Inhibitory effect of secretin and cholecystokinin on Heidenhain pouch responses to gastrin extract and histamine

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EDITORIAL SYNOPSIS Rapid intravenous injections of secretin and cholecystokinin (Vitrum) inhibited the acid response of Heidenhain pouch dogs to continuous intravenous gastrin extract. Cholecystokinin caused greater inhibition than secretin. Responses to small doses of histamine were inhibited by cholecystokinin but not by secretin.

Since Sokolov in 1904 first showed that acid coming in contact with duodenal mucosa inhibited gastric acid secretion, opinion has been divided regarding the mechanism of this inhibition. Although earlier work by Code and Watkinson (1955) had suggested that intact vagal innervation was essential for this form of inhibition, more recent studies by Andersson (1960a, b, c, d, e) have demonstrated marked inhibitory effects where no attempt was made to preserve vagal fibres. The results of these and other studies strongly suggest that a humoral inhibitory substance is released from duodenal mucosa on acidification. It thus seemed reasonable to investigate further the possibility that extracts of duodenal mucosa might display inhibitory properties. Before embarking on the preparation of extracts from fresh material it was decided in the first instance to test those duodenal mucosal extracts already commercially available, namely secretin and cholecystokinin. The results of the present study indicate that when given by rapid intravenous injection the secretin extract can inhibit the response of a Heidenhain pouch to injected gastrin extract and the cholecystokinin extract can inhibit the response of such a pouch both to gastrin extract and to histamine.

MATERIALS AND METHODS

Five dogs with Heidenhain type denervated fundic pouches created more than six months previously were studied.

Secretin and cholecystokinin, prepared by the method of Jorpes and Mutt (1959), were obtained from Vitrum (Stockholm). Gastrin extract was prepared from hog

antral mucosa by a modification of the method of Gregory and Tracy (1961) previously described (Gillespie and Grossman, 1963); doses are expressed as the equivalent weight in grams of wet antral mucosa.

The dogs were deprived of food overnight before each test. The inhibitory action of the secretin and cholecystokinin was tested against a background of secretion stimulated by continuous intravenous infusion of gastrin extract or histamine given throughout each experiment by a Sigmamotor pump, the rate of flow being 20 ml. per hour. The concentration of gastrin extract or of histamine dihydrochloride in 0.9% sodium chloride solution was adjusted to give the desired dose rates. After the response had reached a plateau of at least four approximately equal successive 15-minute collections, a single intravenous injection of secretin or cholecystokinin (75 clinical units = 0.1 mg. for secretin, 3 mg. for cholecystokinin) was given and collections were continued at 15-minute intervals for at least one and a half hours.

The acid concentration was determined by titration with 0.2N sodium hydroxide with phenol red indicator. The results are given as microequivalents per 15 minutes. The control output is the mean of the four 15-minute outputs immediately preceding the secretin or cholecystokinin injection, each subsequent 15-minute output being expressed as a percentage of the control response. In all instances duplicate observations were made on each dog.

RESULTS

Table I shows the effect of secretin on the response to gastrin extract, which was given throughout at a rate of 2.5 g. per hour. Inhibition occurred rapidly after the secretin injection, being present within 15 minutes and statistically significant for 30 minutes, after which there was a return of control levels.

TABLE I

EFFECT OF SINGLE INTRAVENOUS INJECTION OF SECRETIN (75 CLINICAL UNITS) ON ACID RESPONSE OF HEIDENHAIN POUCH TO CONTINUOUS INTRAVENOUS INJECTION OF GASTRIN EXTRACT, 2.5 g. PER HOUR

Dog No.	Control ¹ (μ Eq./15 min.)	Post-injection 15-min. Acid Outputs Expressed as % of Control					
		1	2	3	4	5	6
43	106	71	14	61	125	43	67
43	85	62	21	36	92	74	87
44	102	65	120	192	120	98	64
44	151	56	54	47	36	23	23
45	36	53	0	175	153	139	328
45	18	50	44	167	200	217	150
48	281	59	70	79	61	73	106
48	186	40	75	86	65	51	67
53	197	55	13	45	92	74	53
53	213	25	9	42	89	110	109
Means	138	54**	42**	93	103	90	105

¹Control is mean of four 15-minute outputs immediately preceding injection.

Asterisks indicate statistically significant difference from control
** $P < 0.01$.

Table II shows that inhibition of the response to gastrin extract also followed a single cholecystokinin injection. The inhibition, though statistically significant for 75 minutes after the injection, appeared to develop more slowly, being maximal in the third 15-minute period. The degree of inhibition was also greater, the mean maximal being 78% as compared with 58% in the case of secretin (Table I).

In studying the effect of secretin and cholecystokinin on the responses to histamine two separate dose rates of histamine dihydrochloride were used.

