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SELECTIVE EFFECT OF 1-AMINO CYCLOPENTANE CARBOXYLIC ACID ON PANCREATIC ACINAR TISSUE AND ITS PROTEASE ACTIVITY

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Abstract—A single administration of 375 mg/kg of 1-amino cyclopentane carboxylic acid causes within four days a dramatic atrophy of the pancreas in normal rats. The damage is caused to the acinar cells of the pancreas, leaving intact the islets of Langerhans. A total loss in protease activity in the pancreas corroborates the histological picture.

THE INHIBITORY activity of 1-amino cyclopentane-1-carboxylic acid (ACPC) upon tumor growth has received considerable attention in the last few years.[1–4] The well-known toxicity of this compound has also been the subject of extensive investigation and much contradiction. Ross *et al.*[4] suggested that ACPC, being an amino acid lacking the usual α-hydrogen, may therefore function as an amino acid antagonist. However Michelson,[5] using a bacterial assay, failed to find any evidence of amino acid antagonism. Machlin *et al.*[6] recently reported the results of a nutrition experiment in which they succeeded in alleviating ACPC toxicity in chicken by valine supplementation of the basal diet. It was concluded that ACPC was a valine antagonist. Early results by one of us[7] had presented evidence that ACPC inhibits the incorporation *in-vivo* and *in-vitro* of ^{14}C-valine into proteins of rat liver.

These contradictions might be due to the peculiar pharmacological property whereby ACPC causes an "all or none" type of toxicity, as reported by Ross *et al.*[4] This points to the fact that the mechanism of ACPC toxicity is yet to be understood. While some investigators are examining the possibility of inhibition of amino acid active transport by ACPC,[5,8] our attention has been drawn to the spectacular atrophy of pancreas resulting from ACPC treatment. We had previously shown, using radioautographic techniques,[9] that of all organs the pancreas was the site of greatest accumulation of the substance. Studies of the mode of toxicity of ACPC on this gland were thus undertaken and are reported here.

EXPERIMENTAL

Ten Wistar male rats of mean body weight 147 g were divided in two groups: six animals received 375 mg of ACPC/kg body weight i.p. in one injection, and four served as controls. Body weights were recorded before the injection and at the autopsy, performed four days later. Pancreas weights were recorded and expressed per 100 g body weight.

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Protease assay

For the determination of tryptic and chymotryptic activities, we used the method described by Baker *et al.*[10] with slight modifications. The pancreas is excised immediately after the sacrifice of the animal by guillotine.

We have found after experience that one can successfully remove pancreatic tissue free from mesenteric fat by taking advantage of their differences in consistency, color, and translucence, by using a regular 60-watt bulb placed at an angle about one foot from the animal. The gland is weighed quickly on a Roller-Smith balance and homogenized in saline at pH 4·5. The homogenate is further diluted to obtain a final concentration of 1 g fresh pancreas in 150 ml homogenate.

The homogenate is activated during 4 hr at 37° by addition of 1 ml 0·1% enterokinase (Nutritional Biochemicals Corp.) for each 15 ml homogenate.

Five milliliters of the "activated" homogenate is placed in each of two centrifuge tubes. To the first tube, 5 ml of a hemoglobin substrate solution (2·5% solution of hemoglobin substrate powder, Armour Pharmaceutical) is added. After 10 min the digestion is stopped by addition of 5 ml 10% TCA. In the second tube the TCA is added before the hemoglobin substrate. After centrifugation, 5 ml of the supernatant is placed in 25-ml graduated tubes. To the supernatant are added in order 10 ml 3% NaOH, and 1·5 ml Folin-Ciocalteu phenol reagent. The volume is brought to 25 ml with water. After 10 min the intensity of the color developed is determined with a Leitz-Photrometer at 640 mμ. The reagent blank tube contains the same ingredients, except that distilled water is substituted for the supernatant. The difference between the colorimeter reading for the supernatant from the tube containing the activated homogenate, with hemoglobin as the substrate, and the supernatant from the tube containing the same activated homogenate to which TCA has been added before the substrate, is taken as an index of proteolytic activity and is expressed in milligrams of tyrosine by reference to a standard curve calibrated with the pure amino acid.

Histology

The gland is fixed in formol, and sections are stained with hemalum-phloxine-safran.

RESULTS

Body weight

Table 1 shows the effect of a single injection of ACPC on the growth of the animals.

