N-Hydroxylierung von 2-Aminofluoren durch Lebermikrosomen

Aromatische Amine werden durch Lebermikrosomen von Ratten bei Anwesenheit von TPNH und Sauerstoff am Stickstoff oxydiert[1]. Hierbei entsteht nach unseren Versuchen primär das betreffende Hydroxylaminderivat[2]. CRAMER, MILLER und MILLER[3] konnten im Urin von Ratten, die längere Zeit mit 2-Acetylaminofluoren gefüttert wurden, N-Hydroxy-2-acetylaminofluoren nachweisen. Schon vorher hatte KIESE nach Anilingaben im Blut von Hunden Nitrosobenzol gefunden, wenn das Blut unter Zusatz eines Oxydationsmittels extrahiert wurde[4].

Wir haben untersucht, ob 2-Aminofluoren auch durch Lebermikrosomen am Stickstoff hydroxyliert werden kann. 2-Aminofluoren bildet nämlich im Organismus Methämoglobin. In Ansätzen aus Mikrosomen und TPNH, die verschiedene Amine oxydierten[5], konnte aber nicht sicher N-Hydroxy-2-aminofluoren nachgewiesen werden. Da die Oxydationsgeschwindigkeit ziemlich linear mit der Konzentration an TPNH zunimmt[2], erhöhten wir die TPNH-Konzentration auf 2 μM/ml Ansatz und konnten nun das N-Oxydationsprodukt auffinden und bestimmen.

Tetrachlorkohlenstoffextrakte solcher Ansätze nach Zugabe von Fe^{+++} zeigten dasselbe Spektrum wie synthetisch gewonnenes 2-Nitrosofluoren[6].

Da nach Untersuchungen von HULTIN[7] ^{14}C-markiertes 2-Aminofluoren in Ansätzen von Lebermikrosomen und TPNH wesentlich schneller an Proteine gebunden wird als Anilin, dürfte die wirkliche Hydroxylierungsgeschwindigkeit höher sein, zumal Aminofluoren in den Ansätzen nicht gelöst, sondern als Suspension vorliegt. Das hydroxylierende System in Mikrosomen führt neben der N-Hydroxylierung gleichzeitig Hydroxylgruppen an verschiedenen Positionen von 2-Aminofluoren ein. Solche Phenolgruppen sind aber längst nicht so reaktionsfähig wie die Hydroxylaminverbindung.

Summary. Enzymatic N-hydroxylation of 2-aminofluorene has been achieved with rat liver microsomes in the presence of TPNH and O_2.

H. UEHLEKE

Pharmakologisches Institut der Universität Tübingen (Deutschland), 31. Juli 1961.

[1] H. UEHLEKE, Arch. exp. Path. Pharmak. *241*, 150 (1961).
[2] H. UEHLEKE, V. Int. Congr. Biochem, Moskau 1961, Abstr. p. 399 (Pergamon Press, London 1961).
[3] J. W. CRAMER, J. A. MILLER und E. C. MILLER, J. biol. Chem. *235*, 885 (1960).
[4] M. KIESE, Arch. exp. Path. Pharmak. *235*, 354 (1959).
[5] M. KIESE und H. UEHLEKE, Arch. exp. Path. Pharmak., im Druck.
[6] R. R. HOLMES und R. P. BAYER, J. Amer. chem. Soc. *82*, 3354 (1960).
[7] T. HULTIN, Exp. Cell Res. *18*, 112 (1959).

The Role of Calcium and Zinc in the Electrical and Mechanical Responses of Frog Sartorius Muscle

Much interest has been focussed upon calcium as a link in the excitation-contraction process[1–8]. It is, however, uncertain what aspects of skeletal muscle function are critically dependent upon the presence of calcium, i.e., whether calcium is essential to preserve the excitability of the membrane or whether some later stage in the excitation-contraction process is affected. The present study was aimed at elucidating the relationships between membrane potential, action potential and mechanical responses in single fibres and whole muscle in the absence of calcium. It was also found of interest to employ the same techniques to compare the effects of physiological concentrations of zinc with those of calcium.

Methods. Sartorius muscle of *R. temporaria* was used at a temperature of 0.5–2°C. The isometric contractions of single surface fibres within the intact muscle were recorded by means of an RCA transducer, having a sensitivity of approximately 1 mg. The single fibres were stimulated by passing current intracellularly through a microelectrode, which was also used to measure resting potentials. The action potential was also recorded intracellularly in response to external stimulation with small bipolar platinum electrodes. Phosphate buffered Ringer's solution, containing 1.8 mM Ca was used. All glassware used, after ordinary washing, was treated with 5 M HCl, immediately followed by de-ionised water.

