for the active transport of Ca^{2+} across the membrane. The noradrenaline stimulation of both Ca^{2+} uptake and adenylate cyclase activity in preparations of cardiac sarcoplasmic reticulum suggests an energy relation between Ca^{2+} transported and cyclic AMP synthesized.

This work was supported by a grant to I. M. G. from the British Heart Foundation.

- Butcher, R. W. & Sutherland, E. W. (1962). *J. biol. Chem.* **237**, 1244.
- Gertz, E. W., Hess, M. L., Lain, R. F. & Briggs, F. N. (1967). *Circulat. Res.* **20**, 477.
- Gillibrand, I. M. (1970). *Biochem. J.* **120**, 20P.
- Krishna, G., Weiss, B. & Brodie, B. B. (1968). *J. Pharmac. exp. Ther.* **163**, 379.
- Naylor, W. (1967). *Am. Heart J.* **73**, 379.
- Porter, K. R. & Franzini-Armstrong, C. (1965). *Sci. Am.* **212**, 72.
- Rasmussen, H. & Tenenhouse, A. (1968). *Proc. natn. Acad. Sci. U.S.A.* **59**, 1364.
- Robison, G. A., Butcher, R. W. & Sutherland, E. W. (1970). In *Fundamental Concepts in Drug-Receptor Interactions*, pp. 59-91. Ed. by Danielli, J. F., Moran, J. F. & Trigg, D. J. New York and London: Academic Press.
- Van der Kloot, W. (1969). *Science, N.Y.*, **164**, 1294.

Structural and Functional Changes of Isolated Rat Diaphragm after Treatment with Caffeine together with Local Anaesthetics and β -Receptor Blockers

By GILLIAN BULLOCK. (CIBA Laboratories, Horsham, Sussex RH12 4AB, U.K.) and K. L. MANCHESTER.* (Department of Biochemistry, University College, London, London W.C.1, U.K.)

Caffeine (5mM) enhances the endogenous respiration rate of isolated rat diaphragm and inhibits the incorporation rate of amino acids into protein. This inhibition is not prevented by the local anaesthetics butacaine, cinchocaine and amethocaine (1mM), although these substances decrease the caffeine-induced enhancement of respiration. Local anaesthetics on their own do not lower the respiration rate, but enhance it considerably (Manchester, 1970). The β -receptor blockers D- and L-propranolol, alprenolol, oxprenolol and to a smaller extent practolol exhibit effects similar to those of the local anaesthetics.

PTAH staining demonstrated various degrees of fibre disruption and Z-band alterations with some of the compounds. When the tissue was examined under the electron microscope, it could be seen that the mitochondria showed specific responses to the different drugs used. After incubation of the tissue in caffeine most of the mitochondria were character-

ized by a non-condensed matrix and an increased number of angulations per unit cristal length, the cristae often running in a circular manner. After treatment with the local anaesthetics alone the matrices became very condensed with either very straight cristae giving a rope-like appearance or else the cristae were markedly angulated and frequently in phase with the next crista. Incubation with the two together gave a structure indistinguishable from the control, which could be related to the effect on caffeine-enhanced respiration. Three of the β -receptor blockers (L- and D-propranolol and alprenolol) gave a similar change in structure compared with that of the local anaesthetics; however, instead of reversing the caffeine effect, the tissue was further disrupted. This suggests that the apparent reversal of the caffeine-enhanced respiration by these drugs was caused by a deterioration of the tissue, resulting in decreased Q_{O_2} . Oxprenol and practolol alone produced little or no change, and oxprenolol appeared to modify the caffeine effect.

Where fibres were badly disrupted, some mitochondria showed a non-specific drug response. This was characterized by an electron-dense intracristal material in these structures, formerly only seen in isolated organelles (Vogell, 1962; Bullock, Carter & White, 1970). These were surrounded by a cytoplasm lacking its normal density, and their matrices were also clear. Valinomycin ($1\mu\text{M}$) was able to emulate the non-specific drug effect and at $0.1\mu\text{M}$ produced the local-anaesthetic-like response.

Bullock, G., Carter, E. E. & White, A. M. (1970). *FEBS Lett.* **8**, 109.

Manchester, K. L. (1970). *Biochem. J.* **117**, 39P.

Vogell, W. (1962). *Wiss. Konferenz-ges. deutscher Naturforscher und Ärzte, Rottach-Egern*, p. 56. Berlin: Springer-Verlag.

A Study of the Relationship between Glucocorticoid-Induced Weight Loss in Rats and the Activity of Skeletal-Muscle and Cardiac-Muscle Ribosomes *in vitro*

By P. ELLIOTT, R. F. PETERS and A. M. WHITE. (CIBA Laboratories, Horsham, Sussex RH12 4AB, U.K.)

