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ELEVATED CYCLIC GMP CONCENTRATIONS IN RAT ADRENAL CORTEX AFTER DEXAMETHASONE ADMINISTRATION

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SUMMARY

The levels of cGMP and cAMP were measured in the fasciculata-reticularis zona of the adrenal cortex in both intact and hypophysectomized young adult male rats. After administration of a single dose of dexamethasone to intact rats, cGMP levels were elevated 2-4 fold after 4hr and returned to control level after 8hr. At the same time, cAMP concentrations were moderately lowered. In hypophysectomized rats dexamethasone administration was followed by a similar increase in the cGMP level, the basal cAMP concentrations were not altered by dexamethasone. Our data suggest that dexamethasone might have a direct effect on cGMP concentration in the adrenal cortex of the rat.

Only few reports exist on the effects of steroid hormones upon cyclic nucleotide concentration (1, 2). It has been shown that the administration of synthetic glucocorticoids such as dexamethasone decreases the cGMP level in the skeletal muscle whereas no change in the level of renal cortical or hepatic tissue cGMP was found (3). On the other hands, adrenalectomy brought about an elevation of cGMP in lung and renal tissue (4) which could be reversed by injection of cortisol. In both the adrenal medulla and the superior cervical ganglia dexamethasone induced a decrease in cGMP concentrations whereas the cAMP level remained unaltered (5).

In the present study, we report a transitory elevation of the cGMP level after dexamethasone administration in the adrenal cortex of adult male rats. The cGMP increase is also shown to be present in hypophysectomized animals.The modest (but significant) decrease of cAMP with simultaneous inhibition of steroidogenesis observed in intact rats is practically unapparent in hypophysectomized rats.

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MATERIALS AND METHODS

Animals

Male Wistar rats weighing 180 g were supplied by IFFA CREDO, Saint-Germain-sur l'Arbresle, France. Their diet consisted of dry rat pellet (U.A.R., Villemoisson, France) and tap water ad libitum. They were maintained at 22 ± 1° C. Groups of 8-10 rats chosen at random were used in each experiment. Hypophysectomized male rats of the same origin and of the same age were used seven days subsequent to operation. The effectiveness of hypophysectomy was verified by the atrophy of the adrenals (a ratio value of 4.1 mg dry weight in hypophysectomized rats was obtained against 7.2 mg for intact rats) by growth arrest and by the undetectable blood corticosterone levels (below 0.3 µg/100 ml).

Experimental procedures

The animals were quickly killed by decapitation and trunk blood was collected into iced, heparinized plastic tubes for corticosteroid determination. The two adrenal glands were removed and decapsulated according to the method of Giroud et al. (6). The inner part of each gland, including the fasciculata and reticularis zonae as well as the adrenal medulla, was immediately put on a frozen plate (Pelcool, MSE, U.K.). Next, the adrenal medulla was dissected and discarded.
The efficiency of the fasciculata-reticularis zona isolation was assessed by histological examination.
The adrenocortical tissue was lyophilized and weighed.
Drug administration : a saline solution of dexamethasone 21-phosphate (DXM) kindly supplied by Merck, Sharp and Dohme, U.S.A., was administered by i.m. injection in the thigh.

Assay for cyclic nucleotides

The lyophylized tissues (2-10 mg dry weight) were homogenized by sonication in cold 1N $HClO_4$. After centrifugation the supernatant was alcalinized with 9 M KOH. The precipitate of $KClO_4$ was separated by centrifugation and the supernatant used for cyclic nucleotides assay. Both cyclic nucleotides were determined by a radioimmunoassay (7, 8). The characteristics of this assay are, first of all, preliminary succinylation of the cyclic nucleotides and second, the equilibrium dialysis of the succinylated nucelotide mixed with its ^{125}I cyclic nucleotide derivative against its specific antibody. The excellent specificity of the antibodies used (cross-reactivity between succinyl cAMP and succinyl cGMP less than 0.01 %) allows direct measurement of the cyclic nucleotides without previous separation.

Blood corticosteroid assay

Blood corticosteroids were determined with a commercially available radioimmunoassay (New England Nuclear C°, U.S.A.) and the results expressed as corticosterone. The cross-reactivity with dexamethasone was less than 0.2 %.

Statistical analysis

The statistical difference between the means of two given groups was analyzed by Student's t-test.

