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The secretion of the pancreas upon intraduodenal administration of trypsin and trypsin with its specific synthetic substrate.

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Abstract: Earlier in our laboratory, conclusions were drawn regarding the specific substrates alleviating the selective inhibitory effects of enzymes by interacting at the catalytic center of the enzyme with the substrate. This was evidenced by data using a specific trypsin inhibitor that blocked the inhibitory effect of intraduodenally and intravenously administered trypsin (1), along with our laboratory's findings of alleviating the selective inhibition of amylase secretion by intraduodenal administration of a specific pancreatic amylase inhibitor (2).

However, both protein and lipid emulsion, as substrates, exhibited elements of nonspecific enzyme sorption in their alleviating action.

Keywords:

The research objective.

Therefore, a series of experiments were conducted involving the intraduodenal administration of pancreatic juice and a synthetic specific substrate for trypsin – BAPNA (benzoyl-arginine-p-nitroanilide). Due to the scarcity of this substrate and aiming to minimize its usage, this series of experiments was conducted on rats. This methodological approach wasn't aimed at alleviating the specific inhibitory effect; rather, it was focused on dissecting the secretion enhancement of one of the enzymes against the backdrop of inhibited secretion of amylase, lipase, and proteases induced by the intraduodenal administration of the juice.

Materials and methods of the study

In a series of experiments (7) conducted on 7 rats, the influence of intraduodenal administration of pancreatic juice (Stage II), juice with BAPNA (benzoyl-arginine-p-nitroanilide) (Stage III), and only BAPNA (Stage IV) on the secretion of pancreatic enzymes was studied. Stimulation of pancreatic secretion was achieved by intraduodenal administration of hydrolyzine at a rate of 0.1 ml/100 g body weight every 15 minutes, and pancreatic juice was collected in half-hourly portions.

Similarly, in a series of experiments (5) involving 4 dogs, the effect of intraduodenal administration of pancreatic juice (Stage II), juice with BAPNA (30 mg, Stage III), and only BAPNA (Stage IV) on the secretion of pancreatic enzymes, their activity in the blood plasma, and excretion in urine was investigated.

The results of the study and their discussion

The experiments on rats confirmed that intraduodenal administration of their pancreatic juice inhibits pancreatic secretion: reducing its volume, amylase, lipase, and protease outputs (Figure 1-A). BAPNA, as a substrate-specific to trypsin, restored the proteolytic activity of the juice and significantly reduced the inhibitory effect on the protease output (Figure 1-B).

However, the results from the experiments on rats were not entirely satisfactory, leading to opportunities to conduct a similar series of experiments on dogs. In these experiments, pancreatic juice was also introduced into the duodenum, and the investigated juice, blood plasma, and urine not only determined the overall proteolytic activity but also trypsin levels.

As seen from the experiment results (Table 1), intraduodenal administration of juice reduced the secretion of amylase, protease, and especially trypsin. The introduction of BAPNA with the juice either alleviated or reduced the inhibitory effect of intraduodenal juice administration. The experiments on rats confirmed that

