creates a long-lasting depolarization[2] resulting from a long-lasting change in permeability of the junctional membrane. If this prolonged permeability change is the cause of the necrosis, it should be possible to prevent this by protecting the junctional membrane against the ACh. In 5 DFP-poisoned rats necrosis of the indirectly stimulated gastrocnemius and soleus muscles could indeed be prevented by D-tubocurarine given in a dose of 8 mg/kg, i.p., every 2 h, during the 6 h of stimulation. However, since the muscles in these experiments were inactivated by the curare block, the inactivity of the muscle, as such, might have prevented the necrosis. This was shown not to be the case: if the curare regime was reduced to 0.75 mg/kg each hour, the muscles were still completely paralysed but marked necrosis occurred in the stimulated muscles in 3 out of 4 animals. In these rats the junctional membrane was still too insensitive to ACh for transmission to occur but obviously sensitive enough to allow necrosis to develop.

The results of the experiments show that necrosis occurs in the region of the motor end-plates in fibres of active striated muscles in rats poisoned with cholinesterase inhibitors, probably as a consequence of the presence of abnormal amounts of ACh.

Résumé. Après l'injection d'un toxique anticholinestérasique à dose presque léthale, une nécrose focale des fibres musculaires striées du rat se développe en 24 h. 10 jours après l'injection, la régénération est presque totale. Les parties nécrotiques se trouvent dans les régions des plaques motrices. Les résultats obtenus suggèrent que l'action dépolarisante de l'acétylcholine au niveau des plaques motrices des muscles actifs, augmentée et prolongée par l'intoxication, est responsable de la nécrose.

A. Th. Ariëns, E. Meeter,
O. L. Wolthuis and R. M. J. van Benthem

Roman Catholic Hospital 'De Goddelijke Voorzienigheid', Sittard and Medical Biological Laboratory of the National Defence Research Organization TNO, Rijswijk Z.H. (Netherlands), 5 August 1968.

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Ineffectiveness of Carboxypeptidase B in Inhibiting the Pressor Effect of Incubated Human Plasma

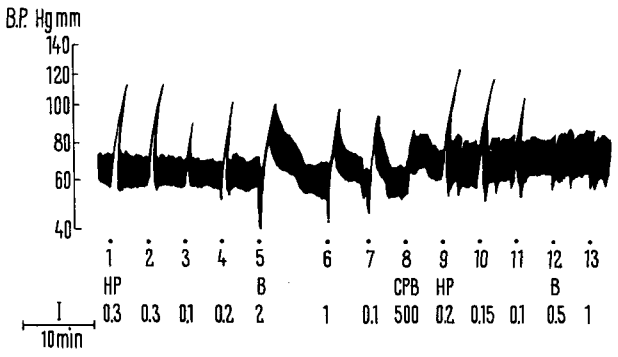
The i.v. injection of 0.1 ml human plasma incubated for several hours at 38 °C into a hypotensive rat produces an increase in the blood pressure equivalent to 5–10 ng of angiotensin II[1,2]. Contrarywise a hypotensive reaction is observed when the same plasma is injected into a cat. These pharmacological features are common to bradykinin[3] and kallidin[4] and suggest that these polypeptides could participate in the action of the incubated plasma.

The demonstration[5] that the carboxypeptidase B in vivo can block the effect of kinins and kinogenases (kallikreins), gives the possibility of investigating whether a liberation of a plasmakinin is involved in the pressor effect of the incubated plasma.

Materials and methods. Nephrectomized rats, operated upon 12–16 h before and anaesthetized with an i.p. injection of dialylbarbituric acid urethane solution (Dial, CIBA) in a dose of 0.1 ml/100 g body weight were used. The blood pressure was measured by introducing a cannula into a carotid artery connected to a membrane manometer (Hürtle type). Heparin was used as anticoagulant.

The incubated plasma and the other substances were injected into the femoral vein through a polyethylene tube. Valyl-5-amide angiotensin II (CIBA) and bradykinin (Sandoz) dissolved in 0.1 ml of NaCl 0.9% were used as standards. Plasma was obtained from healthy adult persons. Enough heparin to get a final concentration of 0.5 or 2 IU/ml, when mixed with the blood, was introduced in sterilized, siliconized bottle, before bleeding. After centrifugation the plasma was transferred to another sterilized siliconized bottle by aspiration, and placed in an incubator at 38 °C, for 80–100 h.

The plasma at the end of the incubation period was submitted to a gel filtration (Sephadex G-100) using the technique already described[6]. Most of the pressor activity of the plasma coincides with the elution volume of the albumin. The solutions having the highest activity were pooled and freeze-dried. The residue containing the pressor substance (VA) after dialysis against 0.9% NaCl was ready for the experiments. Usually 0.1 ml of this solution containing 10 mg of protein/ml was used. Car-



Blood pressure (B.P.) changes in a nephrectomized rat produced by i.v. injections: (1, 2, 3 and 4) of 0.3, 0.3, 0.1 and 0.2 ml, respectively, of human plasma (HP) incubated for 96 h at 38 °C; (5, 6 and 7) of 2, 1 and 0.1 mcg of bradykinin; (8) of 500 U (1 mg) carboxypeptidase B infused in 0.5 ml NaCl 0.9%; (9, 10 and 11) of 0.2, 0.15 and 0.1 ml of incubated human plasma; (12 and 13) of 0.5 and 1 mcg bradykinin.

