The Effect of Electro-convulsive Therapy on the Urinary Excretion of Adenosine 3':5'-Cyclic Monophosphate

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Abdulla & Hamadah (1970) have suggested that depressive illness is due to a severe fall in the intracellular concentration of cyclic AMP (adenosine 3':5'-cyclic monophosphate) in all tissues. They also report that urinary excretion of cyclic AMP is lowered in depressive illness, but an increase in the urinary excretion occurred after administration of tricyclic anti-depressants.

Electrical stimulation has been found to increase the concentration of cyclic AMP in cerebral-cortex slices (Pull & McIlwain, 1972), and electro-convulsive therapy has long been accepted as an effective form of treatment in some types of depression. We have therefore measured the urinary excretion of cyclic AMP in 14 female patients (aged 24–61 years) undergoing electro-convulsive therapy for depressive illness. The administration of antidepressant or tranquillizing drugs and of potential dietary inhibitors of phosphodiesterase (e.g. methylxanthines in tea and coffee) was stabilized (Paul *et al.*, 1971). Patients were well oxygenated through the period of electro-convulsive therapy, as it has been noted that hypoxia in guinea-pig brain slices results in a marked increase in the output of adenosine derivatives (Pull & McIlwain, 1972).

Urines (07:00–07:00h) were collected and stored at -20°C , and the cyclic AMP content was determined by an enzymic radioisotope-displacement technique (Brooker *et al.*, 1968). It has been suggested that this method may lack the specificity inherent in the protein binding assay (Brown *et al.*, 1971), but this has not been our experience with determinations in urine.

Twelve of the patients showed a significant rise in the excretion of cyclic AMP on the day of electro-convulsive therapy (14.2 compared with $4.2\mu\text{mol}/24\text{h}$ before the therapy). Four controls (failed electro-convulsive therapy) showed no significant increase. This finding is not in agreement with the single-patient study made by Brown *et al.* (1972), but we have found that about 10% of our patients showed no significant increase in the excretion of cyclic AMP on the day of electro-convulsive therapy.

The role of cyclic AMP as a transmitter in the central nervous system is under investigation (Greengard, 1972). It is possible that the antidepressant action of electro-convulsive therapy is mediated

through an increased production of cyclic AMP in brain, and that this is reflected in an increased urinary excretion.

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- Abdulla, Y. H. & Hamadah, K. (1970) *Lancet* **i**, 378
 Brooker, G., Thomas, L. J. & Appleman, M. M. (1968) *Biochemistry* **7**, 4177
 Brown, B. L., Albano, J. D. M., Ekins, R. P., Seherzi, A. M. & Tampion, W. (1971) *Biochem. J.* **121**, 561
 Brown, B. L., Salway, J. G., Albano, J. D. M., Hullin, R. P. & Ekins, R. P. (1972) *Brit. J. Psychiat.* **120**, 405
 Greengard, P. (1972) *Biochem. J.* **128**, 75P
 Paul, M. I., Cramer, H. & Goodwin, F. K. (1971) *Arch. Gen. Psychiat.* **24**, 327
 Pull, I. & McIlwain, H. (1972) *Biochem. J.* **126**, 965

The Effect of Glucagon on Adenosine 3':5'-Cyclic Monophosphate Concentrations in an Organ Culture of Adult Rat Liver

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Pieces of adult rat liver have been cultured in basal Eagle's medium, supplemented with glucose (2mg/ml), in $\text{O}_2 + \text{CO}_2$ (95:5) at 37°C (Campbell & Hales, 1971). A zone of cells at the gas/tissue interface appears to remain viable for at least 6 days. This system has been used to study the long-term actions of hormones *in vitro*.

Liver that had been cultured for 1h, 2 days or 6 days was incubated with $12\mu\text{M}$ -glucagon for up to 48h and the tissue cyclic AMP (adenosine 3':5'-cyclic monophosphate) content was measured. The pieces of tissue were frozen in liquid N_2 and homogenized in 0.6M-HClO_4 , and cyclic AMP was measured by radioimmunoassay, by using antibodies prepared by the method of Steiner *et al.* (1969). Tissue contents of cyclic AMP reached a maximum within 15min and decreased to approx. 16% of the maximum within 2h, reaching basal values by 6h. Small amounts of cyclic AMP could be detected in the medium. Changes in the concentration of cyclic AMP in the medium approximately paralleled those in the pieces. It was not possible to account for the loss of tissue cyclic AMP by its release into the medium.

In further experiments concentrations of cyclic AMP were measured after 15min incubation with glucagon. Maximal stimulation with glucagon alone was obtained with a concentration of $12\mu\text{M}$. Concentrations of cyclic AMP were increased further if 1mM-theophylline was also added. Although the

maximum concentration reached varied in different experiments, there was a clear tendency for it to decrease over the 6 days in culture. After 2 days in culture the maximal stimulation was approx. 50% of that at 1 h and had decreased to 20% by 6 days.

