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DIBUTYRYL cGMP: INHIBITOR OF THE EFFECT OF CHOLECYSTOKININ AND GASTRIN ON THE GUINEA PIG GALLBLADDER IN VITRO

Pierre Poitras, Dorina Iacino and John H. Walsh

Center for Ulcer Research and Education (CURE)

V.A. Wadsworth Hospital Center and UCLA, Los Angeles, California 90073*

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SUMMARY

N^2, O^2-di-butyryl guanosine 3':5' monophosphate (Bt_2 cGMP), a known competitive and selective inhibitor of the effect of cholecystokinin on the pancreatic acinar cells in vitro was tested for its effect on the guinea pig gallbladder in vitro. Bt_2 cGMP inhibited competitively the contractile effect of cholecystokinin octapeptide, and also inhibited the contraction induced by sulfated gastrin-17. Bt_2 cGMP failed to inhibit the contraction induced by bombesin, acetylcholine or histamine. The 8-bromo derivative of cGMP and the dibutyryl derivative of cAMP did not affect contraction stimulated by cholecystokinin octapeptide. Since it is specific for gastrincholecystokinin peptides, and not restricted to the pancreas, Bt_2 cGMP could be used to recognize the action of these peptides.

N^2, O^2-di-butyryl guanosine 3':5' monophosphate (Bt_2 cGMP) was shown to inhibit amylase secretion induced by peptides structurally related to cholecystokinin in dispersed acini from guinea pig pancreas. This inhibitory action was characterized by competitive kinetics and appeared specific for cholecystokinin since it was not observed with other pancreatic stimulants such as bombesin, physaelamin, carbachol, secretin or vasoactive intestinal polypeptide (1).

The aim of our study was to verify this action of Bt_2 cGMP on the guinea pig gallbladder in vitro.

METHODS

Gallbladder contraction was studied in a laminar flow superfusion system as previously described (2). Briefly, guinea pigs (200-400 gm) were anesthetized with ether. The whole gallbladder was then removed and suspended along its longitudinal axis in a chamber filled with mineral oil and maintained at 37°C in a water bath. The serosal side of the gallbladder was superfused by dripping oxygenated (O_2 95%, CO_2 5%) Krebs solution (0.2 ml/min) through a fine polyethylene tube (PE-10) attached to the superior portion of the organ. Through this tube, test substances, dissolved in freshly oxygenated Krebs solution, were delivered to the organ in volumes of 100 µl injected over 10 seconds. Bombesin

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was injected with a solution containing 1% protein (Plasmanate, Cutter Laboratories). Measurement by radioimmunoassay of the amount of bombesin delivered to the organ in the presence or absence of protein confirmed the necessity and the validity of the protein addition. Agonists and antagonists were mixed in the same syringe and injected simultaneously. The isometric contraction was recorded through a force transducer (Grass FT03C) on a Beckman rectilinear recorder.

Tested substances included cholecystokinin octapeptide obtained from Squibb, porcine sulfated gastrin-17 from Professor R. Gregory, Liverpool, England, bombesin from Farmitalia, and acetylcholine, $N^{2}O^{2}$ dibutyryl guanosine 3':5' monophosphate, $N^{6}O^{2}$ dibutyryl adenosine 3':5' monophosphate (Bt_2 cAMP), 8 bromoguanosine 3':5'-monophosphate (8 Br cGMP), and histamine dihydrochloride from Sigma Chemical Corporation.

RESULTS

Effect of derivatives of cyclic nucleotides alone: When Bt_2 cGMP and Bt_2 cAMP were injected alone (10^{-4} to 10^{-2} M) they failed to have any action on the gallbladder contraction (not shown).