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boxypeptidase B with isopropylfluorphosphate (Worthington Laboratories, Delaware, USA) was employed.

Result and discussion. The i.v. infusion of 300 units of carboxypeptidase B given to a nephrectomized rat of 250 g produced a complete block of the effect of 1–2 mcg of bradykinin given i.v. This inhibition lasted 60–120 min. The response to bradykinin was restored only very slowly. However, no inhibition whatsoever was observed in the pressor action of incubated plasma or its active fraction (VA). 0.1 ml of the incubated plasma or 2 mg of VA produced before the administration of carboxypeptidase B a pressor effect equivalent to 10 ± 0.12 ng of angiotensin II. An equivalent effect either with VA or incubated plasma was recorded after the enzyme was given, whereas the bradykinin effect was completely blocked (Figure).

These results give support to the concept that the pressor action of incubated plasma is not due to the release of a plasmakinin (bradykinin or kallidin). Either the incubated plasma or its active fraction (albumin) do not provide the substrate (kinogens) or the enzyme (kallikrein) needed to liberate the natural plasmakinins. Since the experiments were performed in nephrectomized rats (16 h) renin can be excluded as an enzyme involved in any step of the pressor mechanism elicited by the incubated plasma.

Résumé. L'action vasopressive chez le rat néphrectomisé, produite par l'injection du plasma humain lors de l'incubation à 38 °C pendant plusieurs heures n'est pas due à la libération de plasmakinines. La carboxypeptidase B in vivo à des doses que produisent l'abolition des effets de la bradykinine et d'autres plasmakinines, ne modifient pas l'action vasopressive soit du plasma incubé soit de sa fraction active.

H. CROXATTO and H. CRUZATT

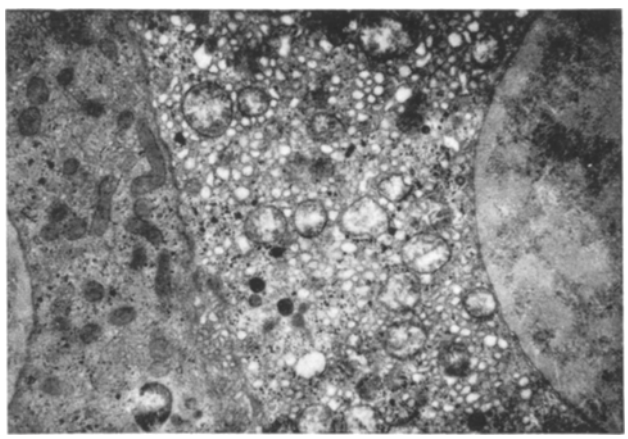
Department of Physiology, Catholic University, Santiago (Chile), 25 July 1968.

Electron Microscopic Studies on Hepatic Cells in Mice After the Administration of Glutathione Mixtures

The present studies were undertaken to determine the changes in hepatic cells in mice after the injection of oxidized and reduced glutathione mixtures. The dd/YF female 90-day-old mice were injected i.p. with 0.5 ml of physiological solution containing 25 mg of synthetic reduced glutathione which had 99.1 % purity and had no possibility of metals being added in the processes of synthesis (Yamanouchi) or the mixture of 6.15 mg of synthetic reduced glutathione and 61.5 mg of oxidized glutathione (Sigma), and with 0.5 ml of physiological solution. Mice were sacrificed under a non-anaesthetic condition 60 min after the injection and a piece of liver tissue was removed to be fixed in 2% of osmium tetra oxide for 2 h, dehydrated in graded alcohol solutions and embedded in Epon. Hitachi HU-11A, HS-7 and Nihon Denshi JEM-7A electron microscopes were used.

No abnormal picture was found in hepatic cells in mice after the injection with physiological solution, the solution of synthetic reduced glutathione, or oxidized glutathione. In hepatic cells in mice after the administration of glutathione mixture, remarkable swellings of mitochondria were observed, including irregular cristae, many vacuoles and many vesicles which appear to result from changes in the smooth endoplasmic reticulum. The parts of some mitochondria were unclear. Some of the vacuoles appeared to be the result of dissolving lipoid granules. The endoplasmic reticulum, especially rough endoplasmic reticulum, could hardly be found in the altered cells. The nucleus, the nuclear membrane, the intercellular space and the cellular membrane were not affected morphologically. Among altered cells, many unaffected or slightly affected cells were found.

The swelling and lysis of mitochondria from hepatic cells in rats are induced in vitro by the addition of the glutathione mixture to the suspending medium through the process of peroxidation of lipid[1–3]. Such a change is not found in the case of administration of reduced glutathione[4] and is modified with additions of very small amounts of various substances to the suspending medium[2,5–8]. The changes in hepatic cells in the present



Remarkable swelling of mitochondria including irregular cristae, the unclear membranes of mitochondria, many vacuoles and vesicles were found in hepatic cells in mice after the administration of glutathione mixture, but the nuclear membrane and the cellular membrane of the altered cell, the intercellular space and the neighbouring cell are not affected. × 8400.

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