Results. Effects of Ca-lack. After removal of calcium from the bathing solution, the twitch and tetanus responses of the whole muscle declined gradually and were abolished in 2–3 h. EDTA (0.1 mM) was always added to the 'Ca-free' solution but it was found that the loss of responses followed a similar time course in 0.01 mM Ca solutions without EDTA. Mean resting potentials of surface fibres were approximately 50 mV (range 25–60 mV) when mechanical responses had disappeared. Membrane potentials declined steadily by approximately 15 mV/h. Thus the loss of mechanical response could be accounted for by a progressive loss of excitable fibres due to the decrease of the resting membrane potentials. In order to test whether this is the sole mechanism, mechanical and electrical responses of single surface fibres were recorded before and after removal of calcium from the bath.

Twitch responses from a large number of ventral surface fibres followed the same time course closely and the variation of peak tensions (5–120 mg) was consistent with the range of fibre cross sections. A mean peak tension of 34 mg per fibre was found in Ca-Ringer's solution. After $^1/_2$ h in Ca-free solution, some fibres failed to give propagated responses. Within the period $^1/_2$–$1^1/_2$ h after removal of calcium, 10% of the tested fibres were inexcitable and the mean peak tension of excitable fibres fell to 30 mg. Within the period $1^1/_2$–$2^1/_2$ hours, 55% of the tested fibres were inexcitable and the peak tension of the responding fibres fell to 14 mg. The time course of the twitches was not significantly altered. When calcium (1.8 mM) was reintroduced to the bath, substantial recovery of twitches occurred. After 2 h, the mean peak tension had recovered to 24 mg per fibre. The single fibres failed to respond to stimulation when their resting potentials were lower than 60 mV. This critical level has been found in Ca-Ringer's solution when fibres were depolarised with potassium[9]. It is concluded that even before individual fibres have

[1] L. V. HEILBRUN, *An Outline of General Physiology*, 3rd. Ed. (Saunders, Philadelphia 1952).
[2] A. SANDOW, Yale J. biol. Med. *25*, 176 (1952).
[3] R. NIEDERGERKE, J. Physiol. *134*, 584 (1956).
[4] A. M. SHANES, Pharmacol. Rev. *10*, 165 (1958).
[5] K. A. P. EDMAN and H. O. SCHILD, Nature *190*, 350 (1961).
[6] P. A. ROBERTSON, Nature *186*, 316 (1960).
[7] G. B. FRANK, J. Physiol. *151*, 518 (1960).
[8] R. P. DURBIN and D. H. JENKINSON, J. Physiol. *157*, 90 (1961).
[9] D. W. GRIEVE, Ph. D. Thesis: London University (1961).

become inexcitable, the strength of their mechanical responses is diminished in the absence of calcium.

It is of further interest to establish whether the diminished response of a single fibre is related to electrical membrane changes. Twitch tensions, resting and action potentials were therefore measured from surface fibres of the same muscle. In Ca-free solution, action potentials steadily decreased in amplitude; the smallest recorded propagated impulses had an amplitude of approximately 30 mV. In contrast with this situation, when membrane potentials are lowered by raising the external potassium concentration (constant $[K]_0 \times [Cl]_0$ product) in the presence of calcium, the mean peak tension of excitable fibres is unaltered and the action potential continues to exhibit an overshoot. Thus calcium lack leads to a disturbance of the mechanisms governing the action potential and the active state which is not simply the result of the decreased membrane potential.

In conclusion we may say that calcium is essential for the maintenance of the membrane potential, development of the action potential and the full development of tension in the single fibre. The alteration of the action potential is closely associated with, and may be the cause of, the decrease in intensity of the active state. The decrease of mechanical response of the whole muscle in calcium free solutions is due in part to inexcitability of individual fibres and partly to a reduction in the intensity of response of excitable fibres.

Effects of zinc. The zinc content of skeletal muscle (0.8 mM/kg wet weight[10]) is similar to that of calcium, although extracellular concentrations (0.1 mM[11]) are less, and free zinc concentrations are very low. In earlier studies[12], the action of zinc as a relaxing factor in the isolated contractile system was investigated. In the present investigation another effect of zinc, an increase in the duration of the active state, has been found when zinc is added to the solution bathing an intact muscle, in confirmation of SANDOW and ISAACSON[13,14]. A distinct increase in the height and duration of twitches of the whole muscle and single surface fibres could be detected at a threshold concentration of $5 \cdot 10^{-6} M$. The tetanic tension was unaffected. Membrane potentials of surface fibres were unaffected over a period of 2 h at concentrations of zinc up to $5 \cdot 10^{-5} M$. There was an increase in the duration of the action potential simultaneously with the increase of duration of the active state. The falling phase was prolonged and the area of the action potential was increased twofold in $5 \cdot 10^{-5} M$ zinc, the rising phase and the overshoot being little affected.