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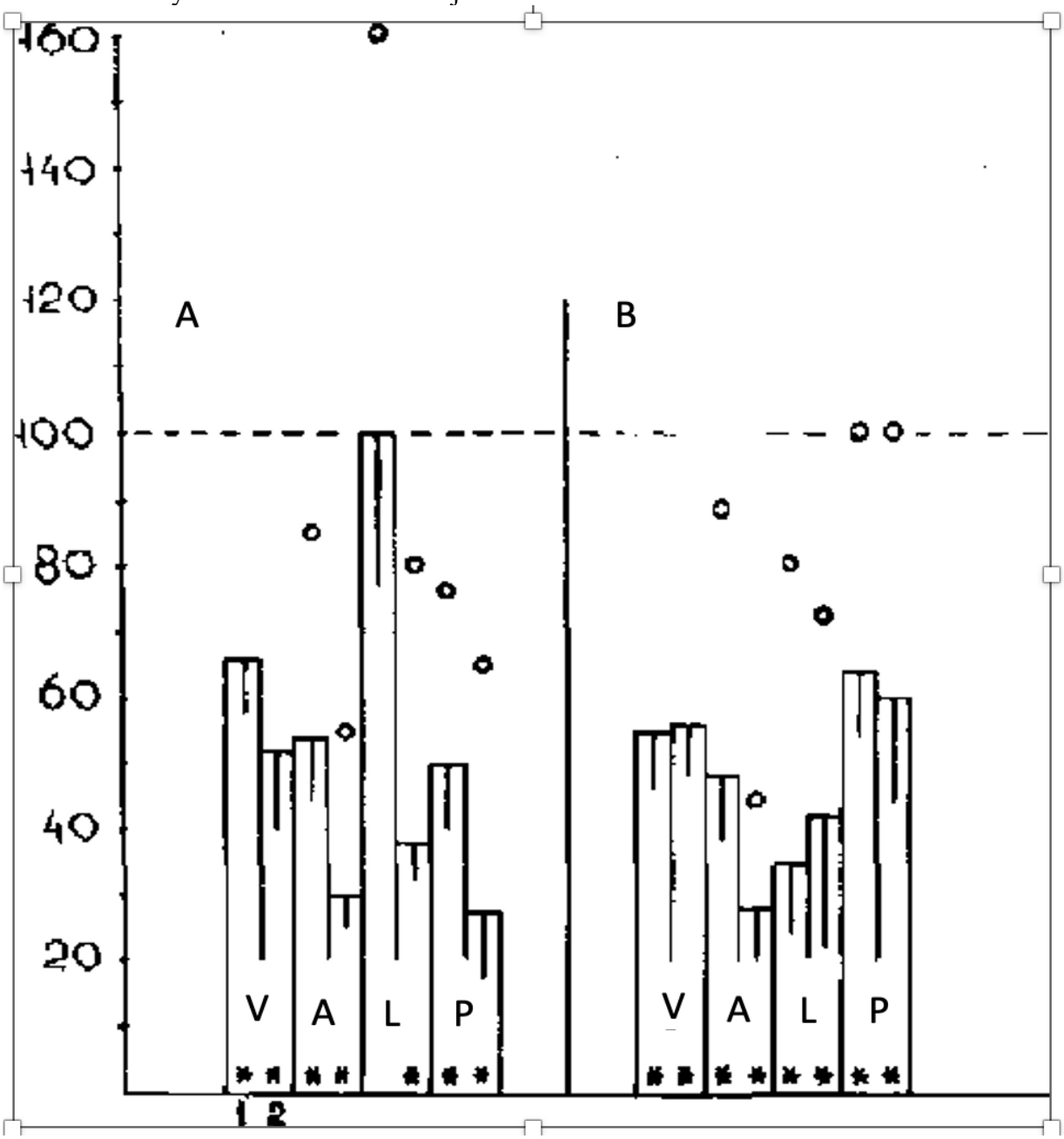

1 - Figure. The influence of intraduodenal administration of pancreatic juice (A) and juice with BAPNA (B) on pancreatic secretion (as a percentage of values before juice introduction).

Legend: V - volume of juice, A - amylase, L - lipase, P - proteases, circles - enzyme activity, bars - their hourly rate, * - significantly different from control (background) indicators, 1 - hour of introducing the substances under study, 2 - subsequent hour.

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1-table.

The effect of intraduodenal administration of pancreatic juice and juice with BAPNA on the hydrolysin-stimulated secretion of the pancreas (as a percentage of the secretion stimulated by hydrolysin before the administration of juice and BAPNA, M±m).

