

INCREASED ^3H -THYMIDINE INCORPORATION INTO DNA OF ORGAN-CULTURED
ADRENAL EXPLANTS FROM RATS INJECTED WITH CORTICOTROPIN AND/OR
CYSTEAMINE

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The effect of a single injection of cysteamine /CySH/
- a sulfhydryl substance, known to deplete tissue content of
somatostatin /SS/ - on ^3H -thymidine incorporation into DNA of
rat adrenal explants incubated in vitro was investigated.
It was shown that: 1/ Single in vivo injection of ACTH or of
CySH increased ^3H -thymidine incorporation into DNA of the
organ-cultured adrenals, 2/ Dexamethasone reduced the ^3H -thymidine
uptake, but that decrease did not attain statistical significance
versus controls. © 1988 Academic Press, Inc.

Presence of somatostatin /SS/ receptors has recently been
demonstrated in the adrenal cortex of the rat and/or other
mammalian species /10/, as well as in human adrenal tumors
/i.e., pheochromocytomas and aldosteronomas/ /16/. Some lines
of evidence are available, suggesting that SS may be a modulator
of the rat adrenal secretory activity /1,2/. This neuropeptide
was shown to inhibit the angiotensin II-induced stimulation of
aldosterone secretion and the growth of rat adrenal zona
glomerulosa cells /11,15/. In turn, chronic SS administration
was found to partially reverse the ACTH-enhanced growth of the
rat adrenal zona glomerulosa /18/.

Cysteamine /2-mercaptoethylamine, CySH/ is known to deplete
the immunoreactive SS content in different organs of the rat
and the mouse /23,24/. Beside SS, CySH is also believed to
deplete prolactin /Prl/ /12,14/ and growth hormone /GH/ /13/
tissue contents.

In the present study we have attempted to examine the
effect of single i.p. injections of ACTH, dexamethasone and/or

CySH on ^3H -thymidine incorporation into DNA of rat adrenals collected 28 h after injection and placed into organ culture.

MATERIALS AND METHODS

Male Wistar rats, weighing 150 ± 20 g each, were donors of adrenals. Twenty eight hours prior to the beginning of incubation the animals were administered one single i.p. injection, as follows:

- Group I - controls, 0.9% NaCl, n=8
- Group II - ACTH /Synacthen Depot, 1-24 ACTH, CIBA/, 120 U/kg BW, n=8
- Group III - Dexamethasone /Decadron phosphate, Merck Sharp and Dohme, The Netherlands/, 2.4 mg/kg BW, n=6
- Group IV - Cysteamine /2-mercaptoethylamine, Sigma/, 300 mg/kg BW, n=7

The rats were killed by decapitation. The adrenals, collected from all the animals under sterile conditions, were divided into two equal parts /equatorial cut/ and immediately placed in a culture vessel on the surface of a stainless grid for the fluid under the grid to moisten them. The halves of adrenals were incubated for 2 hours in RPMI 1640 medium /Gibco/, containing 2 μCi of ^3H -thymidine /Chemapol Prague, Czechoslovakia/ with an addition of 15% fetal calf serum, 20 mM Hepes buffer, penicillin /200 U/ml/ and streptomycin /10 $\mu\text{g}/\text{ml}$ /. DNA was extracted as described by Schmidt-Thannhauser /20/, and determined by diphenylamine method /4/. The results were expressed as the mean counts per minute /cpm/ per 1 μg of DNA. Data were statistically analyzed using a one-way analysis of variance /ANOVA/. The significance of differences among the individual groups was estimated with use of Newman-Keuls' test /7/.

RESULTS

The data are illustrated graphically in Figure 1.

Corticotropin injection significantly increased ^3H -thymidine incorporation into DNA of adrenal explants /ACTH= 305.45 ± 27.27 ; controls= 73.77 ± 6.74 ; $\bar{x} \pm \text{SEM}$ /.

Cysteamine administration also induced a significant rise in the tritiated thymidine uptake by adrenal DNA /CySH= 147.57 ± 15.53 /, when compared to the control group.

In contrast, dexamethasone decreased ^3H -thymidine incorporation into DNA of adrenal explants / 43.55 ± 8.07 /, but that fall did not attain statistical significance.