In the first instance the acid response to the continuous intravenous injection of histamine dihydrochloride, 0.25 mg. per hour, was found to be unaffected by either secretin (Table IIIA) or cholecystokinin (Table IV).

However, it was noted that the mean control acid outputs to this dose of histamine, 280 and 331 μ Eq. per 15 minutes respectively, were approximately twice the mean control acid outputs to 2.5 g. gastrin extract per hour (138 and 166 μ Eq. per 15 minutes). It seemed desirable to test for inhibition against comparable acid responses to the two secretory stimulants. For this reason, and also on account of the possibility that histamine might be more resistant to inhibitory influences than gastrin, the experiments were repeated in the same dogs using 0.125 mg. histamine dihydrochloride per hour, half the previous dose rate. Table IIIB shows that secretin was still without effect on the acid response to this dose of histamine. Table IVB, however, shows that cholecystokinin did cause statistically significant inhibition, of the same intensity and duration as the depression of the gastrin extract response by cholecystokinin.

TABLE II

EFFECT OF SINGLE INTRAVENOUS INJECTION OF CHOLECYSTOKININ (75 CLINICAL UNITS) ON ACID RESPONSE OF HEIDENHAIN POUCH TO CONTINUOUS INTRAVENOUS INJECTION OF GASTRIN EXTRACT, 2.5 g. PER HOUR

Dog No.	Control ¹ (μ Eq./15 min.)	Post-injection 15-min. Acid Outputs Expressed as % of Control					
		1	2	3	4	5	6
43	30	60	17	27	10	93	280
43	71	37	10	4	42	24	23
44	230	72	27	40	91	104	85
44	62	61	13	18	24	100	116
45	152	84	23	11	63	94	86
45	162	82	28	4	9	37	33
48	417	49	11	10	34	57	51
48	121	36	12	11	11	67	31
53	174	97	71	9	5	39	100
53	242	102	93	86	112	130	100
Means	166	68**	30**	22**	40**	76**	90

¹Control is mean for four 15-minute outputs immediately preceding injection.

Asterisks indicate statistically significant difference from control
** $P < 0.01$.

TABLE III

EFFECT OF SINGLE INTRAVENOUS INJECTION OF SECRETIN (75 CLINICAL UNITS) ON ACID RESPONSE OF HEIDENHAIN POUCH TO CONTINUOUS INTRAVENOUS INJECTION OF HISTAMINE DIHYDROCHLORIDE

<i>Dog No. Control¹ (μEq./15 min.)</i>		<i>Post-injection 15-min. Acid Outputs Expressed as % of Control</i>					
		<i>1</i>	<i>2</i>	<i>3</i>	<i>4</i>	<i>5</i>	<i>6</i>
<i>A</i>	<i>Histamine dihydrochloride, 0.25 mg. per hour</i>						
43	70	110	113	154	133	113	167
43	189	83	123	87	87	80	95
44	163	66	102	104	102	101	111
44	217	67	90	93	103	97	90
45	219	79	58	75	101	60	90
45	268	83	121	188	187	132	152
48	456	109	128	113	108	104	103
48	443	100	91	84	94	97	98
53	498	66	73	84	77	65	71
53	272	72	110	101	88	89	103
Means	280	83	101	108	108	94	108

<i>B Histamine dihydrochloride, 0.125 mg. per hour</i>							
43	60	67	127	112	85	45	135
43	103	82	103	98	55	110	75
44	70	103	180	136	115	123	129
44	38	142	187	179	153	116	111
45	31	184	123	55	461	213	216
45	189	61	93	93	110	123	122
48	280	117	94	63	58	64	99
48	280	93	118	120	114	85	98
53	126	94	111	119	138	79	107
53	173	80	53	100	70	70	51
Means	135	104	119	108	131	103	114

¹Control is mean of four 15-minute outputs immediately preceding injection.

In no instance is the difference between post-injection output and control statistically significant.

TABLE IV

EFFECT OF SINGLE INTRAVENOUS INJECTION OF CHOLECYSTOKININ (75 CLINICAL UNITS) ON ACID RESPONSE OF HEIDENHAIN POUCH TO CONTINUOUS INTRAVENOUS INJECTION OF HISTAMINE DIHYDROCHLORIDE

<i>Dog No. Control¹</i> <i>(μEq./15 min.)</i>		<i>Post-injection 15-min. Acid Outputs</i> <i>Expressed as % of Control</i>					
		<i>1</i>	<i>2</i>	<i>3</i>	<i>4</i>	<i>5</i>	<i>6</i>
<i>A Histamine dihydrochloride, 0.25 mg. per hour</i>							
43	190	75	39	95	58	47	47
43	227	51	96	78	36	66	51
44	170	66	54	74	58	47	69
44	198	57	13	12	28	28	38
45	447	81	42	42	62	69	76
45	314	83	54	53	32	76	85
48	663	60	4	8	23	24	21
48	436	31	2	6	11	20	39
53	315	214	273	310	291	224	248
53	353	138	138	155	160	165	156
Means	331	86	71	81	76	77	83
<i>B Histamine dihydrochloride, 0.125 mg. per hour</i>							
43	78	46	72	47	46	35	64
43	87	54	9	3	13	29	37
44	88	51	8	8	7	9	16
44	41	37	0	0	20	44	78
45	80	66	23	21	13	28	41
45	212	52	7	2	3	12	39
48	199	89	10	2	3	3	3
48	266	27	3	0	0	2	6
53	138	183	54	15	37	51	67
53	193	122	58	56	78	88	93
Means	138	73	24**	15**	22**	30**	44**

