Dependence of the Contractility of Coronary Muscle on Substances Favouring Contraction and Relaxation

There is as yet no clear understanding of the mechanism regulating the coronary circulation or of the influence of catecholamines on coronary muscle cells. After we had found two different vasoactive substances, possibly polypeptides, in the cells of the vascular muscle of the cow, which affect either the excitability or the contractility of this muscle – the first substance stimulates contraction (shortening of the latent period after electrical stimulation, increased muscular tension and increased activation of tonoactomyosin ATPase), the second substance depresses contraction[1-3] – it was interesting to find out if and how these substances might control the coronary circulation.

Material and methods. The experiments were carried out on the ramus circumflex of the left coronary artery from cow and horse. Ringshaped vascular segments of 15–18 mm length were set up on our tension measuring apparatus, and as is usual, kept for 45 min in oxygen saturated tyrode solution at 37 °C in order to obtain complete relaxation[4]. Electrical stimulation was applied with a tension gradient of 1 V/mm and a frequency of 20/sec.

Results and discussion. After electrical stimulation there were striking differences between the mechanograms obtained from different segments of the same cow coronary artery. In most cases we obtained mechanograms of the type shown in Figure 1a. After a latent period of about 2 sec, there was an increase in tension respectively a shortening of the specimen. Contrary to the behaviour of other vessels, relaxation set in immediately after the stimulus had ceased and relaxation did not stop when the specimen had regained its initial length, but continued further so that the degree of relaxation might be 2 or 3 times greater than the initial shortening. In other cases (Figure 1b), there was no shortening during stimulation, but a minimal relaxation occurred which increased when the stimulus was stopped.

The coronary vessels of the horse reacted completely differently after electrical stimulation. They showed a steep increase in tension after a relatively long latent period of 5–10 sec. When the maximum level was reached, the tension remained constant for a longer time. Vessels from other animals showed a more gradual increase in tension and, when stimulation ceased, there was a slow decrease in tension until the original level was reached. There were no variations in response in different segments from the same animal.

Adrenalin in a concentration of 0.25–5 γ/ml produced a drop in tension (dilatation) in the cow coronary. When electrical stimulation is followed by dilatation induced by 1–2 γ/ml adrenalin, and the adrenalin is allowed to act for 10 min, after which the preparation is placed in a fresh bathing solution and allowed to stand until a new tension equilibrium is reached, the mechanogram obtained after electrical stimulation will be different. The degree of contraction increases and the relaxation is incomplete. The delayed and incomplete relaxation cannot be due to a lack of ATP because the shortening is even greater after a repeated stimulus. A small increase (beginning from 3 mM/L, i.e. a quantity which can be released in the organism by hypoxia and an increased activity of the heart) in the concentration of potassium in the tyrode bath, produces, like adrenalin, a vasodilatation after a latent period of a few seconds. Such a dilatation can be observed with concentrations up to 30 mM/L potassium. If 5 mM/L potassium are added to the bathing solution after the electric stimulus and the experiment is then continued in the same way as described above after application of adrenalin, the mechanogram shows the same changes as after pretreatment with adrenalin.

Potassium is not effective on the coronary vessel of the horse below a concentration of 30 mM/L. Higher concentrations induce a contraction. As already stated by ROTHLIN[5], adrenalin (0.5–5 γ/ml) also stimulates the contraction of these vessels, in contrast to its effect on cow coronary vessels. We attribute the difference in behaviour between the coronary vessels of cow and horse to the fact that the above-mentioned vasoactive substances are present in extracts of cow coronary vessels but could not be evidenced in extracts of coronary vessels of the horse. The changes after pretreatment seen in the mechanograms of cow coronary obtained after electrical stimulation (i.e. an increase of the degree of con-

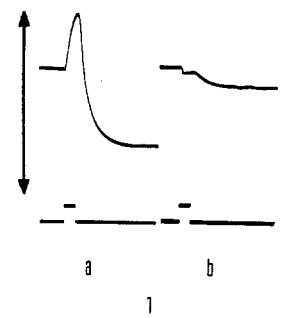

Fig. 1. Tension of cow coronary samples. Under line: signalling of beginning and duration of stimulus (15 sec); ↕, tension interval 30 g; initial tension $\simeq 440 \times 10^3$ dyne/cm^2.

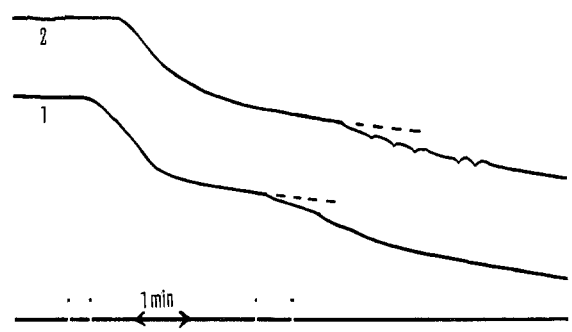

Fig. 2. Tension of cow coronary samples. At first signal given 10 mM KCl to 1, at second signal given 2 γ/ml Adrenalin to 2, at third signal given 50 γ/ml 2-[N-p-tolyl-N-m-hydroxyphenyl-aminomethyl]imidazoline (Regitine® to 1, at fourth signal given 50 γ/ml Regitine® to 2. Tension interval and initial tension are the same as in Figure 1; ↔, time interval 1 min.