Effect of various doses of cyclic nucleotide derivatives on gallbladder contraction induced by stimulating agents: Gallbladder contraction was measured in response to increasing doses of cholecystokinin octapeptide, gastrin II, bombesin, acetylcholine and histamine as shown in Fig. 1. Despite differing

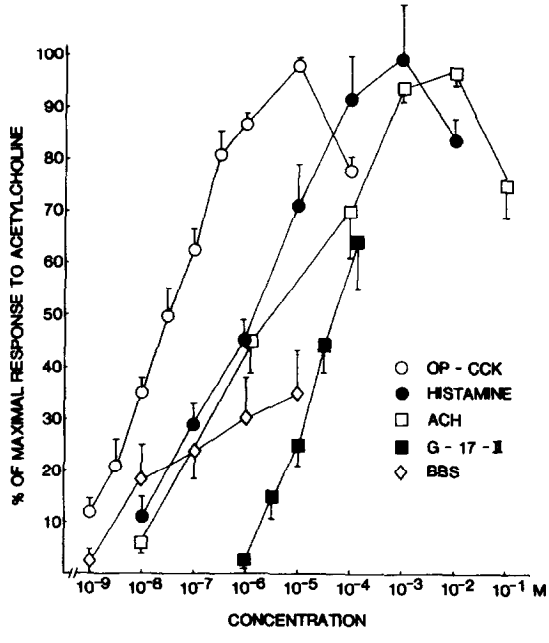

Fig. 1 Dose-response curves for cholecystokinin octapeptide (OP-CCK) (n=12), porcine gastrin II (G-17-II) (n=8), bombesin (BBS) (n=8), acetylcholine (Ach) (n=4) and histamine (n=4). Contractile response is expressed on the ordinate as % of maximal response to Ach (10^{-2} M) obtained before each experiment. A bolus of 100 $l of the concentration on the abcissa was injected.

potencies, cholecystokinin octapeptide, gastrin II, acetylcholine and histamine exhibited parallel dose-response curves. The bombesin dose-response curve however displayed a different slope from the other stimulants. Bombesin was quite potent at low doses but responses to increasing doses were lower than those obtained with the other stimulants. Gastrin II and bombesin were not available in the quantities necessary to obtain maximal responses, but maximal responses (Vmax) obtained with cholecystokinin octapeptide, acetylcholine and histamine were similar.

The response of the gallbladder to these agents was tested in the presence of various amounts of cyclic nucleotide derivatives. From the results obtained in Fig. 1, for each stimulatory agent a dose was selected which produced a contraction approximately equivalent to half the maximal response induced by acetylcholine. This chosen submaximal dose was tested against different doses of the cyclic nucleotide derivatives. Bt_2 cGMP, in doses ranging from 10^{-4} to 10^{-2} M, produced a dose-dependent inhibition of gallbladder contraction induced by submaximal doses of cholecystokinin octapeptide ($5x10^{-8}$ M) or gastrin II (10^{-5} M) as shown in Fig. 2. However, Bt_2 cGMP had no effect on the response elicited by $5x10^{-5}$ M acetylcholine, 10^{-6} bombesin or 10^{-5} M histamine (Table 1). The 8

TABLE 1

STIMULATING AGENT	CYCLIC NUCLEOTIDE DERIVATIVE ADDED		% OF RESPONSE ELICITED BY STIMULUS INJECTED ALONE (NUMBER OF EXPERIMENTS)	
Cholecystokinin octapeptide $5x10^{-8}$ M	8 Br cGMP	10^{-2} M	93.2 ± 2.4	(4)
	Bt_2 cAMP	10^{-4}	98.5 ± 3.1	(8)
		10^{-3}	108.0 ± 6.1	(8)
		10^{-2}	92.1 ± 2.0	(8)
Bombesin 10^{-6} M	Bt_2 cGMP	10^{-3}	91.5 ± 8.5	(4)
		10^{-2}	89.5 ± 4.7	(8)
Histamine 10^{-5} M	Bt_2 cGMP	10^{-4}	98.2 ± 3.6	(4)
		10^{-3}	80.2 ± 7.2	(4)
		10^{-2}	112.0 ± 7.1	(4)
Acetylcholine $5x10^{-5}$ M	Bt_2 cGMP	10^{-4}	88.3 ± 1.3	(4)
		10^{-3}	95.3 ± 7.8	(4)
		10^{-2}	99.6 ± 3.0	(8)
	Bt_2 cAMP	10^{-4}	101.6 ± 1.7	(4)
		10^{-3}	105.3 ± 7.3	(4)
		10^{-2}	103.0 ± 3.2	(4)