Five glucocorticoids, when administered daily to rats for 5-7 days at a dosage of 5mg/kg body wt., were shown to lie in the following order of catabolic potency: corticosterone < prednisolone < triamcinolone < betamethasone < dexamethasone < triamcinolone acetate. Catabolism was measured on the basis of daily body-weight change and the change in weight of a group of white muscles (vastus medialis, vastus lateralis and gluteus medius).

* Present address: Department of Biochemistry, University of West Indies, Mona, Kingston 7, Jamaica.

When ribosomes were isolated from the same muscles 6h after intraperitoneal injection of the steroids it was shown that the minimum effective doses capable of causing a significant ($P < 0.05$) depression in their amino acid-incorporating ability *in vitro* were: corticosterone, 20mg/kg body wt.; prednisolone, 20mg/kg body wt.; triamcinolone, 0.05mg/kg body wt.; betamethasone, 1mg/kg body wt.; dexamethasone, 0.05mg/kg body wt.; triamcinolone acetonide, 0.05mg/kg body wt. Although it is true that the low catabolic potencies of corticosterone and prednisolone were reflected in the high doses required to affect ribosomal activity *in vitro*, the other steroids, on this criterion, were in the wrong order, since betamethasone would be expected to be less catabolic than triamcinolone, which itself would be expected to have a catabolic potential similar to that of its acetonide.

A much more satisfactory correlation between catabolic potential and diminished ribosomal activity was observed on the basis of duration of steroid action. For example, 12h after a single dose (20mg/kg body wt.) of corticosterone muscle ribosomes had regained normal activity, whereas those from animals receiving a similar dose of prednisolone still retained diminished activity. Likewise it was possible to separate, as pairs, triamcinolone and betamethasone from dexamethasone and triamcinolone acetonide. Triamcinolone, the least catabolic of these four, ceased to be effective 18h after injection, whereas the acetonide was still effective 48h after administration.

Cardiac-muscle ribosomes were not affected as a result of steroid treatment, in agreement with the absence of any effect on heart weights.

These results serve to emphasize again the close correlation between changes in ribosomal activity and cellular determinants of catabolic decline in skeletal muscle. Nevertheless the mechanism of the decrease in incorporating ability of isolated ribosomes, which will be discussed, still remains obscure.

The Heterogeneity of Muscle Mitochondria Demonstrated by Discontinuous Density-Gradient Centrifugation

By GILLIAN BULLOCK, ELIZABETH E. CARTER and A. M. WHITE. (CIBA Laboratories, Horsham, Sussex RH12 4AB, U.K.)

Muscle mitochondria in the peripheral region of white fibres enlarge rapidly after the administration of triamcinolone acetonide to rats (Bullock, Christian, Peters & White, 1971). For this reason a method was sought that would separate normal organelles on the basis of size, at the same time preserving functional and morphological integrity.

Mitochondria were released from muscle without a homogenizer by using the method of tryptic lysis (Bullock, Carter & White, 1970) and centrifuged through a discontinuous sucrose density gradient at 105000g for 45min. Broken organelles (band 1) did not penetrate into the gradient, but the remaining mitochondria were collected into three bands at the interfaces of the following sucrose molarities: 1.07M/1.12M, band 2; 1.12M/1.17M, band 3; and 1.17M/1.22M, band 4. Mitochondria in band 2 were smaller than those in bands 3 and 4 and more homogeneous.

For testing with the oxygen monitor, mitochondria from different bands were diluted with medium in the absence of glutamate and pelleted at 11000g for 10min. Oxygen uptake was then measured in the presence of glutamate (10mM) with and without ADP to give the respiratory control ratios. These were 16.0, 10.5 and 3.3 for bands 2, 3 and 4 respectively.

For electron-microscopical examination the mitochondria were diluted and pelleted through medium containing glutamate (10mM) and incubated for 5min at 39°C in the presence of glutamate (10mM) and in the presence or absence of excess of ADP (1mM). They were then re-isolated by centrifugation and the pellet was fixed simultaneously with glutaraldehyde and osmium tetroxide by the method of Hirsch & Fedorko (1970).

Mitochondria in State 4 from all bands assumed a characteristic appearance with parallel cristae and fairly dense matrix, although those in band 4 appeared more swollen. In State 3 there was a shift of a large number of mitochondria to an open-mesh structure, presumably indicative of an energized conformation.

These results demonstrate a heterogeneity according to size among isolated muscle mitochondria and also a morphological change as a response to change in function. The latter could not be demonstrated by Kuner & Beyer (1970), possibly because of a harsher method of tissue homogenization.

Bullock, G. R., Carter, E. E. & White, A. M. (1970). *FEBS Lett.* **8**, 109.

Bullock, G. R., Christian, R. A., Peters, R. F. & White, A. M. (1971). *Biochem. Pharmacol.* **20**, 943.

Hirsch, J. G. & Fedorko, M. E. (1968). *J. Cell Biol.* **36**, 615.

Kuner, J. M. & Beyer, R. E. (1970). *J. Membrane Biol.* **2**, 71.