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Mechanism of Action of Nicotine on Amylase Release by Isolated Pancreatic Acini

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HOSOTANI, R., P. CHOWDHURY, D. McKAY AND P. L. RAYFORD. *Mechanism of action of nicotine on amylase release by isolated pancreatic acini*. PHARMACOL BIOCHEM BEHAV **33**(3) 663–666, 1989.—The effects of nicotine on the pH of acinar suspension, amylase release and on amylase response stimulated by carbachol were examined in isolated rat pancreatic acini. Additions of nicotine at concentrations ranging from 10 μM to 30 mM caused dose-dependent increases in pH of acinar suspension with simultaneous amylase release ($p<0.05$). There was no increase in amylase release when acinar cells were incubated with nicotine adjusted to pH 7.40. Carbachol alone released amylase whereas nicotine (pH 7.40) at a concentration of 10 mM caused a significant and nonparallel inhibition of amylase release in response to graded doses of carbachol. At concentrations ranges between 3 μM and 10 mM, nicotine at pH 7.40 inhibited amylase release stimulated by 1 μM carbachol, with a half maximal inhibition at 0.8 ± 0.2 mM. These results indicate that in isolated rat pancreatic acini nicotine at pH 7.40 has no effect on basal nonstimulated amylase release but it inhibits carbachol-stimulated amylase response in a noncompetitive manner. These observations may have direct implications in underlying mechanism of pancreatic disorders.

Nicotine pH Amylase Isolated rat pancreatic acini Carbachol

NICOTINE in tobacco smoke is known to play a major role in either induction or potentiation of clinically-relevant diseases, including gastrointestinal disorders. Several studies (1, 8, 11, 14) have shown that nicotine or cigarette smoking inhibits pancreatic bicarbonate secretion, suggesting that a possible relationship exists between impaired alkaline secretion by the pancreas and duodenal ulcer development in smokers. In earlier preliminary studies in rats we reported that chronic oral ingestion of nicotine caused suppression of CCK-8-stimulated pancreatic enzyme responses in isolated pancreatic acini (5). These findings suggest that chronic treatment with nicotine might exert a direct influence on pancreatic acinar cells; however, the detailed underlying mechanisms for this phenomenon were not studied. In other studies (10), it has been shown that nicotine stimulates secretion of performed zymogen granules and newly synthesized proteins from dispersed rat pancreatic acini in vitro; however, it is not clear from this study whether or not the pH of the nicotine solution and the pH of the incubation media were controlled. This is important because the pH of the acinar suspension will become more alkaline with addition of higher concentrations of nicotine, and the pH of the incubation media of acinar cell suspension is a crucial factor in the study of pancreatic acinar cell function (13). To our knowledge, the effects of graded doses of nicotine with the resultant changes in pH of the incubation media of isolated pancreatic acini and amylase release have not been reported. The purpose of the current study was to determine whether or not nicotine at different pH levels and at a constant pH affects basal and carbachol-stimulated amylase release by intact rat pancreatic acinar cells.

METHOD

Purified collagenase (Type CLSPA, 675 U/mg) was obtained from Cooper Biomedical (Malvin, PA) and Minimal Eagle's medium amino acid supplement from Gibco (Grand Island, NY). Nicotine (L-1-methyl-2-[3-pyridyl]pyrolidine, free base 98–100%), carbamylcholine chloride (carbachol) and other chemicals were purchased from Sigma Chemical Company (St. Louis, MO).

Isolated rat pancreatic acini were prepared from female Sprague-Dawley rats fasted overnight according to the method of Williams *et al.* (15). The details of this procedure have been described previously (5,6).

Four experimental studies were conducted and each study was repeated four times. The details of each study are as follows: *Study 1:* This study was conducted to determine whether or not nicotine

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at different concentrations when added to acinar cell suspension would alter the pH of the incubation media and thereby effect amylase release. Nicotine diluted in HEPES-buffered Ringer's solution (pH 7.40, HR buffer) to a concentration of 1 M had a pH of 9.50. Further dilutions were made in HR buffer and 20 μl of each diluted nicotine solution were added to 2 ml acinar suspension so that final concentrations of nicotine in acinar suspensions ranged from 10 μM (10^{-5} M) to 30 mM (3×10^{-2} M). In a separate experiment, nicotine was initially diluted to 1 M solution in 0.02 M phosphate buffer at pH 5.40 and neutralized to pH 7.40 by adding HCl. From this 1 M solution, dilutions of nicotine were made in HR buffer and 20 μl of each solution (concentrations ranging from 10 μM to 30 mM) were added to acini. The pH of each acinar suspension was determined at room temperature by the pH meter (Model 701A, Orion Research, Cambridge, MA). *Study 2:* Nicotine (1 M) at pH 9.50 or nicotine adjusted to pH 7.40 at final concentrations ranging from 10 μM to 30 mM were incubated with acinar suspension. As a control, Tris[hydroxymethyl] aminomethane (Trizma base) at a final concentration of 30 mM (pH 8.75) was added to acinar suspension to test the nonnicotine-induced pH effect on amylase release. *Study 3:* Carbachol at concentrations ranging from 10 nM to 100 μM were incubated with acini in the absence or presence of nicotine (pH 7.40) at a fixed concentration of 10 mM. *Study 4:* One μM of carbachol was incubated with acini in the presence or absence of nicotine at pH 7.40 at concentrations ranging from 3 μM to 10 mM.