¹Control is mean of four 15-minute outputs immediately preceding injection.

Asterisks indicate statistically significant difference from control
** $P < 0.01$.

DISCUSSION

Greenlee, Londhi, Guerrero, Nelsen, El-Bedri, and Dragstedt (1957) reported that in dogs intravenous injections of secretin (Lilly) inhibited the response to feeding and to antral pouch stimulation, but not to vagal stimulation or histamine. It was further shown by Kennedy and Hallenbeck (1963) that the inhibitory effect of intravenous secretin was unaffected by removal of the pancreas. Thomford, Kennedy, and Hallenbeck (1963) found that intravenous secretin (Vitrum) failed to reduce the response of a Heidenhain pouch to intragastric ethyl alcohol. As a result of these studies it was concluded that the mechanism by which the inhibition was mediated was suppression of gastrin release. The present studies would seem to indicate interference with the action of circulating gastrin by the secretin and cholecystokinin preparations used.

It is apparent from the experiments with both gastrin extract and histamine that cholecystokinin possesses greater inhibitory properties than secretin. Neither the secretin nor cholecystokinin preparations are pure substances, and the fact that the dose of cholecystokinin contained a larger quantity of protein

material (3 mg. as compared with 0.1 mg. in the case of secretin) suggests that the cholecystokinin extract contained a greater amount of contaminating factors. It is thus possible that a single agent common to both preparations and additional to the pure hormones themselves is responsible for the inhibition.

However, it is known that the cholecystokinin extract exhibits slight secretin activity, and the possibility arises that secretin itself may have inhibitor properties. This view was favoured by Jordan and Peterson (1962), who found similar degrees of inhibition of the response to a meal after the intravenous injection of two separate secretin preparations of widely differing purity. It will be of great interest to ascertain whether a much purer preparation of secretin which has recently become available (Jorpes, Mutt, Magnusson, and Steele, 1962) inhibits the response of the fundic glands to gastrin extract.

The finding that the inhibitory property of cholecystokinin lessened when the stimulating dose of histamine was increased may indicate that the inhibition was relatively weak. On the other hand the increased dose of histamine would have evoked a greater acid response not only from the Heidenhain pouch but also simultaneously from the main stomach remnant. Thus a greater amount of acid would have entered the duodenum during the administration of the larger dose of histamine. It is possible, therefore, that the larger gastric acid responses resulted in the liberation of maximal amounts of endogenous humoral inhibitory agent and that consequently the injection of further inhibitor substance as secretin or cholecystokinin extracts was without additional effect. This hypothesis represents the behaviour pattern which might be expected if secretin were the inhibitory agent, since acid in the duodenum is a well-known potent stimulus for release of secretin.

The differences between the inhibition of the responses to gastrin extract and to histamine suggest that histamine is more resistant to inhibitory influences than gastrin. Comparison has already been made between approximately matched control responses to both gastrin extract and histamine, *i.e.*, to 2.5 g. gastrin extract per hour and 0.125 mg. histamine dihydrochloride per hour. It was seen that secretin inhibited only the response to gastrin extract. It has been previously reported (Gillespie and Grossman, 1963) that in Heidenhain pouch dogs the maximal gastrin response is less than the maximal histamine response. In the present group of dogs separate studies showed that the mean maximal gastrin response was 44% of the mean maximal histamine response. If comparison is made between controls, not as equivalent absolute responses but as comparable proportions of a

maximal attainable by the appropriate stimulant, it might be more relevant to compare the gastrin extract experiments with those in which 0.25 mg. histamine dichloride was given. This approach further emphasizes the lesser susceptibility of histamine to the inhibitory agents.

Andersson found that in dogs acidification of the duodenum inhibited the response of the fundic glands to injected gastrin extract (Andersson, 1960d) but only slightly and inconstantly to histamine (Andersson, 1960e). It is possible that this difference may be accounted for by the relative resistance of histamine to inhibitory influences, and it would be of interest to repeat Andersson's experiments using smaller stimulatory doses of histamine. The overall similarities in the present studies of inhibition by extracts and in those of duodenal acidification raise the possibility that the humoral substance released by duodenal acidification is contained in secretin and cholecystokinin extracts.

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