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COMPARATIVE INHIBITORY EFFECTS OF 3-QUINUCLIDINYL BENZILATE (QNB) AND ATROPINE ON AMYLASE RELEASE FROM RAT PANCREAS

J. MORISSET, K.H. NG & G.G. POIRIER

G.I. Research Unit, Department of Biology, Science Faculty, Sherbrooke University, Sherbrooke, Quebec, Canada, J1K 2R1

1 Secretion of amylase from rat pancreas stimulated by urecholine was studied in relation to its inhibition by atropine and 3-quinuclidinyl benzilate (QNB).

2 Urecholine-induced secretion was completely abolished by atropine at 10^{-6} M and QNB at 10^{-8} M. Significant inhibition could be detected at 10^{-8} M atropine and 10^{-10} M QNB.

3 The secretory response to pancreozymin was not blocked either by atropine or QNB.

4 The inhibitory effect of QNB remained for at least 90 min after its removal from the incubation medium.

5 It is concluded that QNB is 10 times more potent than atropine in inhibiting pancreatic amylase secretion induced by urecholine.

Introduction

Pancreatic enzyme secretion is controlled by gastrointestinal hormones and the neurotransmitter acetylcholine. The nervous control of enzyme release has been emphasized recently by reports showing that atropine can prevent depolarization of the acinar cell (Matthews & Petersen, 1973), cellular cyclic GMP accumulation (Christophe, Frandsen, Conlon, Krishna & Gardner, 1976) and calcium efflux (Matthews, Petersen & Williams, 1973; Heisler, 1974) stimulated by cholinomimetic agents.

In order to evaluate further the importance of the parasympathetic nervous system in the control of pancreatic exocrine physiology, studies were undertaken to investigate the antimuscarinic properties of 3-quinuclidinyl benzilate (QNB). This drug has been previously reported to be a potent central muscarinic antagonist (Albanus, 1970) which also inhibits the acetylcholine-induced contractile response of the guinea-pig ileum (Becker, 1974; Yamamura & Snyder, 1974a). The aim of this work was thus to compare the antimuscarinic effects of QNB with those of atropine on pancreatic amylase secretion stimulated *in vitro* by urecholine and the duodenal hormone pancreozymin.

Methods

Male albino rats of Sprague-Dawley strain were used, weighing 300–350 g; they were maintained on Purina Rat Chow and had free access to water. In order to limit variations in rates of enzyme secretion, the animals were submitted to the following schedule: a 24 h fasting period followed by 15 h of feeding and an additional 24 h fast.

In vitro *incubation and secretion studies*

Rats were killed by decapitation and the pancreas of each removed; fat and excess tissue were trimmed. The tissue was weighed on a Roller-Smith balance and pieces of pancreas (350 mg) were incubated in a tissue culture medium (5 ml) under an atmosphere of 95% O_2 and 5% CO_2 as previously described (Beaudoin, Marois, Dunnigan & Morisset, 1974). A 10 min preincubation period was used to equilibrate the tissue and wash out amylase from damaged cells. The tissue was transferred to a new flask with freshly oxygenated medium containing the various drugs and incubated for a 60 min period. At the end of this incubation period, tissue was homogenized in 5 ml of Krebs Ringer phosphate at pH 7.4 with 2.5 ml of perchloric acid 2.1 N for DNA determination. Amylase activity released into the incubation medium was used to monitor enzyme secretion.

Reversibility of the 3-quinuclidinyl benzilate effect

Reversibility of the QNB effect was studied as follows: after 10 min of preincubation, pancreases were exposed to QNB (10^{-8} M) for 30 min; two successive

15 min wash out periods were then performed in freshly oxygenated medium followed by urecholine (10^{-5} M) stimulation for 60 minutes.

DNA and amylase determinations

DNA was determined according to Volkin & Cohn (1954) with calf thymus DNA used as the standard. Amylase activity was assayed according to Bernfeld (1955) with lintner starch as substrate; a unit of amylase activity is that liberating 1.0 μmol of reducing groups calculated as maltose, per minute at 37°C. Data were analysed by Student's *t* test.

Drugs

Urecholine was purchased from Merck, Sharp and Dohme (Kirkland, Quebec). Pancreozymin was obtained from the Gastrointestinal Hormone Research Laboratories, Karolinska Institute (Stockholm, Sweden). 3-Quinuclidinyl benzilate was obtained from H. Landau (New York, N.Y.) and atropine from Sigma (St. Louis, Missouri).

Results

Amylase release is increased by 4.5 fold over the control in the presence of urecholine 10^{-5} M. Atropine and QNB produced a concentration-dependent inhibition of amylase secretion initiated by 10^{-5} M urecholine. Significant inhibition of urecholine-stimulated amylase secretion could be detected at 10^{-8}M atropine and maximum inhibition was observed at 10^{-6} M. QNB produced a significant inhibition at 10^{-10} M which was maximum at 10^{-8} M (Figure 1, Table 1). From Figure 1, the ID_{50} for QNB was estimated at 4×10^{-10} M and for atropine at 4×10^{-9} M. The ID_{50} represents the concentration of antagonist which inhibits the secretory response to urecholine 10^{-5} M by 50%. QNB is thus 10 times more potent than atropine.

