Inhibitory Action of Calcium on the Inactivation of Antidiuretic Hormone by Rat Kidney Slices

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Some evidence has been presented in favour of the hypothesis that calcium is involved in the mechanism of action of antidiuretic hormone (ADH) on the mammalian kidney (Thorn, *Acta Endocrinol.* **38** (1961) 563). In a series of tests of this hypothesis the effect of increasing the Ca concentration in the medium used for studies of the inactivation of arginine-vasopressin (ADH of the rat) by rat kidney slices was tried.

Slices of 0.4 mm thickness, cut on the Stadie-Riggs microtome, were subdivided into 3 zones: (1) outer cortex, (2) outer medulla and (3) papilla. 5–10 mg (dry weight) of slices from each zone was incubated with 200 mU of a commercial preparation of arginine-vasopressin at 37°C in Warburg vessels for 2 h (after an equilibration period of 1/2 h). For each zone one set of slices was incubated with hormone in the medium of Robinson (*J. Physiol.* **134** (1956) 216) employing the usual Ca conc. (2.5 mM), and a parallel set of slices was incubated in the same medium modified in the way that the strength of the phosphate buffer was reduced to 1/5 whereas the Ca conc. was increased 5 times. The osmolality of the medium was kept unchanged (288 mOsm/kg) by reduction of the conc. of NaCl. An inhibition of the rate of inactivation (mU/2 h/mg dry tissue) of the hormone of 9, 22 and 13 % on the average was found for the 3 zones in the medium with a conc. of Ca 5 times normal. Increasing the Ca conc. in the medium to 10 times the normal conc. consistently resulted in an inhibition of the inactivation to approx. 50 % in zones 2 and 3. The inhibition was not due to any difference in oxygen consumption and was not correlated with the slight changes in pH often found after incubation due to the limited buffer capacity of the high Ca medium. In experiments using 40 mM Tris buffer (pH 7.2) the pH was kept unchanged. Inhibitions of a comparable magnitude were found with high conc. of Ca also with this buffer. — Parallel studies of a somewhat similar nature, carried out independently by Dr. M. W. Smith, Cambridge, have shown similar results.

On the Mechanism of Disulfiram-Ethanol Reaction

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The symptoms of the disulfiram-ethanol reaction, according to Hald *et al.*^{1,2}, are due to an accumulation of acetaldehyde as a consequence of a disulphide inhibition of enzymes involved in the metabolism of ethanol. This explanation necessarily requires that the drug is present intracellularly in its disulphide form. A large body of evidence, however, indicates that disulfiram in the body will be reduced to the corresponding thiol, diethyldithiocarbamate^{3,4}, which appears to be less toxic to enzymes of ethanol metabolism⁵. If the disulfiram-ethanol reaction still should be based on the drug in its disulphide form, a reoxidation of diethyldithiocarbamate must take place.

Recently we have suggested that in the course of ethanol metabolism the reoxidation of diethyldithiocarbamate to disulfiram is promoted, and that disulfiram in turn causes a general "disulphide poisoning" which is considered responsible for the symptoms. This theory is consistent with the fact that disulfiram is a potent and general SH-blocking agent. Furthermore, the paradox that patients may take a potent SH-blocking agent for months with few untoward effects is easily explained.

In an attempt to test the mechanism suggested, the *in vivo* formation of ¹⁴CO₂ from ¹⁴C-*u*-glucose in rats has been studied during disulfiram-ethanol reaction. This assay procedure is based on the observation that hexokinase is inhibited by disulfiram but not by diethyldithiocarbamate³. The results show that the ¹⁴CO₂ output as well as the total CO₂ output by rats receiving disulfiram (0.5 g/kg body weight per day in three days) and ethanol (1.5 g/kg body weight immediately before the test) are decreased with 38 % and 26 %, respectively, as compared to the output by untreated rats (*p* < 0.001). Rats receiving disulfiram only, also demonstrated some decrease in ¹⁴CO₂ output (11 %) and total CO₂ output (11 %), but the decrease obtained with ethanol in addition is significantly larger (*p* < 0.01). The decrease in ¹⁴CO₂ output by rats receiving both compounds is more pronounced than the simultaneous decrease in total

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CO₂ output ($p < 0.05$). Ethanol alone does not significantly interfere with the CO₂ formation.

The results thus suggest that a specific interference with the glucose consumption occurs during the disulfiram-ethanol reaction. This may be due to a disulphide inhibition of the hexokinase as a consequence of a re-conversion of the thiol to its disulphide disulfiram.

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The Mechanism of Lysis of *Halobacterium salinarum* in Hypotonic Solutions

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When the extremely halophilic bacteria of the genus *Halobacterium* are exposed to a lowering of the NaCl concentration in their environment by gradually adding water, the normally rod-shaped cells will change their structure through transition forms to spheres, and the spheres will lyse. In the past it has been believed that the structure changes and lysis in hypotonic solutions were due to a build-up of an osmotic pressure difference between the content and the environment of the cells, leading to a disruption of the cell wall at extreme pressure differences¹. However, recent work on the effect of chemicals and heat on whole cells of *Halobacterium* has shown that osmotic effects probably play a minor role in these phenomena, and has led to the suggestion that NaCl is required by the bacteria to maintain a rigid cell wall^{2,3}.

In the present work *H. salinarum* was grown in a medium containing 25 % NaCl. After grinding the cells, clean cell wall fragments were isolated by fractional centrifugation and repeated washings. All operations were carried out with the cells and the wall fragments suspended in a 25 % NaCl solution. The purity of the wall fragments was assessed by electron microscopy. In subsequent experiments the wall fragments were exposed to a lower NaCl concentration — 2.5 % — by adding water to the suspensions. Upon such dilution no cell wall fragments could be detected by examination in the electron microscope. Exposure of the cell walls to such dilute solutions obviously effected a disintegration of the walls to very small particles which formed a homogeneous layer on the specimens examined.

The cell wall of *Halobacterium* is almost entirely composed of proteins and lipoproteins⁴. The above observations thus strongly support a contention that NaCl is required in such high concentrations by these bacteria to make the proteinaceous sub-units of the wall associate in an orderly array, possibly by reducing electrostatic repulsions between charged groups in the proteinaceous particles or by decreasing the solubilizing interaction between water and polar groups of the wall proteins. By exposure of the cells to hypotonic solutions the protecting effect of the sodium and chloride ions is diminished, leading to a disintegration of the wall — a lysis of the cell — at salt concentrations below a certain limit.

The lysis pattern of the cells was not affected by a number of specific and general enzymic inhibitors, thus ruling out the possibility of enzymes participating in the lysis process.

A detailed account of this work will be published elsewhere⁵. The work was supported by grants from the Royal Norwegian Council for Scientific and Industrial Research.

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