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THE EFFECTS OF ALLOXANATE, NICOTINIC ACID AND IMIDAZOLE ON SECRETORY PROCESSES AND THE ACTIVITIES OF ADENYLATE CYCLASE AND 3′,5′-AMP PHOSPHODIESTERASE IN CAT PANCREAS

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1 Nicotinic acid and alloxanate inhibited water and electrolyte secretion in a dose-dependent fashion when added to the perfusate of the isolated saline-perfused pancreas of the cat stimulated by a supramaximal dose of secretin.

2 There were no changes in the concentration of sodium or potassium secreted into the juice, but the anions exhibited changes which were related to flow rate. As the flow rate declined the chloride concentration increased with a reciprocal decrease in bicarbonate concentration.

3 Nicotinic acid and alloxanate inhibited enzyme secretion stimulated by carbachol.

4 Imidazole inhibited pancreatic electrolyte secretion, but stimulated amylase secretion. Atropine (0.14 μM) reduced the secretion of amylase but did not abolish the effect.

5 Adenylate cyclase prepared from cat pancreas, was stimulated by the octapeptide of cholecystokinin-pancreozymin, secretin and sodium fluoride.

6 Alloxanate strongly inhibited both basal and hormone-stimulated adenylate cyclase activity. Nicotinic acid and imidazole stimulated basal adenylate cyclase activity but had little effect on secretin-stimulated activity.

7 Alloxanate, nicotinic acid and imidazole were all without effect on phosphodiesterase when tested in the presence of micromolar concentrations of adenosine 3′,5′-monophosphate (cyclic AMP). At higher cyclic AMP concentrations (2 mM) alloxanate and nicotinic acid were without effect, whereas imidazole had a slight stimulatory effect at 10 mM which was more marked at 50 mM.

8 Alloxanate (10 mM) strongly inhibited both basal and secretin-stimulated adenylate cyclase activity.

9 It is concluded that the effects of nicotinic acid, alloxanate and imidazole on pancreatic secretion are not mediated entirely through their effects on the adenylate cyclase or phosphodiesterase enzyme systems.

Introduction

The exocrine pancreas is responsible for two major secretory processes, electrolyte secretion and enzyme secretion. Electrolyte secretion is the transport of ions across the cell membrane which, due to the osmotic gradient created, is responsible for water movement in isotonic proportions. In the case of the pancreas, the dominant ions of the primary secretion are Na^+ and HCO_3^-, though the exact nature of the ion transport processes involved remains uncertain. Enzyme secretion is the discharge of digestive hydrolases from pre-formed zymogen granules following fusion of the granule membrane with the cell membrane in the process of exocytosis. There is some evidence also for an extragranular route of enzyme secretion. Enzyme secretion is a function of the acinar cells, which comprise 89% of the gland volume (Bolender, 1974). Most current evidence suggests that electrolyte secretion is a function of ductular elements within the gland, which comprise less than 4% of the total gland volume (Bolender, 1974). The chief stimuli to secretion

are the gastrointestinal hormones secretin and cholecystokinin-pancreozymin (CCK-Pz) and the vagus nerve, via its transmitter substance acetylcholine, although other gastrointestinal hormones (e.g. vasoactive intestinal peptide and gastrin) can also stimulate secretion. Although there is some species variation, generally speaking secretin (and vasoactive intestinal peptide) stimulate largely electrolyte secretion, whereas CCK-Pz and acetylcholine (and gastrin) evoke largely enzyme secretion.

A role for adenosine 3′,5′-monophosphate (cyclic AMP) has been claimed in both secretory processes. However, because of the heterogeneity of the gland and because all, or most, stimuli have multiple sites of action, it has not yet been possible for either secretory process to satisfy absolutely those criteria regarded by Sutherland as the minimum requirement for implicating cyclic AMP in the response to a given stimulus. One approach to this problem is to test the action of agents known to alter the activity of adenylate cyclase or 3′,5′-AMP phosphodiesterase. Cholera toxin is one agent which is thought specifically to activate adenylate cyclase; its actions on the pancreas have been recorded elsewhere (Kempen, de Pont & Bonting, 1975; Smith & Case, 1975). In this paper we correlate the actions of some other agents (alloxanate, nicotinic acid and imidazole) on secretory processes in the isolated pancreas of the cat with their effects on adenylate cyclase and phosphodiesterase activity in cat pancreas homogenates. Alloxan has been reported to inhibit adenylate cyclase of the β-cell of the Islet of Langerhans (Cohen & Bitensky, 1969); nicotinic acid has been shown to lower the concentration of cyclic AMP in adipose tissue (Butcher, Baird & Sutherland, 1968); high concentrations of imidazole have been reported to stimulate mammalian phosphodiesterase (Butcher & Sutherland, 1962). The cat pancreas is a good model of pancreatic secretory processes because there is no basal electrolyte secretion and also because the action of a given stimulus is more specific for a given secretory process than in some other species. The properties of cat pancreatic adenylate cyclase will be described elsewhere (Kempen, de Pont & Bonting, unpublished). Preliminary accounts of some of the work described here have been published (Porter & Scratcherd, 1973; Bonting, Case, Kempen, de Pont & Scratcherd, 1974; Scratcherd, 1974).

