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RATE OF PROTEIN SYNTHESIS AND POLYRIBOSOME FORMATION IN THE FROG PANCREAS AFTER FASTING AND FEEDING

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SUMMARY

1. The rate of incorporation of [^{14}C]leucine into the proteins of the frog pancreas was measured after the animals had been fasted or fed. The incorporation rate increased after feeding, being maximal at about 4 h after the meal.

2. In homogenates of pancreases from fasted frogs only monoribosomes and dimers were present. The absence of polyribosomes in the fasted pancreas was not due to enzymic degradation in the homogenate. After feeding, the appearance of heavy polyribosomal aggregates was observed. When the rate of incorporation was maximal, monoribosomes were almost completely absent.

We have confirmed the observation of HOKIN[1] that the rate of incorporation of labelled amino acids into mammalian pancreatic proteins is not influenced by the extent to which pancreatic cells are filled with zymogen granules.

We found that amino acid incorporation in the rat pancreas was unchanged after a vigorous stimulation of secretion by pilocarpine[2]. Moreover, in the pancreas of a ferret, in which animal feeding causes a considerable loss of zymogen granules, no alteration in the incorporation rate was observed[3].

However, feeding was effective in this respect in a frog awakening from hibernation[4]. The frog can endure prolonged fasting without noticeable harm, and we wondered if, in summertime, the frog could also regulate its rate of protein synthesis in accordance with fasting and feeding. Therefore we studied the rate of amino acid incorporation into the pancreas of frogs caught in July. Fig. 1 shows that the low rate of incorporation during fasting increased after food intake. This increase was maximal at about 4 h after feeding. After 10–14 h the rate returned to the fasting value.

The peak stimulation of the rate of incorporation—about 300 % in winter[4] when normally the animal is hibernating— easily reached 800 % in summer. In the summer this effect can be induced day after day; thus, the frog pancreas seems to offer unique material for studying the events connected with the regulation of protein synthesis.

An increase or decrease in the rate of incorporation of labelled precursors, however, does not necessarily reflect differences in the rate of synthesis because a change in the precursor pool might account for it. However, the small amount of tissue available did not allow us to perform an extensive study of the amino acid

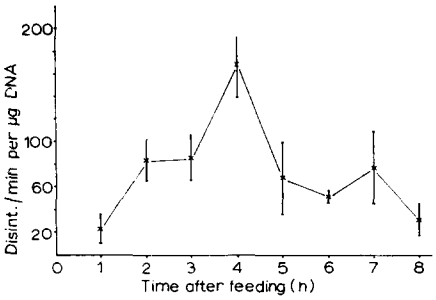

Fig. 1. Rate of incorporation of [^{14}C]leucine into frog pancreas slices after fasting and feeding. The frogs (*Rana esculenta*) were kept for at least 24 h at room temperature without food, then force-fed with about 100 mg of meat. After the indicated periods of time, the animals were killed by decapitation and the pancreas (about 15 mg wet weight) was removed immediately. Slices of the pancreas were incubated in 2 ml of Krebs-Henseleit bicarbonate buffer (pH 7.6) with added glucose (7 mM), 1 μC [^{14}C]leucine per ml (specific activity 312 mC/mmole) and all other unlabelled amino acids (10^{-4} M). Incubation was carried out under an atmosphere of O_2–CO_2 (95 : 5, v/v) at 20° for 10 min. The incorporation was terminated by the addition of ice-cold medium containing a great excess of unlabelled leucine. After two washes the slices were homogenized in 4.5 ml of the same fluid in a Potter homogenizer. The homogenate was filtered, and the filtrate was divided into 4 portions of 1 ml each. An equal volume of 20 % trichloroacetic acid containing an excess of leucine was added. The precipitate was washed twice with 5 % trichloroacetic acid *plus* leucine and twice with 96 % ethanol. Two pellets were used for a duplicate DNA determination[5]. To the others 0.5 ml of Soluene TM-100, (Packard Instruments) was added. The dissolved pellet was transferred to a scintillation vial with the help of 10 ml of a toluene-based scintillation mixture.

pool. Instead we reasoned that during any real increase in the rate of protein synthesis more ribosomes would be engaged in synthesizing proteins. As a result, a shift in the relative numbers of monoribosomes and polyribosomes should be observed.