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traction and the incompleteness of relaxation) we attribute to the release of such vasoactive substances at the onset of contraction. Part of these substances diffuses into the bathing solution. Therefore, changing the bathing solution results in a loss of vasoactive substances. These can be actually identified in the bathing solution by biological tests and paper chromatography. When α-blockers are added to the bath, the contraction due to electrical stimulation is decreased and relaxation favoured. The presence of β-blockers, on the other hand, gives rise to the opposite effect. Furthermore α-blockers will, as shown in Figure 2, intensify the action of potassium much in the same way as adrenalin. All kinds of stimuli, indeed also those which produce contraction such as serotonin, hypertensin, histamin, are influenced by α-blockers which cancel their action. The action of adrenalin on the coronary vessel of the horse is not influenced either by α- nor by β-blockers.

On one side, the ATPase activity of the tonoactomyosin from coronary arteries, as well as from other vessels we have investigated, can be activated by the substance stimulating contraction and this activation hindered by α-blockers. On the other hand, the ATPase activity can be hindered by the substance favouring relaxation, the action of which can be recovered by β-blockers.

On the basis of our investigations, in particular the fact that one of our vasoactive substances is inhibited by α-blockers and the other one by β-blockers, we are led to suggest that these substances might be identical with the α- and β-receptors.

Zusammenfassung. Es werden die Besonderheiten der am isolierten Koronargefäss von Rind und Pferd nach elektrischer Reizung, Verabreichung von Adrenalin oder Kalium registrierten Mechanogramme beschrieben. Es wird darauf hingewiesen, dass die Kontraktilität der Muskelzellen des Rinderkoronargefässes von zwei Stoffen abhängig ist, wovon der eine die Kontraktion und der andere die Erschlaffung fördert.

L. LASZT[6]

Institute for Cardiovascular Research, 1700 Fribourg (Switzerland), 22 January 1969.

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The Response of Frog Taste Cells (*Rana nigromaculata* and *Rana catesbeana*)

In frog as well as in mammals, it has been established that gustatory nerve fibres respond to more than 1 of 4 basic taste solutions applied to the tongue[1]. A recent investigation on the sensory innervation of the frog tongue[2] has suggested that 1 gustatory fibre innervates about 30 receptor cells which are on the average distributed among 6 fungiform papillae. In order to determine whether the multiple sensitivity of one gustatory fibre derives from the multiple sensitivity of individual receptor cells or whether it is due to convergence of receptor cells sensitive to different taste stimuli, in the present study intracellular recordings were obtained from the frog taste cells. These experiments indicated that taste cells exhibit sensitivity to the 4 basic taste qualities. These observations confirm the only previous intracellular recording by KIMURA and BEIDLER[3] on rat or hamster taste cells.

The tongue isolated from the frogs (*Rana nigromaculata* and *R. catesbeana*) was used in this study. Glass capillary microelectrodes filled with 2 *M* KCl (50–150 *MΩ*) were inserted into single taste cells of the fungiform papillae with the aid of a special jolting apparatus designed by TOMITA[4]. NaCl, acetic acid, sucrose and quinine hydrochloride were used as 4 basic taste stimuli. The last 3 substances were dissolved in 0.1 *M* NaCl to exclude the interference of the so-called 'water response'. A small amount of each taste solution was applied very gently to the tongue via a microsyringe to avoid mechanical disturbances. Following each test solution the tongue was always rinsed with Ringer solution.

At a depth of 20–30 μ from the surface of the papilla, the microelectrode tip penetrated a cell of the taste cell layer as signified by a negative resting potential of 10–35 mV. When the resting potential changed with taste stimuli applied to the surface of the tongue, such a cell was assumed to be a taste cell. No cells penetrated at other depths responded at all to taste stimuli. The potential changes to the taste solutions were slow depolarizing potentials: never spike potentials (Figure 1). When the electrode was withdrawn to a just extracellular position, the same taste stimuli elicited no detectable potential change. Therefore, the intracellular recorded slow potential was not simply a physicochemical potential occurring at the fluid interphase, but it was an electrophysiological potential across the cell membrane which will henceforth be referred to as the receptor potential. The amplitude of the receptor potential became larger as the concentration of the taste solution was increased as shown in Figure 2. The 2 curves were obtained from different NaCl-sensitive cells.

It was very difficult to obtain stable intracellular recordings from the taste cells for long periods, because the flask-shaped taste cell is very small with a diameter of about 7 μ in the thickest part. The period of the electrode penetration varied from a few seconds to 10 min. Therefore, in order to complete the sensitivity test to different solutions within a short time, 4 basic taste solutions could be examined only at constant concentrations, e.g. *M*/2 NaCl, *M*/64 acetic acid, *M*/4 sucrose and *M*/256 quinine hydrochloride. These concentrations were 5–10 times threshold for minimal discharges in the whole gustatory nerve, but were lower than the concentrations for maximal discharges.

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