Biological fluids	Components and indicators of biological fluids		Hours of experiments			
			juice + H	H	Juice+ BAPNA+H	H
Pancreatic juice	Volume	-	91,0±14,0	86,3±11,3	94,0±13,4	48,5±4,6^{x}
	Amylase	I	69,3±16,8	50,0±9,5^{x}	90,0±18,5	140,5±22,0
		II	66,5±21,0	40,9±6,0^{x}	76,0±14,6	88,9±10,6
	Lipase	I	110,5±12,0	97,0±11,3	112,4±15,0	127,0±22,7
		II	98,8±11,2	79,5±11,2	123,0±20,3	72,2±19,8
	Proteases	I	104,8±21,6	98,6±10,0	99,5±7,9	111,0±9,4
		II	89,8±3,6^{x}	80,4±6,5	97,8±6,4	58,4±11,3^{x}
	Trypsin	I	74,0±17,4	120,4±26,8	81,3±23,3	876,9±9,7
		II	49,0±7,3^{x}	54,0±8,5^{x}	54,0±8,5x	56,3±11,5^{x}
Blood plasma	Amylase	I	87,8±6,7	91,7±2,6^{x}	93,0±12,0	93,4±6,9
	Lipase	I	89,0±11,8	93,3±3,2^{x}	107,7±3,2	103,6±3,8
	Trypsin	I	102,8±11,8	122,0±16,6	93,5±5,6	92,3±15,0
	Inhibitor of trypsin I		97,7±4,2	73,3±12,4	114,6±14,2	106,0±3,5
Urine	Volume	-	147,0±34,8	145,8±16,1^{x}	115,0±18,0	93,6±13,7
	Amylase	I			99,3±28,2	122,0±24,6
		II	781,0±799,8	3126±1142^{x}	94,0±34,1	79,5±22,3
	Lipase	I	117,5±42,1	106,2±35,0	112,2±18,5	142,7±7,3^{x}
		II	128,7±75,9	117,8±36,5	107,7±24,8	126,7±24,8

The substrate specific to trypsin was reduced without causing significant changes in the enzymatic activity of the blood plasma. Under the influence of introducing pancreatic juice into the duodenum, the renal excretion of amylase increased, which was blocked by BAPNA. The analyzed results left, as in the experiments on rats, some dissatisfaction, although the primary effect of BA-PNA in terms of unleashing trypsin secretion was undeniable. However, the unleashed effects regarding amylase secretion emphasize the prevalence among researchers of the opinion about the leading role of trypsin in inhibiting pancreatic secretion from the duodenum.

The continuation of 1-table

Biological fluids	Components and indicators of biological fluids		Hours of experiments	
			BAPNA + H	H
Pancreatic juice	Volume	-	76,0±11,5	56,0±8,8^{x}
	Amylase	I	107,0±13,0	117,4±10,5
		II	74,0±16,0	59,0±9,7
	Lipase	I	98,0±9,3	105,7±7,1
		II	73,0±5,6	59,5±7,9^{x}
	Proteases	I	103,3±5,1	104,0±2,0
		II	78,8±13,5	58,0±8,6^{x}
	Trypsin	I	96,5±16,3	103,0±10,6
		II	71,5±14,6	58,0±11,3^{x}
Blood plasma	Amylase	I	100,0±4,8	90,5±4,4^{x}
	Lipase	I		
	Trypsin	I	82,0±16,7	103,5±35,4
	Inhibitor of trypsin I			
Urine	Volume	-	61,7±1,7^{x}	57,3±12,1^{x}
	Amylase	I	103,0±0,84^{x}	103,3±2,8

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	II	129,8±42,1	170,7±43,9
	Lipase I	134,8±29,5	119,5±21,7
	II	90,0±24,8	95,4±29,0

Note: I - enzyme activity, II - their hourly rate, x - significantly different from control (background) indicators.

However, considering the varying properties of the pancreatic secretion introduced into the duodenum and, consequently, the effects of this introduction in dogs, another series of acute experiments was conducted. In this series, crystalline trypsin was introduced into the duodenum instead of the secretion.

The results of this series of experiments are distinctive and characterized by great clarity: intraduodenal administration of trypsin significantly inhibited trypsin secretion by reducing the tryptic activity of the secretion (Table 2). Tryptic activity in the blood plasma decreased. The use of BA-PNA - its introduction along with trypsin into the duodenum - reduced these selective effects of trypsin.

2-figure.

The influence of the intraduodenal introduction of trypsin and trypsin with BAPNA on the stimulated secretion of the pancreas (in percentages compared to the indicators of the stimulated secretion by hydrolysine (H) before the application of trypsin andBAPNA, M ±m).

