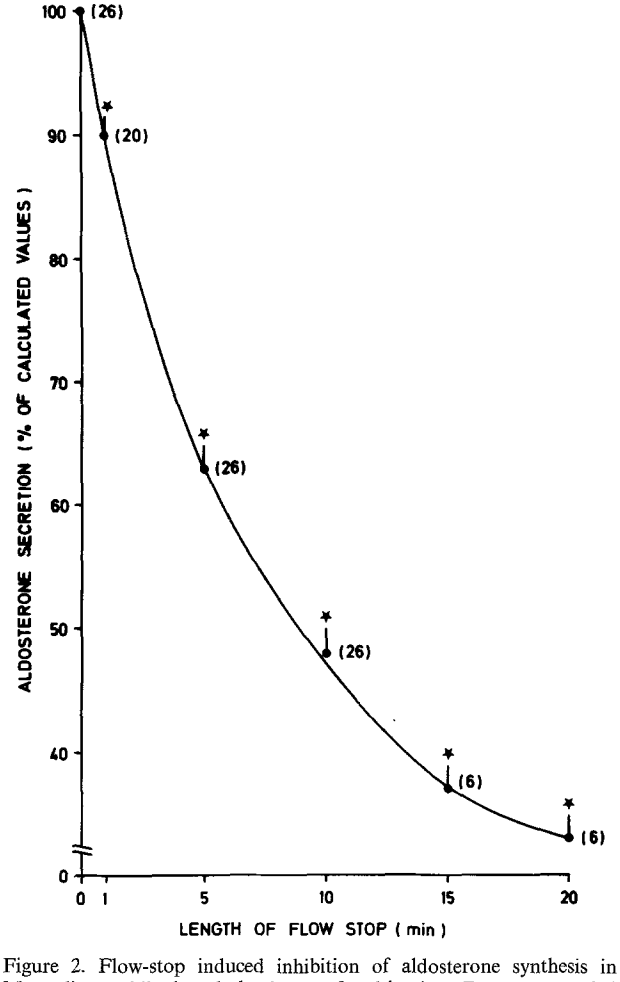

Figure 2. Flow-stop induced inhibition of aldosterone synthesis in Mongolian gerbil adrenal glands superfused in vitro. Data were pooled from 20 experiments with 1-, 5- or 10-min stops (fig. 2, upper part) and 12 experiments with 5-, 10-, 15- or 20-min stops (fig. 2, lower part). Calculated values (%) = basal secretion (100%) × length of flow stop (min). Mean ± SEM, *$\alpha < 0.001$.

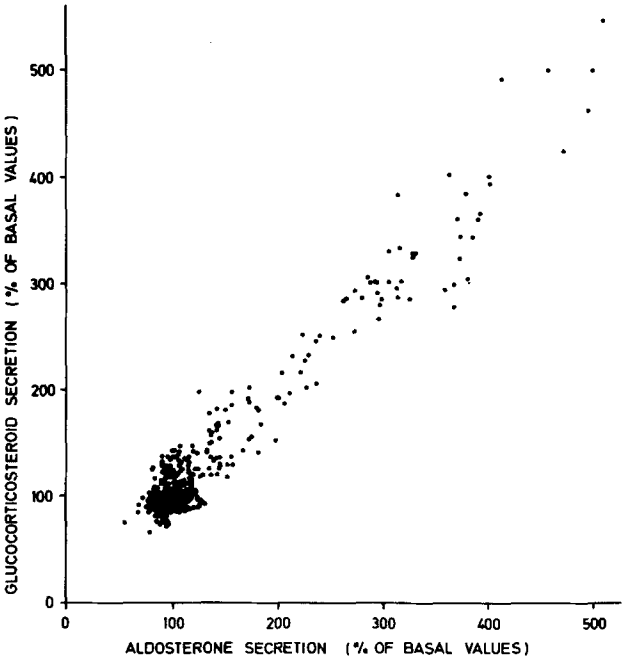

Figure 3. Correlation of aldosterone and glucocorticosteroid amounts released from Mongolian gerbil adrenal glands superfused in vitro. Data for aldosterone (fig. 1) and glucocorticosteroids (data not presented in the text) secretion were pooled from 20 experiments with 1-, 5- or 10-min stops (n = 20) and 6 experiments with 5-, 10-, 15- or 20-min stops. Regression line equation: $y = 0.9x+15.7$, $r = 0.94$, $n = 586$, $p < 0.0001$.

that observed with the superfusion technique (10 min). This discrepancy may be due to the different volumes in which adrenal slices were kept during the experiments (superfusion: 0.2 ml, incubation: 1.0 ml) and/or the much more effective removal of steroids from the extracellular matrix of incubated tissue which was continuously shaken during the 120-min tests.

Unfortunately, the molecular specificity of corticosteroid-induced inhibition of aldosterone and glucocorticosteroid synthesis could not be determined by the technique used in this work since uptake of added steroids into quartered or sliced adrenal tissue is poor and metabolism of exogenous corticosteroids may occur[15,16]. Based on previous studies on the steroidogenic capacity of the Mongolian gerbil adrenal gland[23-25] it may be concluded that 19-OH-11-deoxycortisol, cortisol and corticosterone are the most effective suppressors of corticosteroidogenesis. Although a possible physiological role of aldosterone[7] and testosterone[26] was suggested in earlier studies, recent work demonstrated that aldosterone was an ineffective suppressor at concentrations of 0.5 µg/vial[13].