TABLE 1 EFFECT OF ACPC ON BODY WEIGHT AND ON PANCREAS WEIGHT AND ITS PROTEASE ACTIVITY

	Controls (4 animals)	ACPC* (6 animals	Difference (%)	P
Body weight change† (%)	+12·0	−23·2		>0·001
Pancreas weight (mg/100 g body wt.)	335·6 ± 25·2‡	209·7 ± 7·72	−37·5	>0·001
Protease activity§ (mg tyrosine/g fresh pancreas)	21·60 ± 1·86	None		

* Autopsy performed 4 days after a single injection of 375 mg body weight of 1-amino cyclopentane carboxylic acid/kg.

† Per cent of difference in body weight taken before and after the treatment period.

‡ Mean ± standard error.

§ Tryptic and chymotryptic activities were taken as an index of protease activity.

Four days after the injection the animals had lost 23·2% of their body weight, whereas the controls had gained 12·0%.

Pancreas weight

ACPC produced a marked atrophy of the gland. This is particularly significant since the values reported in Table 1 are expressed in mg/100 g body wt. The difference of 37·5% between the two groups of animals is highly significant. At autopsy the pancreas of the treated animals appeared somewhat darker in color than that of the controls.

Histology

Gross examination of the preparations revealed a marked reduction of weight and volume and a moderate softening. Histologically, a great decrease in size of the acini was noted, with almost complete disappearance of the zymogen granules. In addition, the nuclei appeared pycnotic or partly disintegrated. Inflammatory reaction was absent, and the islets of Langerhans were intact. The definitely greater number of cells per unit area in the acinar portions also points to the atrophy, and confirms the wet-weight measurements (Fig. 1).

Protease activity

No proteolytic activity was observed in the pancreas homogenates obtained from the animals receiving ACPC; this is quite in contrast with the values obtained from all the animals of the control group.

DISCUSSION

Several authors[1–4] have reported the inhibition of body growth produced by ACPC in various species of animals. The pathological and histological findings reported in these studies were of exploratory nature and included stomach ulcers, slight atrophy of the liver, pancreas, and bone marrow, and a fall in the circulating neutrophils, this last effect being common to that of many agents that inhibit tumor growth.[3] In fact, no pathological finding had, so far, pointed to the real cause of the toxicity of ACPC.

Although ACPC also accumulates in bone marrow, it is undoubtedly in the pancreas that one can find the greatest accumulation of the amino acid. Our results are interesting in that they show for the first time a definite pathological damage associated to a biochemical lesion.

Also of interest is the selective nature of the damage wrought by ACPC on the pancreas. We find the acinar cells greatly atrophied, but the islet cells remain unaffected. This is in accordance with some of our yet unpublished experiments in which the results of glucose-tolerance tests were similar in ACPC-treated animals to those in controls.

Other substances are known to produce pancreatic acinar cell damage. In 1950 Farber and Popper[11] produced an acute pancreatitis with ethionine. It was reported that the necrosis of the acinar structure was not accompanied by any alteration of the islets of Langerhans. Since then, the morphological damage wrought by ethionine has been described extensively.[12, 13] It was also claimed in 1957[14] that azaserine and ethionine appeared to be the only two compounds capable of producing pancreatic

lesion by means of the systemic administration of the toxic agent, but ACPC is indeed another substance with this propensity.

The action of ethionine and azaserine on the exocrine pancreas is the only similarity between these compounds and ACPC. Whereas ethionine and azaserine are also responsible for a variety of important physiologic and pathologic effects in the liver, kidney, testis, and other organs,[14, 15] we find that the damage caused by ACPC is mostly confined to the pancreas. Furthermore, most lesions produced by ethionine can be prevented by simultaneous administration of methionine.[16] Azaserine is known to inhibit a variety of glutamine-requiring amination reactions,[17] and this inhibition can be prevented by leucine as well as by aromatic amino acids. As yet, we do not know any substance that has been tried which can prevent the pancreatic damage described here. The partial alleviation in chicken of ACPC toxicity by valine has been reported recently by Machlin *et al.*,[6] but we failed to obtain a similar effect in rats.

An essential difference between ACPC and ethionine is the fact that ethionine is known to inhibit the incorporation of methionine into the proteins of some tissues *in vivo*.[18, 19] According to Levine and Traber[20] this inhibition results because ethionine itself is incorporated into tissue proteins. It is also known[21, 22] that ethionine can participate in metabolic transformations in which the methyl group of methionine and choline are normally involved. It is assumed that this is the explanation of the specific antagonism between ethionine and methionine, since ethionine-induced damages are prevented with concomitant administration of methionine. In contrast, Berlinguet *et al.*[23] have shown that ACPC is completely inert toward most of the general enzymatic reactions common to natural amino acids. By means of the radioactive substance, it has also been found that the molecule remains intact within the cells; it is not decarboxylated, transaminated, or oxidized, and it is not incorporated into tissue proteins.