DISCUSSION

In the present study, a single in vivo injection of CySH increased ^3H -thymidine incorporation into DNA of the rat adrenal explants

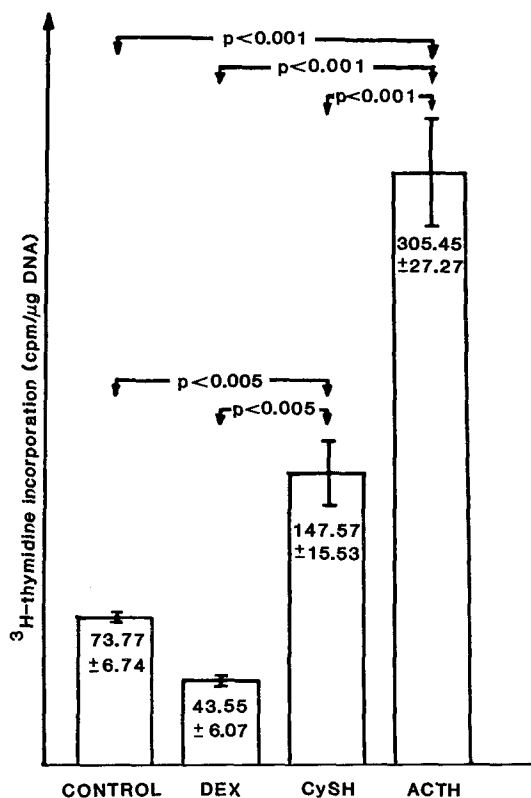


Fig. 1. ^3H -thymidine incorporation into adrenal explants in individual incubations; DEX - dexamethasone, CySH - cysteamine.

Data are means \pm SEM; p - level of significance.

in organ culture. It is possible that this effect of CySH ensues through a selective depletion of SS tissue content in the adrenals. However, the exact mechanism of CySH action on SS depletion is still unclear. It has been suggested that CySH might act by accelerating the intracellular degradation of immunoreactive SS /24/ or, more likely, through a chemical modification of the disulfide bond which would render the molecule unreactive /17/. Gonzalez-Guijarro et al. /5/ have demonstrated that administration of CySH to rabbits depletes both duodenal mucosa and plasma SS and leads to sensitization or up-regulation of SS binding sites in the duodenum.

Beside SS, CySH is also thought to deplete Prl and GH tissue contents /12,13,14/. Since both GH /26/ and Prl /8/ have been shown to stimulate the adrenal cortex mitogenesis, it is unlikely that tissue depletion of these hormones would stimulate adrenal ^3H -thymidine uptake. On the other hand, an assumption can be

offered that the stimulatory effect of CySH, as obtained in the present study, may be related to the depletion of hypothalamic SS and to the increased pituitary GH release which, as mentioned above, stimulates the adrenal mitosis incidence /26/.

As expected, ACTH injection to rats in vivo significantly increased ^3H -thymidine incorporation into DNA of their organ cultured adrenals. It is noteworthy that ACTH in vivo is a potent stimulator of adrenocortical growth /3/ and increases DNA content in adrenals /9/. It was demonstrated by us that ACTH increased the mitotic activity of adrenocortical cells in rats /22,25/ and in mice /21/ in vivo.

In contrast to the stimulating effect of ACTH, we found that dexamethasone - a synthetic glucocorticoid - decreased DNA synthesis in vivo. Our results are in compliance with previous studies reporting the inhibitory effect of dexamethasone on the cortical cell division and on ^3H -thymidine incorporation into DNA of the rat adrenal gland /19,27/. The mechanism by which glucocorticoids act on DNA synthesis and cell division remains unclear. It is possible that dexamethasone depresses adrenocortical cell divisions, acting via an inhibition of phospholipase /6/.

In conclusion, CySH-induced increase of adrenal ^3H -thymidine uptake, suggests that SS, directly and/or indirectly, may participate in the control of adrenocortical growth. On the other hand, ACTH-induced enhancement and dexamethasone-induced decrease of ^3H -thymidine incorporation into the adrenal DNA confirm previous results of other authors.

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