Direct Effect of Histamine on the Production of Adrenocortical Hormone by Guinea-pig Adrenal Cells

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Summary In trypsin-dispersed adrenal cells of the guinea-pig, the production of 11-hydroxycorticosteroids *in vitro* was found to be significantly stimulated by 10^{-7} – 10^{-4} M histamine and the maximum steroidogenic response (1.7-fold increase in 11-hydroxycorticosteroid production) was observed at 10^{-6} M. The stimulatory action of histamine on steroidogenesis was blocked by cycloheximide treatment.

It has been observed in earlier studies that the systemic administration of histamine in hypophysectomized dogs elicits a small but significant increase in adrenocortical secretion (HIROSE *et al.*, 1976, 1977) and that administration of histamine to collagenase-trypsin dispersed adrenal cells in dogs brings about an increase in cortisol and corticosterone production *in vitro* (HIROSE *et al.*, 1978, 1979). These findings demonstrate a direct stimulatory effect of histamine on adrenocortical secretion, though the mechanism has not yet been defined. In contrast, it was reported by DE WIED (1961) that histamine administered to radically hypophysectomized rats failed to affect corticoid production of the excised adrenal gland *in vitro*, suggesting the ineffectiveness of histamine in directly stimulating the rat adrenal cortex. This discrepancy may suggest that there is species difference in the direct effect of histamine on corticosteroidogenesis. It has been established that an inhibitor of protein synthesis, such as cycloheximide, interferes with ACTH-induced steroidogenesis (GARREN *et al.*, 1965; DAVIS and GARREN, 1968; SCHULSTER *et al.*, 1970; KOWAL, 1970; RUBIN *et al.*, 1973). The present investigation was undertaken to evaluate the direct stimulatory action of histamine on corticoid production of trypsin-dispersed adrenal cells of guinea-pigs and the effect of cycloheximide on histamine-induced steroidogenesis.

The dispersion and incubation of adrenal cells of the guinea-pig were carried out using a modification of the method of SAYERS *et al.* (1971). Male guinea-pigs weighing 250–300 g were sacrificed by decapitation under ether anesthesia. The adrenals were removed and cut into small blocks (≤ 1 mm³) in Krebs-Ringer pho-

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sphate buffer containing 0.2% glucose (KRPB). The small blocks of adrenal tissue were treated with trypsin solution (0.25% in KRPB, Worthington Biochemical Co.) and agitated with a glass paddle at 450 rpm for 15 min at 37°C. The cell suspension was pipetted and pooled at 4°C. Trypsin treatment was repeated several times. The pooled cell suspension was centrifuged at 100 *g* for 30 min at 4°C. The pellet was resuspended in fresh KRPB, the incubation medium used containing 0.5% bovine serum albumin (Wako Co.), 0.1% Lima bean trypsin inhibitor (Worthington Biochemical Co.) and 7.65 mM Ca^{2+} . The final concentration of dispersed cells was adjusted to approximately 1×10^5 cells/ml and 1 ml of the cell suspension was incubated in a shaking water bath (66 oscillations/min) with various concentrations of histamine (10^{-8} – 10^{-4} M) and/or cycloheximide (10^{-4} M) for 120 min at 37°C. All glass apparatus used in this experiment were previously coated with siliconizer (Fuji Co.). Estimation of 11-hydroxycorticosteroids (11-OHCS) produced was performed by the fluorimetric method of SILBER *et al.* (1958). The production of 11-OHCS was expressed as ng/ 10^4 cells and Student's *t*-test was used for statistical analysis of the results.

The effect of increasing concentrations of histamine on 11-OHCS production by trypsin-dispersed adrenal cells is shown in Fig. 1. The stimulatory effect of histamine on steroidogenesis was detectable at 10^{-8} M. A significant increase of steroid formation was first observed in the presence of 10^{-7} M of histamine and the maximal steroidogenic response (1.7-fold increase in 11-OHCS production) at 10^{-6} M. When the concentration of histamine was increased beyond 10^{-6} M, the steroidogenic responses to histamine were found to be significantly decreased in comparison with maximal steroidogenic response.

In order to study the effect of an inhibitor of protein synthesis on steroidogenesis induced by histamine, the suspended cells were incubated with 0.1 mM cyclohexi-

Fig. 1. Production of 11-hydroxycorticosteroids (11-OHCS) by trypsin-dispersed adrenal cells of guinea-pigs in response to histamine. Vertical lines indicate S.E. of the mean. Numbers in parentheses show the number of experiments. * $P < 0.02$, ** $P < 0.01$, *** $P < 0.001$ compared with control.

mide and 10^{-6} M histamine for 2 hr at 37°C . The results are shown in Table 1. An increase in 11-OHCS production in response to histamine was found to be abolished by the cycloheximide treatment.

Table 1. Effect of cycloheximide on histamine-induced steroidogenesis in dispersed adrenal cells of guinea-pigs.

Compound tested		Production of 11-hydroxycorticosteroids (ng/ 10^4 cells)	P
a. None (control)	(8)	6.9 ± 0.29	
b. Cycloheximide	(8)	4.8 ± 0.34	0.001 (a vs. b)
c. Histamine	(12)	11.6 ± 0.63	0.001 (a vs. c)
d. Histamine+cycloheximide	(12)	6.3 ± 0.26	0.001 (c vs. d)

Numbers in the parentheses indicate the number of experiments. Values indicate mean $\pm$ S.E.M.

The normal values for the plasma histamine concentration in guinea-pigs were previously determined by radio-enzymatic or fluorometric assay to be 0.5–50 ng/ml (BEAVEN *et al.*, 1972) corresponding to 4.5×10^{-9} – 4.5×10^{-7} M. It is well known that endotoxin shock (HINSHAW *et al.*, 1961) or anaphylactic shock (PARROT *et al.*, 1977) causes a marked increase in blood or plasma histamine. In the present study, 11-OHCS production was significantly stimulated by histamine at concentrations ranging from 10^{-7} to 10^{-6} M, which might be within the range of plasma levels of histamine under certain pathological conditions. These data indicate that the adrenocortical secretory response to histamine in guinea-pigs, although mainly dependent on ACTH, might depend at least in part on the direct stimulatory action on the adrenal cortex.

The possible participation of histamine in the control of adrenocortical secretion as a local hormone at the adrenal level remains unresolved in the present study.

The data of cycloheximide treatment indicate that the steroidogenic action of histamine on the adrenal cortical cells in guinea-pigs is cycloheximide-sensitive.

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