As shown in figure 3, aldosterone and glucocorticosteroid amounts secreted in vitro were significantly correlated, irrespective of the length of flow-stop ($r = 0.94$, $n = 586$, $p < 0.0001$). This close relationship suggests that synthesis and secretion of aldosterone and glucocorticosteroids changes synchronously. This is compatible with the view that in the intact tissue aldosterone and glucocorticosteroids are secreted after de novo synthesis[17,21] and are not dependent in part on the release from sequestered steroid reserves[27,28].

From the rapid and short-lasting changes in corticosteroidogenesis during flow stops it may be concluded that the suppression of cortisteroidogenesis may function as a very rapid and fine adjustment of corticosteroidogenesis within adrenocortical cells, and that a fine balance may exist between the self-suppressing effects of corticosteroids and stimulation by secretogogues.

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Hypophysectomy and the sympatho-adrenal system in cold acclimation

M. C. Laury, M. Beauvallet, L. Zizine, P. Delost and R. Portet

Laboratoire d'Adaptation Energétique à l'Environnement (UA CNRS no 645), Ecole Pratique des Hautes Etudes, Collège de France, 11 Place M. Berthelot, F-75231 Paris Cedex 05 (France), 10 February 1986

Summary. Hypophysectomy does not impair the increase in weight of brown adipose tissue and adrenals following cold acclimation of the rat. In brown fat, the cold-induced increases in NE and 5 HT contents are not modified by hypophysectomy. In adrenals, hypophysectomy does not change the NE content, but a fall in epinephrine content was observed.
Key words. Cold acclimation; hypophysectomy; brown adipose tissue; adrenals.

Hypophysectomized rats, progressively exposed to a cold environment, are able to produce some heat by non-shivering thermogenesis (NST) and to survive in moderate cold (15 °C)[1]. In spite of the lack of pituitary hormones, cold acclimation also stimulates the development of some characteristics generally induced by cold. Brown adipose tissue (BAT) increases in size and undergoes changes in composition[1] and enzymatic activity[2]. Moreover, hypophysectomy does not prevent the hypertrophy of adrenals which is commonly observed in cold acclimation[3] of the intact rat. BAT is an important site of NST regulated by the sympathetic system and its mediator, norepinephrine (NE)[4] which is found in high concentration in this tissue[5]. It has been reported that serotonin (5 HT), an agent which is also present in high concentration in BAT[5], may also promote thermogenesis[6,7]. It is possible that BAT partly compensates for the absence of pituitary hormones and allows homeothermy at low temperatures. This study was undertaken to determine the levels in BAT and in adrenals of biogenic amines which may have a role in the survival of the hypophysectomized rat in a cold environment.

Materials and methods. Male Long-Evans rats, 6–7 weeks old, were hypophysectomized by the parapharyngeal route. To check the completeness of hypophysectomy, the following criteria were verified: no weight gain, testicular atrophy, examination of the selle turcica in the sphenoid bone under binocular microscopy post mortem. The operation was simulated in another group. After surgery, the rats were maintained at 23 °C for 3 weeks. It was thus possible to check, in vivo, the completeness of hypophysectomy by the absence of resumed body growth. Then both the sham-operated (SO) and hypophysectomized (H) rats were separated into 2 groups, one of which was maintained at 28 °C and the other at 15 °C for 5–6 weeks. Cold acclimation was attained progressively over a 7-day period. The animals were sacrificed by decapitation, adrenals and interscapular brown adipose tissue (IBAT) were excised. All animals were killed between 10.00 and 12.00 h to minimize the effects of circadian changes[8]. Catecholamines and serotonin were extracted from tissues by Bertler's method[9]. Epinephrine (E) and NE contents were determined using the method described by von Euler and Lishajko[10], and 5 HT according to Maickel and Miller[11].

Results and discussion. Hypophysectomy impairs body growth and in hypophysectomized rats, cold acclimation did not modify the body weight. The weight of IBAT decreased after hypophysectomy (by about 40%), but cold acclimation led to an increase of 50% in IBAT weights for both H and SO rats. The amount of IBAT expressed in terms of body weight was greater in the two groups of H rats, 40% for 15 °C, 60% for 28 °C. Adrenal weights were also greatly decreased following hypophysectomy, but were doubled by cold exposure of H animals. These results were in accordance to the previous observations[1]. Thus hypophysectomy does not impair the cold-induced growth of brown adipose tissue and adrenals. The fact that IBAT and adrenals can be increased by gradual cold acclimation of H rat and that these animals are able to produce non-shivering thermogenesis indicates that pituitary dependent hormones are not necessary for the initiation of cold-induced non-shivering thermogenesis. However, deficiency of these hormones may impair the development of the capacity to produce heat at a normal level.

Interscapular brown adipose tissue (table). As previously observed[12,13] the content of NE was higher in cold acclimated rats (50%) than in control rats; hypophysectomy did not influence this cold effect appreciably. Expressed in relative values, there are no significant differences between the four groups.