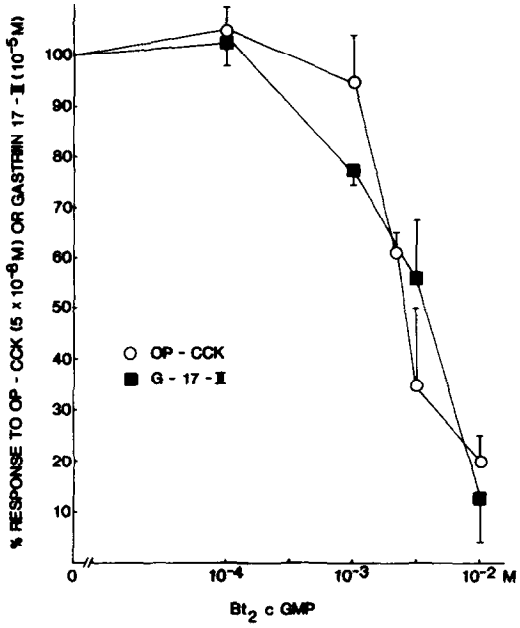

Fig. 2 Bt_2 cGMP administered at increasing doses (10^{-4}, 10^{-3}, $5x10^{-3}$, $3.7x10^{-3}$, 10^{-2} M) produced a dose-related inhibition of the effect of OP-CCK and G-17-II. Contractile response was expressed on the ordinate as a percentage of the response elicited by OP-CCK ($5x10^{-8}$ M) or G-17-II (10^{-5} M) alone.

bromo derivative of cGMP (10^{-2} M) failed to inhibit the response to $5x10^{-8}$ M cholecystokinin octapeptide (Table 1) and Bt_2 cAMP, in in doses ranging from 10^{-4} to 10^{-2} M also failed to inhibit the response elicited by $5x10^{-8}$ M cholecystokinin octapeptide or by $5x10^{-5}$ M acetylcholine.

Effect of a submaximal dose of Bt_2 cGMP on the contraction induced by increasing doses of cholecystokinin octapeptide. In order to describe further the characteristics of Bt_2 cGMP inhibition of cholecystokinin octapeptide action, the contractile effect of increasing doses of cholecystokinin octapeptide ($5x10^{-9}$ to 10^{-5} M) injected alone was tested in six gallbladders and compared to the contraction obtained when Bt_2 cGMP ($3.7x10^{-3}$ M) was added. An inhibitory effect of Bt_2 cGMP was observed with the lower doses of cholecystokinin octapeptide but was surmountable at the highest dose tested (Fig. 3). The Vmax's obtained for cholecystokinin octapeptide in the absence or presence of Bt_2 cGMP were similar. As shown in Fig. 3, the dose-response curves obtained in each case were not hyperbolic but sigmoidal and prohibited the use of Michaelis-Menten kinetic analysis

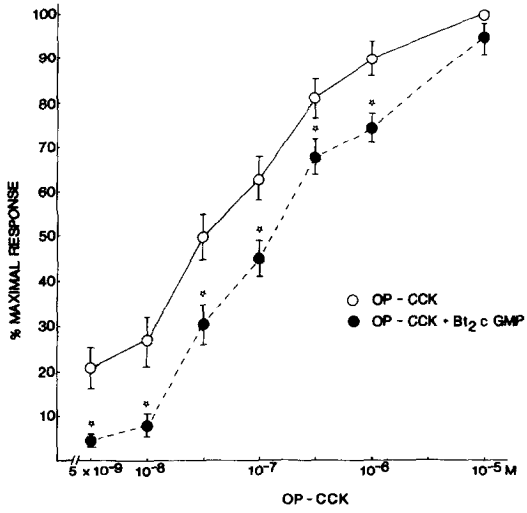

Fig. 3 Bt_2 cGMP (3.7×10^{-3} M) produced significant inhibition of the effect of OP-CCK in the dose range of 5×10^{-9} to 10^{-6} M. The inhibitory effect of Bt_2 cGMP was surmountable when 10^{-5} M OP-CCK was injected. * indicates $p<.01$.

