J. Pharm. Pharmacol. 1981, 33: 551
Communicated January 12, 1981

0022-3573/81/080551-01 $02.50/0

Species differences in response to two naturally occuring α-amylase inhibitors

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The presence of naturally occurring α-amylase inhibitors from wheat and kidney beans was reported by Bowman (1945) and by Sandstedt & Beckard (1946). In 1968, Jaffe & Letti isolated and partially purified an α-amylase inhibitor from red kidney beans. This protein was further purified and characterized by Marshall & Lauda (1975) and designated phaseolamin.

In our work, using inhibitors from these two sources, differences in species sensitivities were encountered. We now describe these differences using pancreatic α-amylase obtained from seven species.

Materials and Methods

Wheat α-amylase inhibitor was obtained from Nutritional Biochemical Corporation, Cleveland, Ohio. Kidney bean α-amylase inhibitor (phaseolamine) was isolated from red kidney beans (*Phaseolus vulgaris*) according to Marshall & Lauda (1975).

Sources of pancreatic α-amylase. Pancreatic α-amylase preparations from mouse, rat, hamster, guinea-pig, rabbit, and dog were obtained by homogenizing the pancreas with 0·9% NaCl. Pig pancreatic amylase purchased from the Sigma Chemical Co., St. Louis, Missouri. Amylase activity was assayed using whole homogenates diluted with phosphate buffer which would give 50% hydrolysis of starch in 10 min.

Assay of α-amylase inhibitor activity. α-Amylase activity was measured by use of the iodine-starch staining reaction. The reduction of starch hydrolysis produced by an inhibitor was expressed as a percent change from control. A 30-min pre-incubation of the inhibitors with the amylase preparations was carried out at 37 °C using 0·4 ml of digest which consisted of 0·1 ml of pancreatic α-amylase homogenate, 0·1 ml of phosphate buffer containing 10 or 100 μg of the inhibitor and 0·2 ml of phosphate buffer pH 7·4. Final incubation was for 10 min at 37 °C after the addition of 0·1 ml of buffered soluble starch.

The absorbance of the starch-iodine complex was obtained by addition of 0·1 ml of the digest to 5 ml of 0·02% I_2 −0·2% KI solution and measured at 680 nm.

Results and discussion

The data in Table 1 indicate that the inhibitor from wheat is active in inhibiting crude pancreatic α-amylase from all species tested. There appeared to be a clear difference in sensitivity between the mouse (least sensitive) and the rat, hamster, and dog (most sensitive) with the guinea-pig, pig, and rabbit showing intermediate sensitivity.

In contrast, phaseolamin, at a concentration 10 times that of the wheat inhibitor, showed clear species differences. Inhibitory activity was marked for the preparation from the dog, moderate for the pig, weak for the rabbit, and absent for the mouse, rat, hamster, and guinea-pig. There is no apparent explanation for the species differences in sensitivity.

Table 1. The inhibitory effects of wheat α-amylase inhibitor and phaseolamin on pancreatic amylases from seven species.

	Wheat α-amylase inhibitor			Phaseolamin	
Sources of Amylase	Control	10 μg	% Change from control	100 μg	% Change from control
Mouse	553·3*	266·5	50	500·0	6
Rat	466·5	83·0	82	533·0	14
Hamster	566·5	133·0	77	516·5	9
Guinea-pig	600·0	216·5	64	600·0	0
Rabbit	611·0	250·0	59	472·0	23
Dog	611·0	138·5	77	0	100
Pig	531·5	215·0	60	280·5	47

* μg starch hydrolysed in 10 min at 37 °C.

The beneficial effect of amylase inhibitors in diabetes mellitus has been reported by Puls & Keup (1973), Schmidt et al (1977) and Caspary (1978). A knowledge of species differences in sensitivity to known α-amylase inhibitors might prove useful in the development of such agents.

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