ACTION OF FINOPTIN ON cAMP DYNAMICS IN THE ISOLATED GUINEA PIG HEART DURING CONTRACTION AND RELAXATION

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A key role in the regulation of cardiac contraction and relaxation is played by calcium ions and cyclic adenosine monophosphate (cAMP) (secondary mediators), by means of which the various inotropic influences on the myocardium are realized. Many investigations have been devoted to the study of calcium and cAMP metabolism [1, 5, 6], but their interaction in the working heart remains unexplained. We know that positive inotropic influences leading to an increase in the cAMP concentration caused an increase in the amplitude of oscillations of ionized calcium in the myoplasm (agonists of β-adrenoreceptors etc.) [2, 7]. Another mechanism of positive inotropic influences is an increase only in the amplitude of oscillations of ionized calcium with no change in the cAMP concentration (cardiac glycosides) [3, 12]. It was shown in [9, 11] at the molecular level that calcium-calmodulin-dependent forms of adenylate cyclase and phosphodiesterase (enzymes, the first of which synthesizes while the second hydrolyzes cAMP, are present in heart muscle. This suggests that a link may exist in the working heart between the ionized calcium level and the cAMP concentration. The aim of the present investigation was to determine the effect of Finoptin on the cAMP dynamics in the isolated heart.

EXPERIMENTAL METHOD

Experiments were carried out on the isolated heart of male and female pigs weighing 200-350 g, and perfused by Langendorff's method with Krebs–Henseleit solution at 25 ± 0.5°C. The heart contracted under isotonic conditions with a constant load of 5 g and a constant frequency of stimulation of 2 Hz. There were three series of experiments. In series I (control) isoproterenol ($3 \cdot 10^{-8}$ M) was added to the perfusion solution in the course of 2-3 min. In series II, the calcium concentration in the perfusion solution was reduced to 0.15 mM, after which isoproterenol was added in the same concentration as in the control series. In series III, 10 min before the addition of isoproterenol ($3 \cdot 10^{-8}$ M, the calcium blocker Finoptin ($3 \cdot 10^{-8}$ M) was added to the perfusion solution. In all the series of experiments, after the above procedures the heart was quickly frozen by means of a special device, described in [10], and including blocking synchronization with the phases of the cardiac cycle and special aluminum forceps, cooled in liquid nitrogen. Freezing was carried out at five times of the cardiac cycle, separated by equal time intervals of 100 msec; the first point was before the beginning of the cardiac cycle, the second corresponded in time to the maximal rate of contraction. Allowing for the thickness of the frozen heart, the time taken to lower its temperature to 0°C, according to our own data and results obtained by other workers, was under 150 msec [10]. The cAMP concentration was determined by radioimmunoassay using kits from "Chemapol" (Czechoslovakia), and with ^{125}I-cAMP. The isoproterenol was obtained from "Sigma" (USA) and the Finoptin from "Orion" (Finland).

EXPERIMENTAL RESULTS

In the control series of experiments, during stimulation of β-adrenoreceptors by isoproterenol the cAMP concentration was minimal at the beginning of the cardiac cycle (Fig. 1b). During contraction (points 2 and 3) values of the cAMP concentration rose by more than 50% (relative to the 1st point), while during relaxation (points 4 and 5) in cAMP concentration was close to its original value. Despite the wide dispersion, the cAMP concentration was

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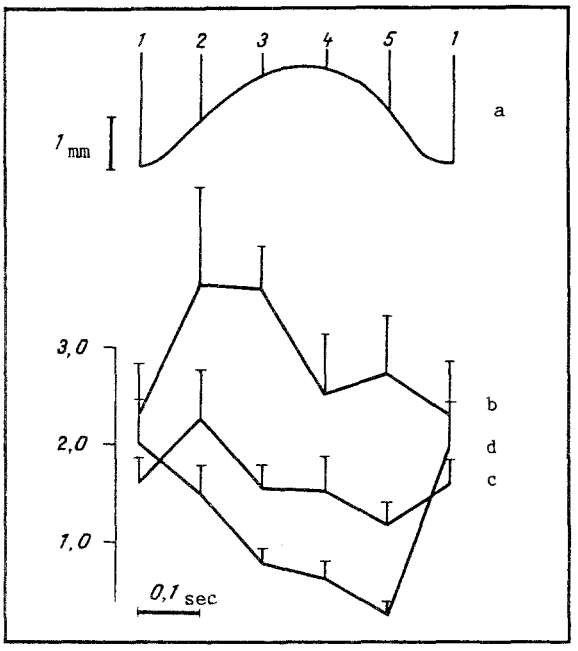

Fig. 1. Effect of low-calcium solution (0.15 mM) and of calcium entry blocker Finoptin ($3 \cdot 10^{-8}$ M) on cAMP dynamics (in nmoles/g wet weight of tissue) in isolated guinea pig heart during contraction and relaxation. a) Curve of contraction and relaxation of heart. 1-5) Times during cardiac cycle at which heart was frozen; b) isoproterenol $3 \cdot 10^{-8}$ M (control); c) simultaneous action of low-calcium solution and isoproterenol ($3 \cdot 10^{-8}$ M); d) simultaneous action of Finoptin and isoproterenol ($3 \cdot 10^{-8}$ M). Each value of cAMP obtained as mean of 7-11 experiments.

significantly greater during contraction than at the remaining points of the cardiac cycle taken together ($p < 0.05$).