RESULTS

The adrenocortical levels of cGMP and cAMP were evaluated before and at different times following DXM administration. In intact rats the mean basal

values were around 5 pmoles/mg dry weight for cGMP and 0.5 pmoles/mg dry weight for cGMP with a cAMP/cGMP ratio of about 10. In a first experiment the cyclic nucleotide levels in intact rats administered a single dose of DXM (1 mg/kg body weight) were followed for up to 36 hr after injection. The results are shown in Fig. 1, 2, 3. Within 2hr after a single DXM injection the cGMP level increased and then reached a maximum of two fold the basal level declined and returned to the control level after 12 hr ; cAMP decreased from 50-70 % of the control value after 4 hr and remained depressed for 20 hr. Inhibition of steroidogenesis persisted for 28 hr.

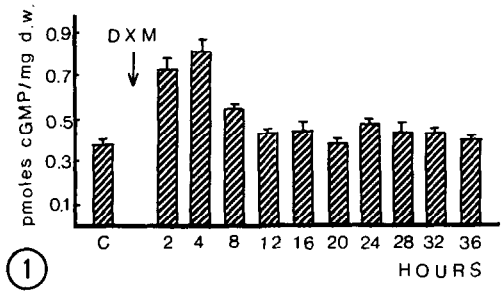
In a second experiment (Fig. 4) DXM injection (1 mg/kg body weight) was repeated each day for 9 days.

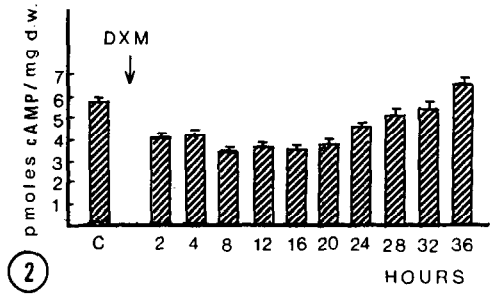
cAMP and cGMP were measured at days 1, 2, 3, 5 and 9, 4 hr after each DXM injection ; cAMP and blood corticosterone (not represented because practically undetectable) were depressed throughout whereas cGMP after an initial increase (4 fold the control level) as that observed in the first experiment, decreased and remained the following days within the control limits.

Effects of DXM on adrenocortical cyclic nucleotide levels in hypophysectomized rats are shown in Fig. 5, 6. The cGMP concentration presented a significant increase 2, 4 and 8 hr after DXM injection. cAMP showed only a modest decrease at 4 hr.

DISCUSSION

The most remarkable effects of steroids on cyclic nucleotide tissue levels described to date mainly concern an increase in cGMP in the rat uterus (1) and the chick oviduct (2) induced by estrogen administration. Such increase starts 1-2 hr after estrogen injection and reaches a peak at 4 hr. The peak values are about 7 fold the control ones in the rat's uterus. A simultaneous decrease of cAMP levels accompanies the increase of cGMP. In this study the pattern of adrenocortical cyclic nucleotides after DXM administration appears very similar, although the cGMP peak was not as high. The published experiments





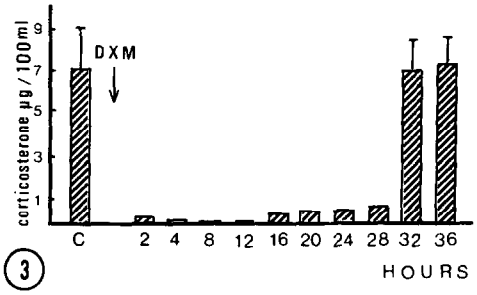


Figure 1. Time course of changes in levels of adrenocortical cGMP after DXM administration in intact adult male rats.
cGMP was measured at various time intervals after a single i.m. injection of DXM (1 mg/kg body weight).
Each value represents the mean ± S.E.M. of 8-10 subjects.
$p < 0.001$ at 2, 4, 8 hours.
Each group was compared with the controls C.

Figure 2. Time course of changes in levels of adrenocortical cAMP after DXM administration.
$p < 0.001$ at 2, 4, 8, 12, 16 hours.
$p < 0.01$ at 20 hours.
$p < 0.01$ at 36 hours.
Each group was compared with the controls C.

Figure 3. Time course of changes in levels of blood corticosterone after DXM administration.

(3, 5) concerning the effects of DXM on cGMP tissue levels (muscle, adrenal medulla) were not repeated at different times following drug administration, and furthermore, the effect observed was a decrease instead of an increase, as in the present study.