In all studies, rat pancreatic acinar cells were incubated in duplicate flasks at 37°C for 30 min. Amylase activity was measured using procion yellow starch substrate (7). Amylase release was calculated either as percentage of the initial content in the acinar pellet or as percentage of maximal release after subtraction of basal release.

Results are expressed as the mean ± SEM. Slopes of dose-response curves were determined by regression analysis using log-logit transformations, and regression curves were compared using Fisher and Scheffe F-test. The ANOVA followed by Duncan's multiple test was used to analyze the data for statistical difference and a difference of $p<0.05$ was considered significant.

RESULTS

Effects of pH on Amylase Secretion

Changes in pH of the acinar suspension and amylase release in response to different concentrations of nicotine are shown in Fig. 1. When different concentrations of nicotine were added, the pH of acinar suspension changed from 7.40 to 8.65 at nicotine concentrations ranging 1 μM from (10^{-6} M) to 30 mM (3×10^{-2} M). When nicotine solutions were adjusted to pH 7.40 before being added to the acinar suspension, the pH of the acinar suspension after addition remained relatively constant up to 1 mM (10^{-3} M) and was 7.56 at 30 mM (3×10^{-2} M) (Fig. 1A).

Amylase release in response to nicotine was increased dose-dependently at concentrations higher than 1 mM and reached 23.1 ± 2.0% of initial content at 30 mM (Fig. 1B). When pH of the nicotine solution was adjusted to 7.40, amylase release was not different at different nicotine concentrations (Fig. 1B). When pH of the acinar suspension was changed to 8.75 by 30 mM of Trizma base (the value of pH was close to that found by 30 mM original nicotine), amylase release was significantly increased to 15.5 ± 4.6% of initial content (data not shown in figure). This value is not significantly different from the value obtained with 30 mM of nicotine. These results indicate that pH has an effect on amylase release of nicotine.

Effect on Carbachol-Stimulated Amylase Release

Carbachol alone caused dose-dependent increase in amylase release (Fig. 2). Maximal response was obtained at 3 μM and amylase release was submaximal at higher concentrations. Addition of 10 mM of nicotine (pH 7.4) resulted in a significant inhibition of carbachol-stimulated amylase release. The slopes of dose-response curves of carbachol alone was 1.01 and for carbachol plus nicotine (pH 7.4) was 0.51 ± 0.08 ($p<0.05$).

Nicotine (pH 7.40) at concentrations ranging from 3 μM to 10 mM caused a dose-dependent inhibition of amylase release stimulated by a fixed dose of carbachol (1 μM), with a half maximal inhibition of 0.8 ± 0.2 mM (Fig. 3).

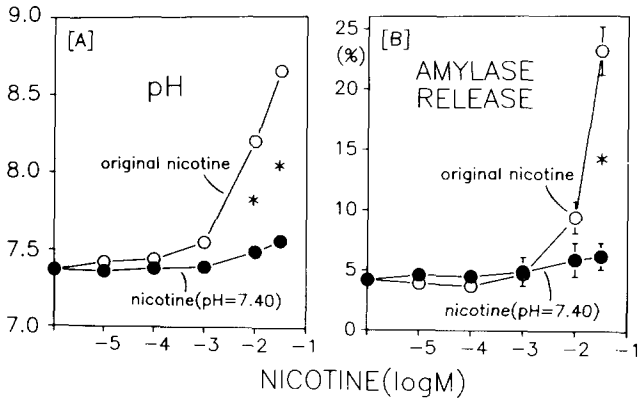

FIG. 1. (A) Changes of pH of the acinar suspension (initially pH 7.40) in response to different concentrations of original nicotine (○) and the nicotine solution which was adjusted to pH 7.40 before being added to the acini (●). (B) Changes in amylase release in response to nicotine. Amylase release was calculated as percentage of initial contents. Points are the mean ± SEM of four separate experiments. *$p<0.05$, between original nicotine vs. nicotine (pH 7.40).

DISCUSSION

It has been known that in man and experimental animals nicotine inhibits basal and secretin-stimulated pancreatic juice volume and bicarbonate secretion (1, 8, 11, 21); however, the effect of nicotine on pancreatic enzyme secretion has not been well studied, particularly in isolated cell systems. It was recently reported that nicotine at concentrations ranging from 3 to 25 mM

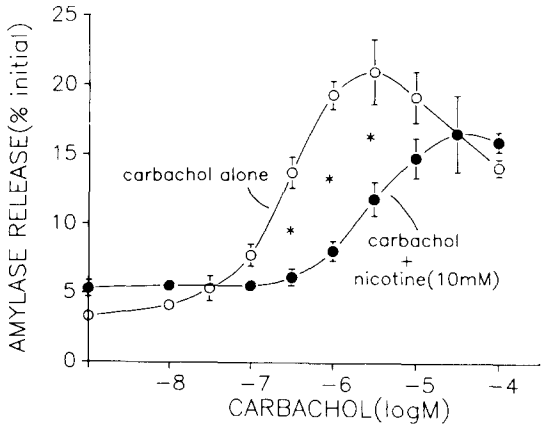

FIG. 2. Effect of 10 mM nicotine (pH 7.40) on dose-response curve for carbachol-stimulated amylase release. *$p<0.05$, between carbachol alone vs. carbachol plus nicotine.