It was found that zinc cannot replace calcium in the excitation-contraction process. The loss of tetanic response of the whole muscle in Ca-free solution could not be restored by adding zinc, the only effect upon the residual mechanical activity being an increase in the duration of the active state. The low membrane potentials were not affected by zinc.

It would appear that extracellular zinc is not an essential ion for contraction over a period of hours, unlike calcium, but may nevertheless be of physiological significance by modifying the duration of the active state.

Zusammenfassung. Membranpotential, Aktionspotential und Spannung der Einzelzuckung einer Einzelfaser des M. sartorius von *Rana temporaria* nehmen in der calciumfreien Badeflüssigkeit progressiv ab. Extrazelluläres Zink hingegen ist für die Kontraktion nicht notwendig, beeinflusst aber in physiologischen Konzentrationen die Dauer der Aktivität.

K. A. P. EDMAN[15] and D. W. GRIEVE[16]

Department of Physiology, University College, London (England), August 9, 1961.

[10] K. A. P. EDMAN, Acta physiol. scand. *46*, 209 (1959).
[11] B. L. VALLEE and M. D. ALTSCHULE, Physiol. Rev. *29*, 370 (1949).
[12] K. A. P. EDMAN, Acta physiol. scand. *49*, 330 (1960).
[13] A. SANDOW and A. ISAACSON, Biochem. biophys. Res. Comm. *2*, 455 (1960).
[14] A. ISAACSON and A. SANDOW, Fed. Proc. *20*, 301 (1961).
[15] Present address: Pharmacology Department University of Uppsala (Sweden).
[16] Present address: Human Biomechanics Laboratory, Medical Research Council, Hampstead, London (England).

Activity of some Enzymes in Experimental Hypertension in the Rabbit[1,2]

In 1945, PICKERING[3], demonstrated two phases of renal hypertension in rabbits by clamping one renal artery after removing the contralateral kidney. After 7 to 8 days, the clamp was removed and normal blood pressure restored, whereas hypertension still persisted if the clamp was removed after 7 to 8 weeks. This work has been repeated and confirmed by others[4,5].

It has been postulated that in the early phase, the kidney produces a renal pressor substance, probably renin, which is responsible for the maintenance of the hypertension. There seems to be agreement that in rabbits, dogs and sometimes rats, the early phase is different from the chronic phase of experimental hypertension[6]. The interpretation of the late phase of hypertension has led to many hypotheses, all of which suggest the possibility of an extra renal regulatory pressor mechanism[6]. However, there is evidence that the kidneys participate even in the pathogenesis of chronic hypertension.

The present work was designed to test the importance of the renal pressor mechanism in the various stages of hypertension in the rabbit, by observing the ability of renal tissue to produce renin and to produce and/or metabolize other pressor substances. In hypertensive rabbits, we have followed renin levels and the response to rabbit antirenin during the acute and chronic phase of hypertension. Other kidney enzymes, which might be involved in the formation and metabolism of pressor substances, have been studied, i.e., renal dopadecarboxylase (DDC) which forms dopamine from dopa and monoamine-oxidase (MAO) which forms the corresponding aldehydes by oxidation.

Material and Methods. Rabbits of both albino and black strains were used. Under nembutal anesthesia, right nephrectomy was performed through a loin incision. Two weeks later, the contralateral left renal artery was clamped with a silver clamp 10–14 mm long and 0.4–0.8 mm lumen

[1] This investigation was aided in part by grants from the Office of the Surgeon General, United States Army (no. DA-49-007-MD-429), and from the National Health Institute, United States Public Health Service (no. H-444 (C 10)).
[2] A preliminary report appears in Fed. Proc. *19*, 100 (1960).
[3] G. W. PICKERING, Cl. Sci. *5*, 229 (1945).
[4] H. A. FLEMING, J. Path. Bact. *65*, 441 (1953).
[5] P. M. DANIEL, M. M. L. PRICHARD, and J. N. WARD-MCQUAID, Cl. Sci. *13*, 247 (1954).
[6] M. A. FLOYER, Brit. Med. Bull. *13*, 29 (1957).