THE COUNTERACTION AGAINST THE DEXAMETHASONE-INDUCED ADRENAL INHIBITION BY COLD STRESS

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Received for publication February 21, 1967

It has been noted that administration of adrenocortical hormones as well as synthetic adrenal steroids give rise to a marked inhibition of adrenal function. However a few reports referring to a counteraction against this depressive effect of adrenal steroid have been presented. Onuki *et al.* (1) have reported that, although xylitol seems to slightly protect a depression of corticoid biosynthesis in the adrenals resulting from prednisolone administration, the effect is not statistically significant. Recently, it has been reported that reserpine obviously counteracts a inhibitory effect of dexamethasone on the adrenals, and this effect is presumably not due to a depression of corticotrophin secretion by reserpine but due to its stimulative effect on the hypophysial-adrenal axis (2).

These results are elicited of some interest in view of counteraction between the corticoid-induced adrenal inhibition and the stress which is proposed to stimulate the hypophysial-adrenal system.

MATERIALS AND METHODS

The experiments were undertaken using male Donryu rats ranged in weight from 170 to 200 g and maintained on standard laboratory chew. These animals were reared in a room kept at $24^{\circ}\text{C} \pm 1^{\circ}\text{C}$.

Cold stress was achieved by moving rats to a cold room kept at $4^{\circ}\text{C} \pm 1^{\circ}\text{C}$.

Adrenal weight was expressed as total weight of the both adrenals.

The blood samples were collected from the inferior vena cava at just below diaphragm, and the values were expressed as corticosterone (μg) in the plasma (100 ml).

Corticoid biosynthesis in the adrenals was determined by the method which was essentially similar to that described by Saffran *et al.* (3-5). The both adrenals from each rat were contributed to one Warburg flask containing 1.5 ml of Krebs-Ringer-phosphate buffer with added glucose. Preincubation and incubation were in an atmosphere of oxygen at 38°C . The flask was shaken for 30 minutes, then the medium was exchanged for the ACTH (1 I.U.) (N.V. Organon) contained medium. After incubation period of 1 hour, corticosterone in the medium was estimated, and the values were expressed as corticosterone (μg) released from the adrenals (100 mg).

Corticosterone was assayed by Zenker and Bernsteins method (6).

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Electron microscopical observations were performed initially by removing the adrenals from rats immediately after anesthesia. The tissues were quickly sliced into 1-2 mm cubes, and these materials were pre-fixed by 2.5% of glutaraldehyde, which was adjusted to PH 7.4 with 0.2 M of Sørensen phosphate buffer, for 1 hour at 4°C. These blocks were washed several times with phosphate buffer for more than 12 hours, and post-fixation with 1% of buffered osmium tetroxide was continued for 3 hours at 4°C. Dehydration was undertaken using a graded series of chilled acetone solution. The blocks, then, were embedded in Epon 812 infiltrated through acetone, and sectioned by Porter-Blum MT-1 and Reidhert Om-U₂ ultramicrotomes after polymerized in incubator. The ultrathin sections were mounted on carbon supported copper grids, stained with uranyl-acetate-lead citrate, and examined by a JEM7A electron microscope.

RESULTS

A) *Effects of cold stress on the adrenal inhibition induced by dexamethasone*

Dexamethasone was injected intraperitoneally in a dose of 0.1 mg/kg for 4 days and the rats were used for experiments 1 day later.

As seen in Table 1, administration of dexamethasone at a temperature of 24°C was found to bring about a reduction of adrenal weight, a decrease of corticosterone biosynthesis in the adrenals and a fall of corticosterone level in the plasma. However, when cold exposure to 4°C had been started from 1 hour prior to the administration of dexamethasone, the changes induced by dexamethasone was shown to be less marked.

TABLE 1. Effects of cold stress on the adrenal inhibition induced by dexamethasone.

	Adrenal weight (mg)	Corticosterone released from adrenals ($\mu\text{g}/100\text{ mg}$)	Corticosterone level in plasma ($\mu\text{g}/100\text{ ml}$)
Normal	23.5 $\pm$ 1.1 (n = 25)	27.3 $\pm$ 1.5 (n = 23)	12.3 $\pm$ 0.9 (n = 30)
at 24°C	17.9 $\pm$ 0.64 (n = 13)	17.9 $\pm$ 1.67 (n = 8)	6.1 $\pm$ 0.51 (n = 10)
Dexa. at 4°C	21.6 $\pm$ 0.53 (n = 13)	24.2 $\pm$ 2.02 (n = 8)	9.7 $\pm$ 0.75 (n = 8)

mean $\pm$ standard error, n : experimented number

In electron microscopic observation, there were no remarkable changes in the nucleus, endoplasmic reticulum, ribosomes and cell membrane. Although the mitochondria were not changed conspicuously by such dose of dexamethasone, the matrix were less electron dense and long tubular cristae were increased in the rats treated with dexamethasone at 24 and 4°C than in the normal rats. Vesicles, which were regarded as extruding vacuoles, were observed more abundantly in both the normal rats and rats treated with dexamethasone at a temperature of 4°C. Lamellar structures, which were adjacent to the mitochondria, were observed in each three groups but more frequently in rats treated with dexamethasone at 22°C. Large homogeneous vacuoles, which were considered to be lipid droplets, were most abundant in rats treated with dexamethasone at 4°C.

a) Normal rats

The honeycombed mitochondria which are characteristic in the adrenal cortex are shown. Vacuoles, which are close to the mitochondria, are seen in places. The upper left mitochondrion contains crystalline substance. At upper right of the figure, the nucleus is observed.

b) Rats treated with dexamethasone at 22°C

Lamellar structure, which are close to the mitochondria, are observed at lower right of the figure. Long tubular cristae are seen in the mitochondria. The lysosome is shown at upper left part.

c) Rats treated with dexamethasone at 4°C.

