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THE ACTION OF ATROPINE ON PANCREATIC SECRETION

BY

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The action of atropine, in preventing pancreatic secretion in response to a parasympathomimetic drug, is analysed. Atropine does not appear to affect the uptake of glycine by the pancreatic cell, or the incorporation of radioactive amino acids into the total pancreatic tissue proteins, or into the proteins of the zymogen granules. The rate of amylase resynthesis in stimulated glands is not affected by atropine. It is suggested that atropine blocks pancreatic secretion in rats by blocking the extrusion of zymogen granules.

Although the action of atropine in inhibiting cell secretion has been the subject of several papers (for a review see Henderson and Sweeten, 1943), we could find no references to experimental studies on the mechanism of action of this drug. We present here the results of a series of experiments undertaken in order to analyse this problem.

The net effect of atropine on the pancreas is to block the secretion elicited by parasympathomimetic drugs. Cell secretion is a complex phenomenon where one can distinguish at least three distinct phases: (1) *ingestion,* during which the raw material penetrates the cell; (2) *synthesis,* consisting of the building up of the secretion product; and (3) *extrusion,* a process in which this material is extruded from the cell (for a detailed analysis of cell secretion see Junqueira and Hirsch, 1956). Atropine might act on any one of these stages, or on more than one, to block secretion.

To analyse the action of atropine on ingestion, the rate of penetration of a radioactive amino acid into the pancreatic cell and its proteins was studied in control and atropine-treated animals. If atropine blocks ingestion, a decrease in the rate of penetration would be expected.

To study the action of atropine on the phase of synthesis the following estimations were made in atropine-treated and control animals: (a) the rate of incorporation of amino acids into the total pancreatic proteins; (b) the rate of incorporation of amino acids into the zymogen granule proteins; and (c) the rate of resynthesis of a pancreatic digestive enzyme after its depletion by parasympathomimetic stimulation. If atropine acts by inhibiting the synthetic processes in the cells, a decrease of the rate of amino-acid incorporation into the total proteins and zymogen granule proteins, and also of the rate of resynthesis of the enzyme, would be expected.

If, however, none of the above mechanisms operate one would be led, by exclusion, to postulate that atropine probably acts mainly on the phase of extrusion. This, indeed, is suggested by the results presented in this paper.

MATERIAL AND METHODS

Adult male Wistar rats were used throughout. To study the rate of penetration of an amino acid into the rat pancreatic cell, ether-anaesthetized animals (200 to 250 g.) were stimulated by subcutaneous injection of a 2.5% solution of methacholine bromide (acetyl-β-methylcholine bromide, 30 mg./kg.). After 5 min., a 10% atropine sulphate solution (100 mg./kg.) was administered, also subcutaneously, followed 3 min. later by an intravenous injection of glycine-1-[^{14}C] (10 μc./100 g.) in saline. In this experiment one group of animals (5) received only saline; the second (6) methacholine and saline, and the third (5) methacholine and atropine. Pancreatic biopsies were taken 2, 5, 15, and 30 min. after the glycine injection. Total and protein-bound radioactivity were assayed in 25 λ aliquots of 8% aqueous homogenates of the biopsy material according to the technique described by Junqueira, Hirsch, and Rothschild (1955) and Rothschild, Hirsch, and Junqueira (1957), with thin mica-window Geiger tubes (TGC 2 of Tracerlab, Boston, U.S.A., with 1.7 mg./sq. cm.).

To study the rate of incorporation of phenylalanine and glycine into the proteins of the rat pancreas, and into the zymogen granule proteins, rats (250 to 300 g.) were anaesthetized and their pancreases stimulated as mentioned above. After 55 min. a 10% atropine sulphate solution (100 mg./kg.) was administered subcutaneously, followed 5 min. later by an intravenous injection of 7 μc./100 g. of DL-phenylalanine-3-[^{14}C] or 5 μc./100 g. of glycine-1-[^{14}C] in saline. In the control group the animals were treated likewise.