Methods

Experiments on perfused cat pancreas

Experiments were performed on a saline-perfused preparation of the cat's pancreas prepared, with slight modifications, as described in detail elsewhere (Case, Harper & Scratcherd, 1968). Briefly, the pancreas was surgically isolated from cats of either sex weighing 1–3.4 kg, which had been denied food 18 h before the experiment. Perfusion fluid was led from a reservoir, through a heat-exchange coil (maintained at 38°C) and, by means of a roller pump, infused into the arterial supply of the gland (either the coeliac and superior mesenteric arteries via a cannula in the aorta, or in some experiments the coeliac artery alone). The effluent from the gland was drained from the superior mesenteric vein after occlusion of the portal tract. The standard perfusion fluid, isosmolal with cat plasma, had the following composition (mM): NaCl 125, KCl 4.3, $NaHCO_3$ 25, $MgSO_4$ 1.0, NaH_2PO_4 1.0, $CaCl_2$ 2.5 and glucose 5. Unless otherwise stated, drugs (nicotinic acid, alloxan (BDH, Chemicals Ltd.) and imidazole (Sigma Chemicals Ltd.)) were added to this perfusion fluid at concentrations of 3, 8 and 13 mM without compensatory reduction in NaCl concentration since osmotic changes of this magnitude have relatively little effect on secretory rate from this preparation (Case *et al.*, 1968). Perfusion fluids were filtered before use and gassed continuously with a mixture of 95% O_2 and 5% CO_2 to maintain pH at 7.4. A bank of four reservoirs allowed rapid changes in the composition of the perfusion fluid to be made. Throughout all experiments pancreatic electrolyte secretion was stimulated maximally by infusing secretin (prepared by the method of Crick, Harper & Raper, 1949) into the arterial cannula by means of a motor-driven syringe. Enzyme secretion was evoked by injecting either acetylcholine, carbachol (Sigma) or CCK-Pz (GIH Laboratory, Stockholm, Sweden) in a small volume of 0.9% w/v NaCl solution (saline, 0.5–1.0 ml) over 10 seconds. Pancreatic juice samples were collected in weighed plastic tubes. Bicarbonate concentrations were measured immediately after collection with a Natelson microgasometer; sodium and potassium concentrations were measured by flame photometry (Mark II, Evans Electroselenium Ltd., or Corning-EEL Model 430); chloride was measured potentiometrically (Model 92 chloridometer, Evans Electroselenium Ltd. or Buchler Digital chloridometer). Where necessary, perfusate osmolalities were checked with conventional osmometers (Osmet Precision Osmometer, Precision Systems Ltd., or Kanuer osmometer, Herber Knauer & Co.). As an index of total enzyme secretion, amylase activity was determined by digestion of starch at 30°C for 15 min using either the method of Nørby (Lagerlöf, 1942) or the method of Bernfield (1955); amylase activity is expressed as international units (iu).

Activity of adenylate cyclase and phosphodiesterase in cat pancreas

Cats of either sex, weighing 2–3 kg and fed *ad libitum*, were killed by rapid intracardial injection of pentobarbitone (90 mg/kg). The pancreas was

removed, freed from adhering fat, minced with scissors and homogenized with a Polytron homogenizer (Kinematica, Lucerne, Switzerland) in 15 ml of a solution containing: 10 mM Tris-HCl buffer, 2.5 mM $MgCl_2$, 2.5 mM disodium edetate (Na_2 EDTA) and 0.2 mg/ml soybean trypsin inhibitor. For adenylate cyclase experiments the buffer was adjusted to pH 7.4 and for phosphodiesterase experiments to pH 8.5. In the latter case the homogenate was stored without further treatment in 1 ml aliquots at −70°C to await assay. For adenylate cyclase studies the homogenate was centrifuged at 8000 *g* for 5 min in the cold, the pellet washed twice in the same homogenization medium and finally resuspended in 5 ml. The particulate suspension was divided into 0.5 ml aliquots and stored at −70°C to await assay. No decrease in enzyme activities was detected after 3 months storage. Adenylate cyclase activity was determined according to Rutten, de Pont & Bonting (1972). The assay medium had the following final composition (pH 7.4): α-[^{32}P]-adenosine triphosphate 0.4–0.6 mM, Tris-HCl 45 mM, $MgCl_2$ 5.5 mM, theophylline 10 mM, phosphoenolpyruvate 10 mM, pyruvate kinase 0.2 mg/ml, Na_2EDTA 0.5 mM and soybean trypsin inhibitor 40 μg/ml. The adenylate cyclase was tested with or without the following substances: secretin (donated by Dr M. Wünsch, Max Planck Institute for Biochemistry, Munich, G.F.R.), the C-terminal octapeptide of cholecystokinin-pancreozymin (PzO, a gift of Dr M. Ondetti, The Squibb Institute for Medical Research, Princeton, N.J., U.S.A.) and sodium fluoride. When hormone effects were studied, 1 mg/ml phosphatidylserine was also included in the assay medium to maintain a constant rate of cyclic AMP formation during the 10 min incubation at 37°C (Kempen, DePont & Bonting, 1974).