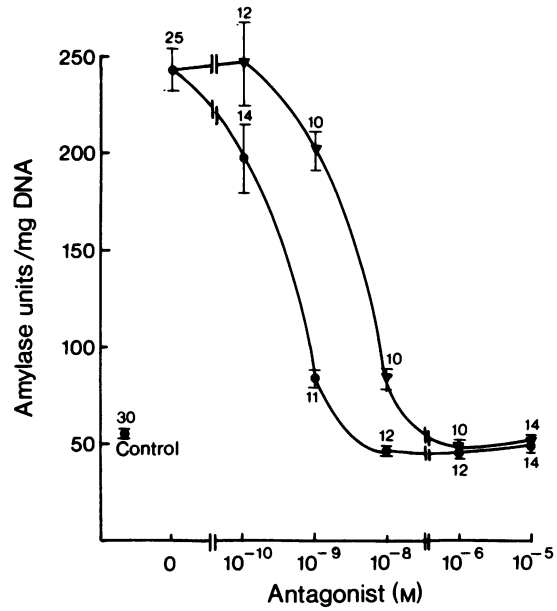

Figure 1 Effects of 3-quinuclidinyl benzilate (QNB) and atropine on urecholine-stimulated amylase release from rat pancreatic tissue. Pancreatic tissue was incubated in tissue culture medium as described in the methods section. For each experiment, urecholine concentration was 10^{-5} M. Each point is the mean value; the figure beside each point indicates the number of tissue pieces in each group. Vertical lines show s.e. mean. The controls represent the sum of all controls for each separate experiment. (●) Urecholine + QNB; (▼) urecholine + atropine.

Table 2 shows that the secretory effect of pancreozymin (0.4 IVY unit/ml) was not impaired by QNB 10^{-5} M; this same concentration of the anti-muscarinic agent did not modify basal release of amylase. The inhibitory effect of QNB (10^{-8} M) remained for at least 90 min after its removal from the incubation medium (Table 3).

Table 1 Percentage inhibition of urecholine-stimulated amylase release from rat pancreatic tissue by atropine and 3-quinuclidinyl benzilate (QNB)

	% inhibition			
Concentration (M)	*Atropine*	P *value*	*QNB*	P *value*
10^{-5}	100	<0.001	100	<0.001
10^{-6}	100	<0.001	100	<0.001
10^{-8}	85	<0.001	100	<0.001
10^{-9}	22	>0.05	85	<0.001
10^{-10}	0		24	<0.05

Pancreatic tissue was incubated in tissue culture medium as described in the methods section. For each experiment, the concentration of urecholine was 10^{-5} M. The percentage inhibition was calculated after subtraction of basal amylase release. *P* value was evaluated between means of amylase release from urecholine and those of both antagonists.

Table 2 Effects of 3-quinuclidinyl benzilate (QNB) on basal and pancreozymin stimulated amylase release from rat pancreatic tissue

Groups	No. of samples	Amylase units/ mg DNA
Control	30	55.5 ± 3.4
Pancreozymin	5	209.7 ± 11.5
Pancreozymin plus QNB	5	212.6 ± 12.6
QNB	9	68.5 ± 6.2

Pancreatic tissue was incubated in tissue culture medium as described in methods. Pancreozymin (GIH) was added at a concentration of 0.4 IVY unit/ml and QNB at a concentration of 10^{-5}M. Results are the mean ± s.e.

Table 3 Irreversible inhibition by 3-quinuclidinyl benzilate (QNB) of urecholine-stimulated amylase release from rat pancreatic tissue

Groups	No. of samples	Amylase units/ mg DNA
Urecholine	5	432.4 ± 40.9
Urecholine plus QNB	9	79.3 ± 7.1

Pancreatic tissue was incubated in tissue culture medium as described in methods. Urecholine was added at a concentration of 10^{-5} M and QNB at 10^{-8} M. Results are the mean ± s.e.

Discussion

Atropine has been shown to abolish several reactions related to the exocytosis process of pancreatic enzymes stimulated by cholinergic agents. These include depolarization of the acinar cells (Matthews & Petersen, 1973), cyclic GMP accumulation in the cytosol (Christophe *et al.*, 1976), calcium efflux (Matthews *et al.*, 1973; Heisler, 1974) and enzyme release (Morisset & Webster, 1970; Heisler, 1974; Williams, 1975). The present study, using pieces of rat pancreatic tissue, shows that atropine produces a concentration-dependent inhibition of amylase release initiated by urecholine. This inhibition curve (Figure 1) correlates very well with those previously obtained by Christophe *et al.* (1976) on cyclic GMP accumulation and by Gardner, Conlon, Klaeveman, Adams & Ondetti (1975) on calcium efflux.

The pharmacological effects of 3-quinuclidinyl benzilate and its derivatives have been studied in dog, mouse and cat (Albanus, 1970) and in guinea-pig (Yamamura & Snyder, 1974a); these compounds elicited anticholinergic activities i.e. tachycardia, mydriasis, inhibition of salivation and inhibition of acetylcholine-induced contractions of the longitudinal muscle of the guinea-pig ileum. Our data indicate that QNB is 10 times more potent than atropine as an inhibitor of pancreatic enzyme secretion *in vitro*. The observation that QNB does not affect pancreozymin-stimulated amylase release is similar to what was found with atropine (Morisset & Beaudoin, 1977) and shows its antimuscarinic specificity. The irreversible inhibition of amylase release noticed 90 min after QNB removal from the medium demonstrates its long-lasting action; similar results were obtained *in vivo* on salivation and light reflex of the eye in dogs (Albanus, 1970) and on contractions of the guinea-pig ileum (Yamamura & Snyder, 1974a).

Studies are now in progress (Ng, Poirier & Morisset, 1977) to characterize the pancreatic muscarinic cholinoceptor from plasma membranes using [^{3}H]-QNB, as in rat brain (Yamamura & Snyder, 1974b; Snyder, Chang, Kuhar & Yamamura, 1975). Such studies will be helpful to evaluate further the role of the parasympathetic nervous system in the control of pancreatic enzyme secretion.

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