Finally, ACPC exerts the same selective damage on the exocrine pancreas at a much smaller dose than ethionine. The acinar lesions are obtained after 7 or 8 daily administrations of 1 g ethionine/kg body wt., whereas the same damage was obtained 4 days after a single administration of 375 mg ACPC/kg body wt.

Experiments are now in progress using close structural analogs of ACPC in order to determine how specific is the action of ACPC. But even now the practical implication of the exclusive destruction of acinar cells appears obvious. We are led to believe that the effect of ACPC on the exocrine pancreas is analogous to the effect of alloxan on the endocrine pancreas.

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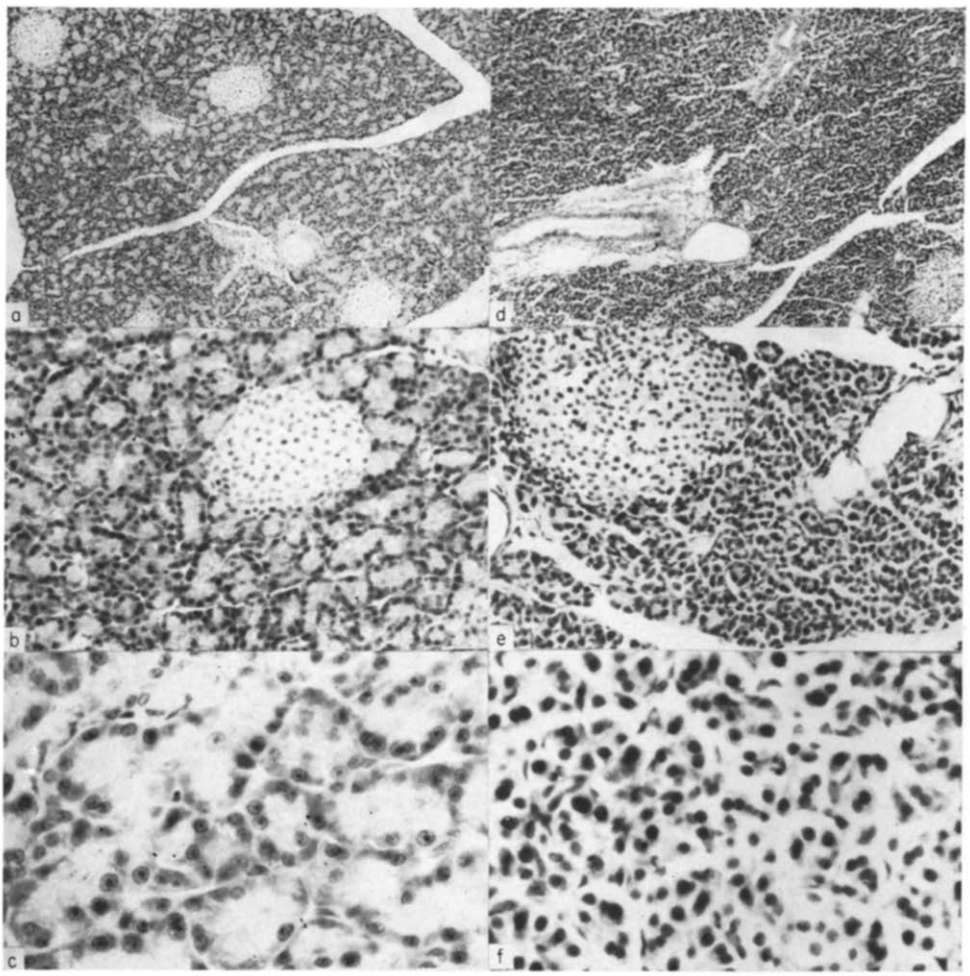

FIG. 1. a, b, and c. Pancreas from control rat; × 70, × 160, and × 400 respectively. d, e, and f. Pancreas from rat, four days after a single intraperitoneal administration of ACPC (375 mg/kg body weight). Same magnification as above, respectively. Note the dissappearance of the acinar arrangement of the exocrine pancreas, the greater number of cells per unit area in the acinar portion, and the integrity of the islets of Langerhans.

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