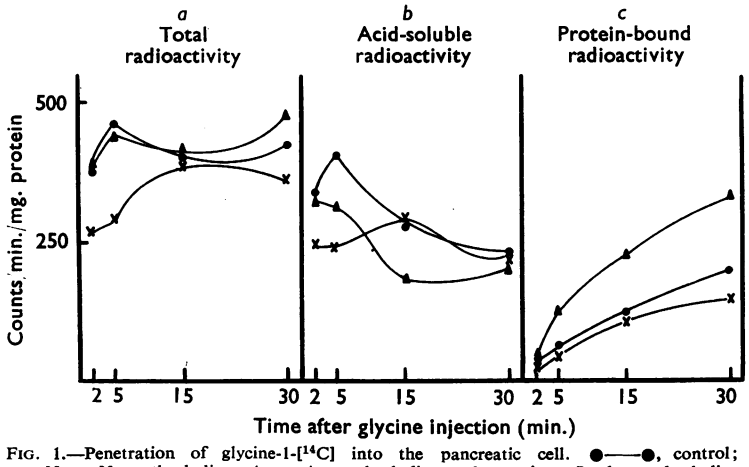

FIG. 1.—Penetration of glycine-1-[^{14}C] into the pancreatic cell. ●——●, control; X——X, methacholine; ▲——▲, methacholine and atropine. In the methacholine experiment each point is the average of 6 rats, whereas for the control and atropine experiments 5 animals were used in each group. Note that the total radioactivity shows no definite trend to increase or decrease, and that, while the acid-soluble radioactivity decreases, the protein-bound radioactivity increases.

but received saline injections instead of atropine. Five and three animals in each group were used for the glycine and phenylalanine experiments respectively. Pancreatic biopsies were performed under ether anaesthesia 30, 150, and 540 min. after giving the radioactive amino acid. The biopsy material was homogenized in 0.25 M-sucrose and the zymogen granules isolated by the technique of Hokin (1955). Measurements of protein-bound radioactivity of the total pancreatic proteins, as well as of the zymogen granules, were performed as described above.

Amylolytic activity was assayed in 200-fold diluted 1% aqueous homogenates of the pancreatic biopsy material of animals treated as described for the incorporation experiments by the method of Smith and Roe (1949).

To visualize the entrance of radioactive glycine into the pancreatic cells, autoradiography was performed on 4 μ thick histological sections fixed with 10% formol solution in M/150 phosphate buffer at *p*H 7.2. The stripping film technique described by Doniach and Pelc (1950) was followed, using Kodak Scientific Plates emulsion and an exposure time of 75 days.

The results of all experiments were analysed by Student's *t* test.

RESULTS

The results obtained on the penetration of glycine into the pancreatic cells—that is, on the ingestion phase—are shown in Fig. 1. The rate of penetration follows a similar curve in the 3 groups of animals studied (Fig. 1*a*). Therefore, it seems that atropine does not act on cell secretion by depressing the uptake of the amino acid used. Furthermore, Fig. 1*b* shows that the decrease of

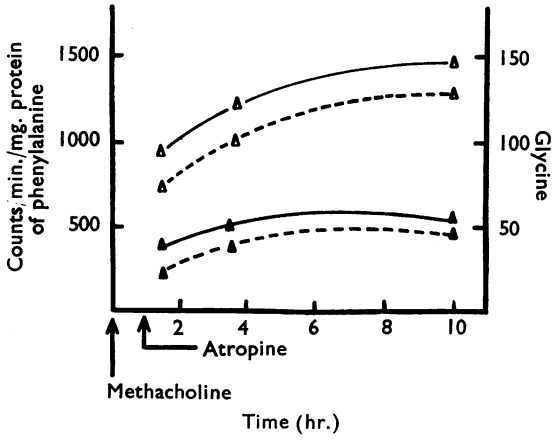

FIG. 2.—Incorporation of phenylalanine-3-[^{14}C] and glycine-1-[^{14}C] into the pancreatic proteins. Full lines are control experiments while the broken lines denote atropine experiments. The open triangles refer to glycine and the closed triangles to phenylalanine. In the phenylalanine experiment each point is the average of 3 rats, while in the glycine experiment 5 animals were used for each group. These curves suggest that atropine does not influence the incorporation rate.