In intact rats, the possibility of an indirect effect of DXM cannot be completely excluded. Generally the inhibitory action of glucocorticoids upon steroidogenesis and adrenal growth is considered to be due to a suppressive effect on ACTH secretion (9, 10). The site of action of glucocorticoids may

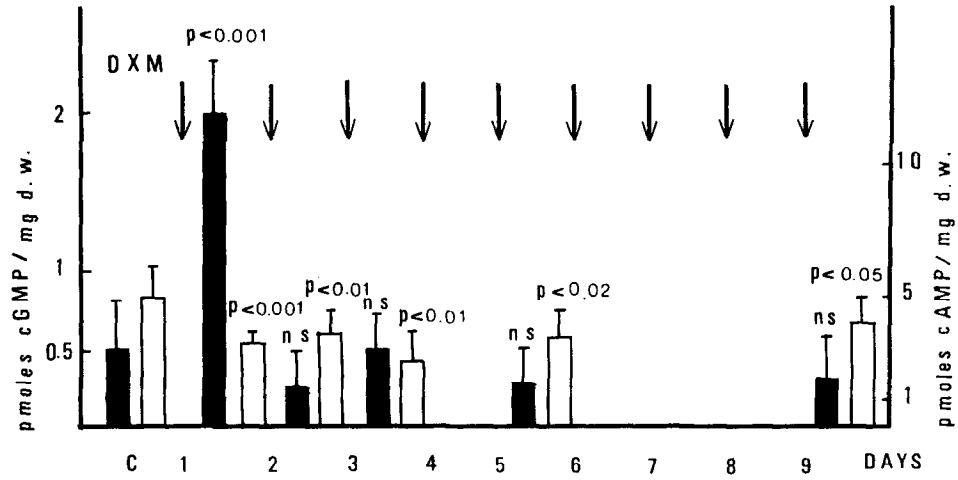
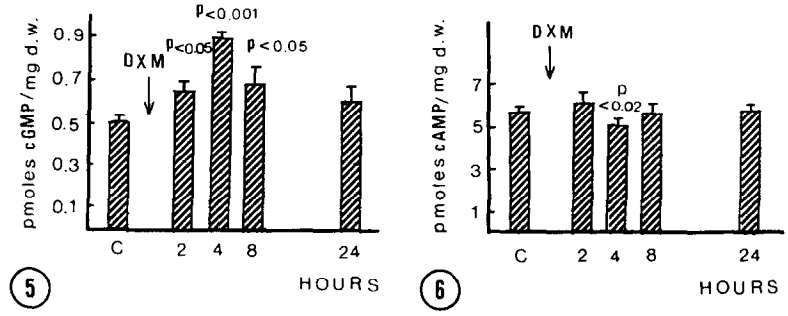


Figure 4. Effects of daily repeated DXM i.m. injections (1 mg/kg body weight) for 9 days. cGMP ■ and cAMP ▭ concentrations were measured 4 hr after DXM administration at days 1, 2, 3, 5 and 9. Each value represents the mean ± S.E.M. of 8-10 subjects for the controls and at day 1, and of 3-4 subjects at days 2, 3, 5 and 9.



Figures 5-6 Time course of changes in levels of adrenocortical cyclic nucleotides after DXM administration in hypophysectomized adult male rats. cGMP Fig. 5 and cAMP Fig. 6 were measured at various time intervals after a single i.m. injection of DXM (1 mg/kg body weight). Each value represents the mean ± S.E.M. of 10-11 subjects.

be in both the pituitary and the hypothalamus (11, 12). Moreover, Steiner et al (13) have shown that the adrenal cGMP level rises in rats shortly after hypophysectomy whereas adrenal cAMP falls. Furthermore, physiological doses of ACTH produce an increase in adrenal cAMP and a simultaneous suppression of adrenal cGMP. These studies indicate that the control of adrenal cGMP is regulated at least partially by ACTH. In our experiments we used ACTH suppressing DXM doses and the data presented in Fig. 1 could be explai-

ned by the inhibition of ACTH release. Nevertheless, the results obtained in hypophysectomized rats Fig. 5, 6 suggest that DXM acts directly, at least in part, on the adrenal cortex independently of the inhibition of ACTH release. This hypothesis is strengthened by the existence of glucocorticoid receptors in the adrenal cortex of the rat (14). Steroidogenesis inhibition and cGMP increase can be dissociated, since steroidogenesis inhibition persists when the cGMP levels have already returned to the control value(Fig. 1, 3).

The significance of this cGMP increase remains unclear. A relationship with an arrest of the adrenal cell division has been described after DXM administration (15) and cAMP and cGMP might act as cell cycle regulators (16). Additional studies are required to determine whether the cGMP increase plays a role in the control of growth in the adrenal cortex.

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