At the lower part of the figure, the cell membrane is seen. Long tubular cristae is observed in the lower left mitochondrion. Large homogeneous vacuoles, which are considered to be lipid droplets, are shown in places.

FIG. 1. Electron micrograph of the zona fasciculata of adrenal cortex. (Phosphate buffered glutaraldehyde fixation, osmium tetroxide post-fixation, Epon embedding and uranylacetate-lead citrate stain. $\times 40,000$.)

B) Effects of cold stress on recovery from the adrenal inhibition induced by dexamethasone

After administration of dexamethasone in a dose of 0.15 mg/kg for 4 days, it was shown to be 15.8 mg ($n=3$) in adrenal weight and $2.1 \mu\text{g}$ ($n=3$) in plasma corticosterone levels 1 day later, however, the values were 18.4 mg ($n=3$), $9.3 \mu\text{g}$ ($n=3$) 7 days later, and 19.8 mg ($n=3$), $10.8 \mu\text{g}$ 12 days later respectively. These results suggested that the adrenal function had been recovered progressively from its inhibition. So the rats were divided into two groups 1 day after administration of dexamethasone for 4 days: the one group had been reared at a temperature of 24°C and the other had been reared at 4°C, then, the both group were used for experiments 10 days later.

As seen in Table 2, the each values were higher in the latter group than that in the former group.

TABLE 2. Effects of cold stress on recovery from the adrenal inhibition induced by dexamethasone.

	Adrenal weight (mg)	Corticosterone level in plasma ($\mu\text{g}/100 \text{ ml}$)
at 24°C	19.2 ± 0.81 ($n=8$)	10.9 ± 0.68 ($n=8$)
at 4°C	23.6 ± 0.83 ($n=8$)	19.8 ± 1.12 ($n=8$)

DISCUSSION

When dexamethasone, which is potent synthetic adrenal steroid and has been proposed to induce a marked inhibition of adrenal function, is administered, it is observed to bring about a reduction of corticosterone quantity released from the adrenals to the medium following addition of ACTH, and this result suggests a suppression of corticosterone biosynthesis in the adrenal glands by dexamethasone. In addition, a depletion of ACTH stores in the pituitary inducing by prolonged administration of adrenal steroids has been reported (7-9). The results might imply that dexamethasone administered repeatedly reduces a release of ACTH and a synthesis of new ACTH in the pituitary. These changes in the hormonal system seem to result in a decrease of adrenal weight and a fall of plasma corticosterone levels as observed.

On the other hand, the cold stress has been proposed to stimulate the hypophyseo-adrenal hormonal system. Maickel *et al.* (10) have demonstrated that exposure of rats to a temperature of 4°C evokes a fall in adrenal ascorbic acid, a rise in plasma corticosterone and free fatty acid, and an increased activity of liver tryptophane pyrrolase.

Consequently, the prolonged administration of dexamethasone and cold stress are thought to be inverse in effects on the hypophyseo-adrenal hormonal system.

It has been shown, by the values in adrenal weight, adrenal biosynthesis and plasma level of corticosterone, that the adrenal inhibition induced by dexamethasone is apparently weaker in rats exposed to cold than in rats reared at 22°C.

Morphological changes in the fine structure of rat adrenal cortex after the hypophysectomy have been reported (11), and dexamethasone, likewise, is thought to induce a decrease of ACTH release from the pituitary as mentioned. Although the result obtained from the electron microscopical observation did not allow firm conclusion regarding the changes in the adrenal cortex, the alterations appeared to be similar to those seen in the hypophysectomized rats to some extent after prolonged administration of dexamethasone at 22°C, and there appeared to be a little counteraction between dexamethasone and cold stress. Vesicles, which were adjacent to the mitochondria and regarded as extruding vacuoles, were observed more abundantly in both the normal rats and rats treated with dexamethasone at 4°C, however, lamellar structures which were close to the mitochondria, were observed more frequently in rats treated with dexamethasone at 22°C. It is conceivable that the vacuoles are emerged as a result of corticoids synthesis and secretion, and, if the secretion is suppressed, the lamellar structures remain unchanged.

The cold stress after the repeated administration of dexamethasone has revealed that the recovery from inhibition in adrenal weight and plasma levels of corticosterone are likewise accelerated by the stress.

From these results, it has been demonstrated that the cold stress obviously counteracts the inhibitory effect of dexamethasone on the adrenals, and, presumably, the counteractive effect of reserpine on the dexamethasone-induced adrenal inhibition is due to its stimulative effect on the hormonal system as proposed by Giuliani *et al.* (2).

SUMMARY

The experiments were undertaken using male Donryu rats. Administration of dexamethasone at a temperature of 24°C induced a reduction of adrenal weight, a decrease of corticoid biosynthesis in the adrenals and a fall of corticosterone levels in the plasma, however, these changes were less marked when the rats were exposed to 4°C. In electron microscopical observation, there appeared to be a little counteraction between the rats treated with dexamethasone at 22°C and at 4°C. The cold stress, likewise, stimulated a recovery from the dexamethasone-induced adrenal inhibition.

It has been demonstrated that cold stress counteracts the inhibitory effects of dexamethasone on the adrenals, and, presumably the counteractive effects of reserpine on the adrenal inhibition induced by dexamethasone is due to its stimulative effects on the hormonal system as proposed.

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