Phosphodiesterase activity was determined by the method of Rutten, Schoot & de Pont (1973), using substrate ([^{3}H]-cyclic AMP) concentrations of 1 or 4 μM for the low K_m activity and 2 mM for the high K_m activity. The other assay constitutents were: Tris-HCl (pH 8.5) 66 mM, $MgCl_2$ 1.0 mM and soybean trypsin inhibitor 40 μg/ml. The amount of substrate conversion was kept below 30%. Incubations were for 15 min at 37°C.

Results

Effects on the perfused cat pancreas

Nicotinic acid and alloxan. When these two drugs were added to the perfusate the solution becomes more acid. The pH was therefore adjusted to 7.4 at 37°C by addition of sodium hydroxide. Alloxan is a very unstable compound and in neutral solution is rapidly converted into alloxanate. The half life of alloxan at pH 7.4 and 37°C is about 1 min (Webb, 1966). Therefore, effects of addition of alloxan are probably due to alloxanate. Exposure of the pancreas to nicotinic acid or alloxanate at neutral pH caused a dose-dependent inhibition of secretion. Whereas both drugs had identical effects at 3 mM, nicotinic acid was more effective at concentrations of 8 and 13 mM (Figure 1). The responses were qualitatively similar at all concentrations tested, so only the electrolyte changes at 13 mM will be documented. The output of pancreatic juice and the concentration of electrolytes secreted in the third 10 min test period during perfusion with nicotinic acid or alloaxan were compared with the control period immediately before the addition of the drugs. In the case of alloxanate the volume of secretion fell by a mean of 0.28 ± 0.015 g/min ($n=6$) (a fall to 63.3% of the maximal rate), but the concentrations of the monovalent cations were almost unchanged. The concentration of sodium was 163.3 ± 1.26 mM ($n=6$) in the control period and 160.8 ± 0.75 mM ($n=6$) in the test period. The corresponding figures for potassium were 5.18 ± 0.03 mM ($n=6$) and 4.95 ± 0.02 mM ($n=6$) respectively. Nicotinic acid reduced secretion rate by a mean of 0.43 ± 0.045 g/10 min (a fall to 47% of the maximal rate) and as in the case of alloxanate, the monovalent cation concentrations of sodium in control and test period were 161.7 ± 0.5 mM ($n=6$) and 161.8 ± 1.3 ($n=6$) respecitvely and the corresponding figures for potassium were 4.9 ± 0.10 mM ($n=6$) and 5.13 ± 0.06 mM ($n=6$). The changes in anion concentration were very variable, the absolute values depending upon the rate of secretion. Inhibition with either alloxanate or nicotinic acid caused the

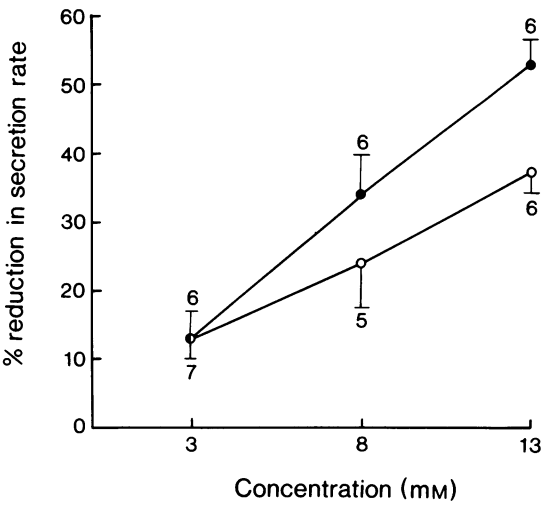

Figure 1 The percentage by which the maximum secretion rate was reduced by nicotinic acid (●) and alloxan (○), at the concentration stated. Bars indicate s.e. mean; no. of experiments beside each point.