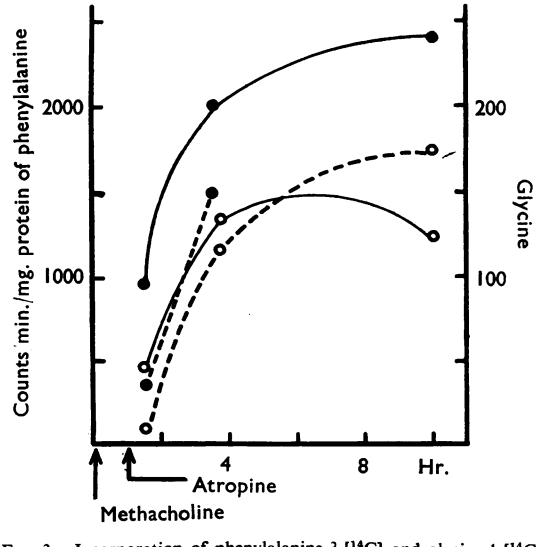

FIG. 3.—Incorporation of phenylalanine-3-[^{14}C] and glycine-1-[^{14}C] into the proteins of pancreatic zymogen granules. The number of animals used is the same as in Fig. 2. Atropine does not influence the incorporation rate. ○——○, control; ○ - - - - ○, atropine; ●——●, phenylalanine; ● – – ●, glycine.

acid-soluble radioactivity in this material is not different for the various groups. The protein-bound radioactivity (Fig. 1*c*) in the atropine-treated animals is, however, significantly ($P<0.05$) higher than in the methacholine-treated group; but no plausible explanation can be given at present for this.

Negative results were also obtained when studying the influence of atropine on the synthesis of the zymogen granule and total pancreatic proteins. Figs. 2 and 3 show similar curves for the incorporation rates of phenylalanine and glycine in both atropine-treated animals and their controls.

As for the results obtained on amylolytic activity, it is obvious (Fig. 4) that atropine did not decrease the rate of amylase resynthesis in the treated animals when compared with their controls.

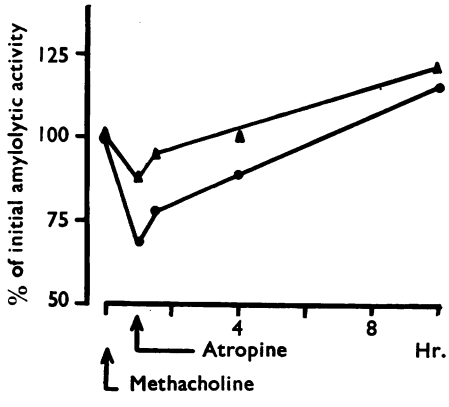

FIG. 4.—Amylolytic activity in successive pancreatic biopsies after parasympathomimetic stimulation followed by saline (●) or atropine (▲) injection. Each point is the average of at least 7 animals. Atropine does not affect significantly the rate of amylase resynthesis (Student's *t* test).

The results of these experiments showed no statistically significant difference between the control and atropine-treated groups.

From the examination of the autoradiographs of the pancreas of the atropinized animals and their controls, it was impossible to distinguish one group from the other. Thus, under the conditions of our experiment, the administration of atropine did not inhibit the penetration of glycine into the cells of the pancreas. The intensity of the autoradiographs varied from animal to animal, irrespective of whether atropine had been given or not.

Our results suggest, therefore, that the main action of atropine on the rat pancreatic exocrine secretion is to block the extrusion of zymogen granules elicited by parasympathomimetic stimulation.

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REFERENCES

Doniach, I., and Pelc, S. R. (1950). *Brit. J. Radiol.*, **23**, 184.
Henderson, V. E., and Sweeten, M. O. (1943). *Amer. J. dig. Dis.*, **10**, 241.
Hokin, L. E. (1955). *Biochim. biophys. Acta*, **18**, 379.
Junqueira, L. C. U., and Hirsch, G. C. (1956). *Int. Rev. Cytol.*, **5**, 323.
—— —— and Rothschild, H. A. (1955). *Biochem. J.*, **61**, 275.
Rothschild, H. A., Hirsch, G. C., and Junqueira, L. C. U. (1957). *Experientia*, **13**, 158.
Smith, B. W., and Roe, J. H. (1949). *J. biol. Chem.*, **179**, 53.