A concentration of approx. $0.05 \mu\text{M}$ -glucagon caused a half-maximal stimulation in liver cultured for 2 and 6 days, whereas a concentration of $0.6 \mu\text{M}$ was required to cause half-maximal stimulation in liver cultured for 1 h. The different sensitivities of liver cultured for 1 h, 2 days and 6 days and of the perfused liver (Exton *et al.*, 1971) or isolated liver membranes (Pohl *et al.*, 1971) may be due to slow diffusion or destruction of the hormone in this culture system.

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- Campbell, A. K. & Hales, C. N. (1971) *Exp. Cell Res.* **68**, 33–42
 Exton, J. H., Robison, G. A., Sutherland, E. W. & Park, C. R. (1971) *J. Biol. Chem.* **246**, 6166
 Pohl, S. L., Birnbaumer, L. & Rodbell, M. (1971) *J. Biol. Chem.* **246**, 1849
 Steiner, A. L., Kipnis, D. M., Utiger, A. & Parker, C. W. (1969) *Proc. Nat. Acad. Sci. U.S.A.* **64**, 367

Permeability of Human Buccal Mucosa to an Amino Acid Analogue, the Antibiotic D-Cycloserine

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Absorption of organic amines and organic acids from the mouth has been reported. Such permeability was maximal at the pH value corresponding to the un-ionized form of the compound. Absorption was also proportional to the partition coefficient of the compound between a lipophilic solvent and water (reviewed by Beckett & Hossie, 1971).

D-Cycloserine (D-4-amino-3-isoxazolidone) is an antibiotic inhibiting the incorporation of D-alanine into bacterial cell walls (Strominger *et al.*, 1960). It has an amino group and a phenolic hydroxyl group giving an isoelectric pH (pI) of 5.9. Absorption from the mouth was measured as with other drugs (Beckett & Hossie, 1971), and D-cycloserine was determined colorimetrically (Jones, 1956).

Loss from the mouth was proportional to initial concentration at pH 5.9 over the range 1–5 mM with a 5 min test period. The linear transport kinetics excluded metabolism of the D-cycloserine by buccal mucosa accounting for its loss from the buccal cavity. This result suggested passive diffusion or, conversely, a carrier-mediated process with a very high affinity constant.

D-Cycloserine transfer at a concentration of 1 or 2 mM was not inhibited by equimolar L-serine, D-serine, L-alanine, D-alanine, β -alanine or L-methionine. These results are compatible with passive diffusion of the un-ionized molecule. This type of transport was also found for D-cycloserine with rat small intestine *in vitro* (Wass & Evered, 1971). Further, buccal transfer was not inhibited by the presence of a metabolic inhibitor, Amytal, at an equimolar concentration (2 mM).

Permeability of the human buccal mucosa to D-cycloserine was also shown by L-proline and L-cycloserine. The lack of stereospecificity is also compatible with passive diffusion.

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- Beckett, A. H. & Hossie, R. D. (1971) *Handb. Exp. Pharmacol.* **28**, 25–46
 Jones, L. R. (1956) *Anal. Chem.* **28**, 39–41
 Strominger, J. L., Ito, E. & Threnn, R. H. (1960) *J. Amer. Chem. Soc.* **82**, 998–999
 Wass, M. & Evered, D. F. (1971) *Life Sci.* **10**, 1005–1013

Micro Methods of Osmometry Based on the Equilibrium Swelling of Gels

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Edmond *et al.* (1968) and Ogston & Wells (1970) showed that the changes of diameter of a single bead of Sephadex can be used to measure colloid osmotic pressure in solutions of solutes that do not penetrate the gel. Because of its low sensitivity (about 1500 dyn/cm²), this method is not suitable for measuring low osmotic pressures, e.g. for measuring molecular weights of proteins. Also, Sephadex G-50 is too permeable to proteins of molecular weight less than 100000.

Several-fold increase of sensitivity, accompanied by decreased permeability to solute, can be achieved by use of a thin (0.5 mm or less) strip of polyacrylamide gel, of length greater than the diameter of a Sephadex bead, and of concentration 18% (w/v) or higher.

A far greater increase of sensitivity is achieved by use of a thin layer of polyacrylamide gel cast on to a narrow strip of cellulose tissue, about 15 mm long. This curls into an almost closed loop, whose ends separate in proportion to the osmotic pressure of a solution of non-penetrating solute. Sensitivity of 20 dyn/cm² can be achieved, with impermeability to relatively small molecules. For example, the molecular weight of cytochrome c (12384) measured in