Fig. 2 shows that the pancreas of the fasted frog contained mostly monoribosomes and dimers. Then, at about the time when the incorporation rate rises after feeding, the size and amount of polyribosomes increased, suggesting that the in-

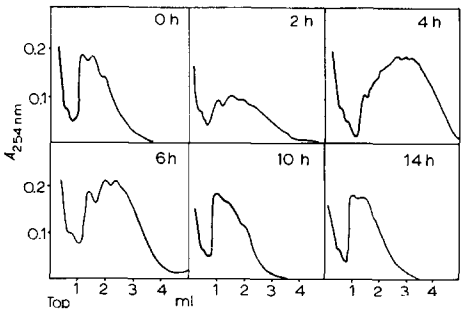

Fig. 2. The effect of feeding on the polyribosomal profile of the frog pancreas. Pancreases were cut into small pieces and homogenized in a Potter homogenizer with a loose fitting plunger in 0.3 ml of ice-cold Tris–Mg^{2+}–KCl buffer (0.05 M Tris–HCl, 0.01 M magnesium acetate and 0.025 M KCl).Cycloheximide (0.1 mg/ml) was added to the homogenization medium to prevent the formation of run-off ribosomes. Debris, nuclei and some mitochondria were removed by a short centrifugation (5 min at 3000 × *g*), and to the supernatant 0.1 vol. of a 10 % sodium deoxycholate solution was added. The resulting suspension was layered onto a 10–40 % linear sucrose gradient in Tris–Mg^{2+}–KCl buffer. An 0.2 ml intermediate layer of 5 % sucrose in Tris–Mg^{2+}–KCl buffer was used to prevent proteins from entering the 10–40 % gradients. The tubes were spun at 39 000 rev./min in a Spinco type SW-39 rotor with the timer set for 45 min and the brake off, the supernate was analyzed at 254 nm with a continuous flow monitoring system.

crease in the incorporation rate is the result, at least partially, of a more frequent reading out of the messengers, *i.e.* of an increase in the rate of protein synthesis. Similarly, the disappearance of the polyribosomes 10–14 h after feeding is in line with the assumption that the observed decrease in the rate of amino acid incorporation indeed reflects a slowing down of protein synthesis.

Before this conclusion is drawn, however, it should be shown that the observed relative scarcity of polyribosomes in the pancreatic cells of the fasted frog does not result from the breakdown during homogenization of polyribosomes by the secretory ribonuclease accumulated in these cells. We found no significant differences, though, in the amounts of acid-extractable ribonuclease from pancreases (about 2–3 μg ribonuclease per g of pancreas (J. J. BEINTEMA, personal communication)) from fed and fasted frogs. For a more conclusive test we mixed homogenates of "fed" and "fasted" pancreases. Fig. 3 shows that in the mixed homogenates the polyribosomes of the fed pancreas were undegraded. Thus, the absence of polyribosomes in the fasted pancreas was not caused by enzymic breakdown of polyribosomes during the isolation procedure.

In conclusion, our results suggest that the frog pancreas, unlike the mammalian pancreas is able to adjust its rate of protein synthesis to fasting and feeding. When the rate of protein synthesis goes up, more ribosomes initiate to a messenger RNA to form heavy polyribosomal aggregates.

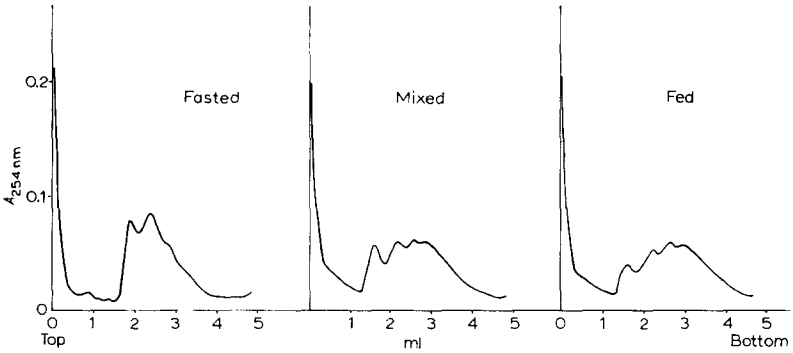

Fig. 3. The effect of mixing the homogenates of fasted and 4-h-fed pancreases on their polyribosomal profiles. Homogenates were prepared as described in the legend of Fig. 2. Part of the fasted and part of the fed homogenate were mixed, and the polyribosomes were prepared and analyzed as for Fig. 2.

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