Biological fluids	Components and indicators of biological fluids	Часы опытов			
		I H+trypsin	2-3 H	I H+trypsin +BAPNA	2-3 H
Pancreatic juice	Volume -	106,0±11,2	98,0±14,3	78,5±14,3	73,5±9,5^{x}
	Amylase I	93,0±10,7	68,9±9,2^{x}	80,0±21,0	89,7±10,9
	II	96,0±8,4	68,0±10,5^{x}	71,4±21,0	73,2±17,2
	Lipase I	105,0±7,0	102,7±5,5	99,0±8,9	101,8±4,8
	II	112,2±17,0	109,4±16,5	78,0±10,1	76,9±12,9
	Proteases I	113,4±13,1	100,0±6,5	99,0±3,4	99,9±3,5
	II	107,8±14,2	105,2±14,2	81,0±3,4	72,8±9,2^{x}
	Trypsin I	41,3±15,2^{x}	57,4±11,6^{x}	91,7±31,5	96,4±14,5
	II	50,5±16,3^{x}	74,8±22,5	81,5±24,0	73,5±17,0
	Protein I	101,3±18,5	124,8±25,1	104,5±15,0	123,8±26,0
	II	98,7±11,5	130,6±24,0	81,5±11,8	110,3±19,3
	Bicarbonate I	110,6±12,8	103,8±10,0	95,5±10,8	97,1±9,9
	II	99,5±12,6	96,0±13,4	74,0±7,8^{x}	71,6±14,2
Blood plasma	Amylase I	95,0±5,8	107,9±7,0	92,2±8,5	92,8±6,6
	Lipase I	104,0±10,7	117,6±14,5	116,0±11,8	100,9±11,3
	Inhibitor of trypsin I	87,0±21,5	112,4±12,4	82,5±10,8	111,0±15,2
	Trypsin I	67,3±14,8	54,8±12,7	120,0±30,2	80,6±18,9
Urine	Volume -	161,4±10,8	185,7±23,7	89,3±14,3	80,5±11,6
	Amylase I	115,6±14,2	196,1±30,5^{x}	107,0±9,8	356,6±84,9^{x}
	II	134,8±29,7	407,0±79,7^{x}	105,8±28,3	712,5±98,5^{x}
	Lipase I	136,3±42,4	146,0±41,1	141,0±11,3^{x}	155,6±24,3
	II	151,7±5,5^{x}	342,0±62,3^{x}	135,8±29,8	105,8±20,5

Note: I - enzyme activity, II - their hourly rate, * - significantly different from control indicators.

The presented results indicate, firstly, the possibility of selectively inhibiting the secretion of trypsin from the duodenum by trypsin itself, conclusively establishing this fact. Secondly, regarding the resolution of one of the set tasks, they demonstrate that the relief of enzyme inhibition might involve not only their sorption and modification of the regulatory center but also the interaction of a specific substrate (including highly specific synthetic ones) with the enzyme's catalytic center.

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Regarding the renal excretion of enzymes in the analyzed experiments, the introduction of trypsin increased it. However, the effects of BAPNA were highly unstable across different indicators and at various points during the experiments.

The material presented in this work illustrates the complexity of the mechanisms involved in correcting the secretion of the pancreas, depending on the enzymatic properties of duodenal contents. The regulatory systems of the duodenal mucosa and the secretory apparatus of the pancreas face intricate challenges, emphasizing the specificity and intricacy of visceral system behavior. These challenges are highlighted in the analysis of the enzymatic activity of duodenal contents, the correlation with specific and non-specific substrates, and how pancreatic enzymes behave in solubilized or immobilized form within the duodenal chyme (3, 4, 5).

Conclusions:

The material presented in this study allows for the following summary conclusions:

1. Secretion of pancreatic enzymes is selectively inhibited by pancreatic enzymes present in the duodenal content.

2. The deactivating effect is induced not only by specific but also nonspecific substrates introduced into the duodenum alongside enzymes. Moreover, it might involve modifying the impact of the introduced substrate on the enzyme spectrum of pancreatic juice.

3. The specific synthetic substrate for trypsin - BAPNA - selectively alleviates the selective inhibitory effects of trypsin from the duodenum on its secretion by the pancreas.

4. Not only does the physicochemical sorption of pancreatic enzymes in the duodenal content exclude them from the reverse inhibition of enzyme secretion by the pancreas but also the interaction of their catalytic center with the substrate. Modification by nonspecific substrates on the regulatory properties of enzymes influencing the enzyme spectrum of pancreatic juice is also possible.

5. Judging from the changes in the hydrolytic properties of blood plasma and urine, their dynamics depending on the enzymatic properties of the duodenal content not only influence the excretion of pancreatic enzymes but also their synthesis by pancreatic cells.

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