(3). For this reason, data were analyzed according to Hill method [log r/R-r (where r = observed response and R = maximal response) on the ordinate and log cholecystokinin octapeptide dose on the abcissa] and results from mean values are

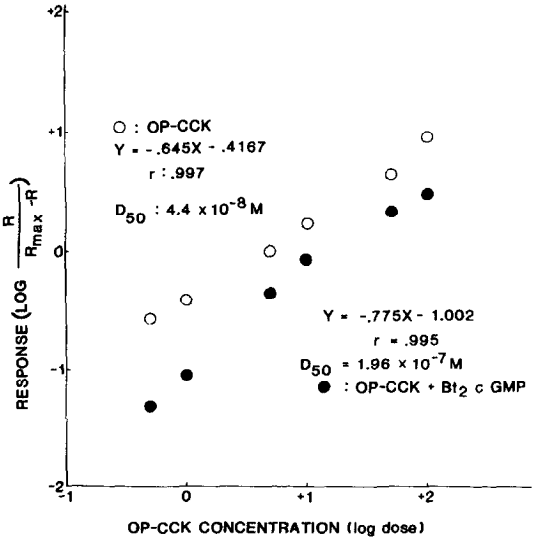

Fig. 4 Hill plot of the mean data obtained from 6 different experiments when OP-CCK (5×10^{-9} M to 10^{-6} M) was administered in the absence or presence of Bt_2 cGMP (3.7×10^{-3} M). Equations and D_{50} respectively displayed for OP-CCK alone (o) and OP-CCK + Bt_2 cGMP (●) were obtained from the linear regression lines constructed with the mean data for each series of experiments.

shown on Fig. 4. When, with the same method, each gallbladder was analyzed separately, 6 individual linear regression lines were obtained and a D_{50} (log r/R-r = 0) in the presence or absence of Bt_2 cGMP was calculated for each experiment. Paired-t-test analysis of these data revealed that the D_{50} for cholecystokinin octapeptide administered with Bt_2 cGMP ($2.1 \pm 0.4 \times 10^{-7}$ M) was significantly higher ($p < .001$) than the D_{50} obtained with cholecystokinin octapeptide alone ($4.7 \pm 1.0 \times 10^{-8}$ M).

DISCUSSION

Our results indicate that Bt_2 cGMP competitively inhibited the contractile effect of cholecystokinin octapeptide on the guinea pig gallbladder *in vitro*. Moreover, this effect appears selective for gastrin and cholecystokinin peptides.

The effect of Bt_2 cGMP on cholecystokinin was described before for the *in vitro* pancreas (1); the action of Bt_2 cGMP on gastrin is not unexpected since cholecystokinin and gastrin share a common COOH-terminal pentapeptide sequence and have similar effects in several organs including the guinea pig gallbladder.

The mechanism of action of Bt_2 cGMP was previously analyzed by Peikins *et al* (1). The inhibitory action appeared to be peculiar to butyryl derivatives of cyclic GMP since Bt_2 cGMP was more potent than monobutyryl cyclic GMP in inhibiting the effect of cholecystokinin, whereas cyclic GMP or Bt_2 cAMP did not alter the increase in amylase secretion caused by cholecystokinin in the *in vitro* pancreas. In our experiment, the failure of Bt_2 cAMP to affect the response to cholecystokinin octapeptide confirms that the pharmacological effect of Bt_2 cGMP is not caused by the butyryl compound alone. The failure of 8 Br cGMP to inhibit the effect of cholecystokinin octapeptide suggests that the action of Bt_2 cGMP was not due to the intracellular penetration of cGMP. Competitive kinetics is compatible with the possibility that the antagonist substance is acting on the same receptor as the agonist. Receptor assay studies with gallbladder cells would be required to demonstrate such an action. Such studies have been done in the guinea pig pancreas; Jensen *et al* (4) demonstrated

that the binding of ^{125}I-labeled cholecystokinin to acinar cells was inhibited by Bt_2 cGMP.

Specific antagonists of peptides are expected to be very useful for elucidating these peptides' physiological role (5). Naloxone, for example, a competitive inhibitor of enkephalins and endorphins is used as a criterion to determine the physiological action of these peptides (6-7). Because of its selectivity for gastrin-cholecystokinin peptides and its apparent action on many organs, Bt_2 cGMP could be used in vitro to inhibit and thereby recognize the specific actions of gastrin and cholecystokinin.

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