In the experiments of series II, after addition of isoproterenol to the low-calcium solution the cAMP concentration also was increased during contraction (Fig. 1c), but only at the 2nd point and compared with all the remaining points of the cardiac cycle ($p < 0.05$). Compared with the control series, the average cAMP concentration in series II was reduced by 45% ($p < 0.01$). In the experiments of series III (Fig. 1d), after addition of isoproterenol preceded by the calcium blocker Finoptin, the mean cAMP concentration was even lower compared with the control series (by 64%, $p < 0.01$). The trend of the cAMP concentration in this series differed qualitatively from the control series, for starting with the 2nd point the cAMP concentration fell steadily, and by the end of the cardiac cycle it was only 14% of its level at the 1st point.

We know that influences used in this investigation (the low-calcium solution and the calcium entry blocker) lead, by different mechanisms, to a decrease in the amplitude of oscillations of ionized calcium in the myoplasm [2, 8]. However, despite this difference, they gave rise to a similar change – a decrease in the mean cAMP concentration. The most probable mechanism of the link between the fall of the ionized calcium concentration in the myoplasm and the fall of the cAMP concentration, taking account of biochemical data [4, 9, 11], could be a change in calcium-calmodulin-dependent activation of adenylate cyclase and phosphodiesterase. The observed difference in the intracellular localization of these enzymes makes this suggestion more likely to be true, and provides an explanation for the significant fluctuations the cAMP concentration in the course of the cardiac cycle by differences in the dynamics of the ionized calcium concentration in different parts of the myoplasm during contraction and relaxation.

The use of the calcium entry blocker Finoptin thus leads to changes in the dynamics and to lowering of the average concentration of cAMP in the isolated guinea pig heart.

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ROLE OF CHOLINERGIC STRUCTURES AT DIFFERENT LEVELS IN RAPID ADAPTATION OF THE PANCREAS TO FOOD QUALITY IN ONTOGENY

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The ability of the digestive glands to adapt the enzymic spectrum of their secretions to the character of the food stimulus is an important mechanism of efficient hydrolysis of the components of the food [1, 3]. The leading role in adaptation of synthesis and secretion of pancreatic enzymes is played by duodenal hormonal mechanisms [2, 6].

In the investigation described below the participation of cholinergic mechanisms of regulation in the formation of rapid enzymic adaptations of the pancreas in early postnatal ontogeny was studied.

EXPERIMENTAL METHOD

Rapid adaptation of the pancreatic enzyme spectrum to a predominantly protein (uncooked meat), fat (butter), and carbohydrate (bread) diet was studied in rats aged 23 days (the change to definitive feeding), 30 days (weaning), and 90 days (adult rats). The animals aged 30 and 90 days were starved beforehand for 14-16 h, and the rats aged 23 days were fed only on their mother's milk during the same period. For the next 30 min the rats were allowed free access (depending on the aim of the experiment) to meat, buffer, and bread and to the ordinary mixed animal house diet. Activity of lipase [5], and of a combination of proteases and amylase [4] was deterined in pancreatic homogenates 2 h after feeding. The same experiments were carried out after preliminary (30 min before feeding on the above-mentioned diets) intraperitoneal injection of the peripheral muscarinic acetylcholine receptor blocker atropine (1 mg/kg), the ganglion blocker benzohexonium (1 mg/kg), or the central muscarinic acetylcholine receptor blocker benactyzine (0.6 mg/kg). In control groups, activity of the pancreatic enzymes were studied in the fasting state. Animals of all groups had unrestricted access to water.

EXPERIMENTAL RSULTS

The experiments showed that at the time of switching to definitive feeding (at the age of 23 days) rapid specific adaptation to food stimuli with predominance of one particular component was absent in the intact rats. At that age, activity of proteases and lipase in intact rats on a protein diet was unchanged and amylase activity was depressed; on a carbohydrate and fat diet the pancreas reacted by an undifferentiated stimulation of synthesis of all enzymes, in the same way as in a mixed diet.

On the 30th day of life (Table 1) the response to food stimuli became more differentiated: protein induced an increase mainly in synthesis of proteases, but in response to fat or carbohydrate stimuli at this age there was an undifferentiated increase in hydrolase synthesis. On a mixed diet, amylase activity fell but activity of proteases and lipase increased.

In adult rats (aged 90 days) increased synthesis of proteases was observed after feeding on meat and of lipase after feeding on butter, i.e., adaptations of pancreatic enzymes to protein and fat stimuli took place.

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