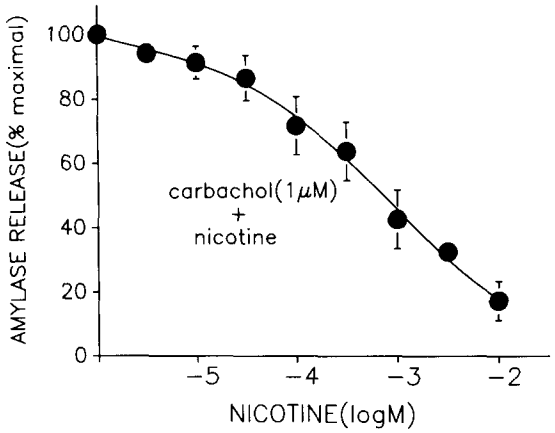

FIG. 3. Effect of nicotine (pH 7.40) on amylase release stimulated by 1 μM carbachol. Amylase release was calculated as percentage of maximal release by 1 μM of carbachol alone after subtraction of basal release.

caused dose-dependent stimulation of amylase and trypsinogen secretion by isolated rat pancreatic acini, and trypsinogen-to-amylase ratio was altered during incubation (10). It was not clear from this study whether or not the pH of nicotine solution and the incubation media after addition of nicotine was controlled. The current study demonstrated that nicotine at concentrations higher than 1 mM caused increases in amylase release that were related to changes in the pH of the acinar suspension (Fig. 1). To further substantiate the effects of pH, increases in amylase release were measured when the pH of the media was increased by adding Trizma base. However, when the pH of nicotine solutions were kept constant at pH 7.40, the increases in amylase release were significantly lowered. According to Preissler and Williams (11) who studied the relationship between pancreatic acinar cell function and pH changes, this cell system is sensitive to extracellular and intracellular pH changes. Our findings indicate that nicotine alone has no effect on amylase release and that amylase release is primarily due to changes in pH of the incubation solution (Fig. 1A and B).

Acinar cells from rodents have been known to possess several different classes of receptors that mediate the actions of secretagogues in enzyme secretion (4). Among these receptors, cholinergically-stimulated amylase release is considered to be mediated through muscarinic receptors, rather than by nicotinic receptors, because the action of carbachol is inhibited in a competitive fashion by specific muscarinic receptor antagonists, such as atropine and pirenzepine (9,12). In the current study, nicotine (pH 7.40) caused a significant and dose-dependent inhibition of carbachol-stimulated amylase release (Figs. 2, 3). The slopes of dose-response curves by carbachol alone and carbachol plus 10 mM nicotine were significantly different from each other, suggesting that the inhibition of carbachol stimulated amylase release by nicotine is noncompetitive. Thus, nicotine does not seem to act via cholinergic receptors on the acinar cell membrane, but might directly inhibit the intracellular pathway such as mobilization of intracellular calcium or an increase in cAMP production (4).

Earlier preliminary studies conducted in our laboratory indicated that chronic treatment of rats with nicotine resulted in significant and dose-dependent inhibition of amylase release by isolated pancreatic acinar cells stimulated by cholecystokinin (2). Solomon *et al.* (14) in their studies on in vitro rabbit pancreas demonstrated that 10 mM of nicotine suppressed pancreatic bicarbonate secretion and cellular ATP levels. In studies in rats utilizing ^{3}H nicotine infusion, we have found an increased uptake of nicotine by the pancreas (3). Plasma concentration of nicotine in smokers will vary depending on the number of cigarettes smoked per day in addition to the individual's smoking pattern. The mean plasma nicotine concentration of average smokers could range from 1.2 to 28 ng/ml. The dose levels we used as compared to the levels expected in smokers are higher in isolated acinar cell systems and may be considered as an acute dose since peak plasma levels of nicotine during and within 20 minutes of cigarette smoking could range to 10 to 15 mM of nicotine concentration. Taken together, the results suggest that nicotine might have a direct toxic effect on acinar cell function at higher concentrations, and thereby have the potential to impair pancreatic enzyme secretion when chronically administered.

In conclusion, in isolated rat pancreatic acini, nicotine alone has little or no effect on amylase release, but it does inhibit carbachol-stimulated amylase response in a noncompetitive manner. Thus, chronic exposure to nicotine, may be a factor in the underlying mechanism of the clinically relevant pancreatic disorders in man.

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