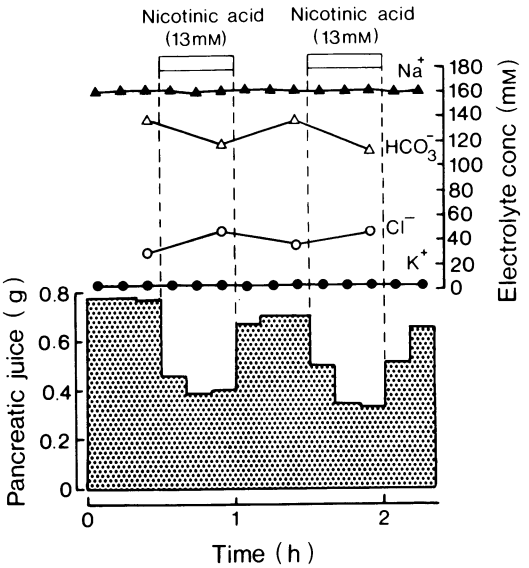

Figure 2 The effect of nicotinic acid 13 mM on the volume and electrolyte secretion of the maximally stimulated perfused pancreas.

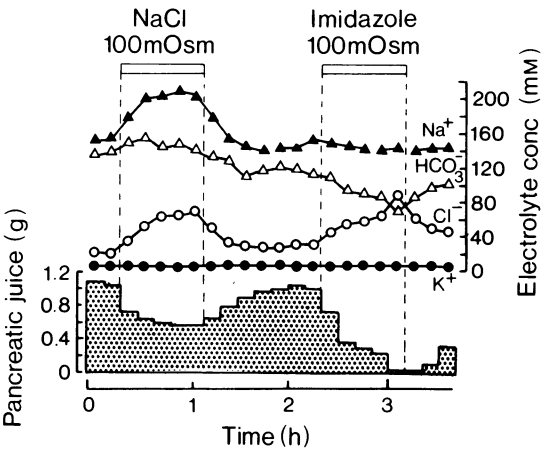

Figure 3 Comparison of equiosmolar solutions of sodium chloride and imidazole on the volume and electrolyte secretion in the perfused pancreas. Note the absence of an effect on the sodium concentration of the juice when imidazole 100 mM was added to the perfusate.

bicarbonate concentration to fall, accompanied by a reciprocal rise in chloride concentration (Figure 2). However, these changes were not different from those caused by reducing secretion rate to similar values by varying the dose of secretin.

The amylase secretion in response to a standard dose of 100 ng carbachol given during the third 10 min test period was expressed as a percentage of the mean response before the test period. A dose of 100 ng of carbachol was chosen because it evokes a consistent amylase secretion from the gland; with larger doses the response declines with repeated stimulation.

An inhibition of enzyme secretion occurred so that at 13 mM the response was reduced by $36 \pm 6\%$ ($n=5$) in the presence of nicotinic acid and by $54 \pm 11\%$ ($n=5$) in the presence of alloxanate.

Imidazole. In order to stimulate purified beef heart phosphodiesterase with imidazole, Nair (1966) had to use concentrations of the order of 10–100 mM. Since addition of imidazole to the perfusate at such concentrations would be likely to cause inhibition by an osmotic effect (Case *et al.*, 1968), in 4 experiments the effects of 100 mM imidazole addition were compared with the effects of adding an osmotically equivalent amount (50 mM) of sodium chloride. All experiments gave similar results; one is illustrated in Figure 3. The inhibition due to imidazole was greater than that produced by sodium chloride. More significantly, unlike the inhibitory effects of alloxanate and nicotinic acid, that due to imidazole was largely irreversible even after prolonged return to perfusion with normal fluid (2.5 h being the longest time tested).

The lack of any increase in $(Na^+ + K^+)$ and $(HCO_3^- + Cl^-)$ concentrations during exposure to 100 mM imidazole suggests that imidazole was appearing in the pancreatic juice at approximately the same concentration as in the perfusate.

Because of the lasting inhibition of electrolyte secretion by high concentrations of imidazole (which effectively precluded observations on enzyme secretion), concentrations of 20 mM or less were tested in six other experiments. At such concentrations, imidazole was usually without effect on electrolyte secretion. However, basal enzyme secretion was stimulated; and stimulation was detectable at concentrations as low as 2.5 mM imidazole. This stimulation of enzyme secretion was largely, but not entirely, abolished by atropine at concentrations (0.14 μM) known to block the effect of acetylcholine released endogenously from nerve terminals within the gland by excess potassium (Argent, Case & Scratcherd, 1971) (Figure 4).

Effects on cat pancreas adenylate cyclase and phosphodiesterase activities

Adenylate cyclase is stimulated by the addition of pancreozymin-c-octapeptide, secretin and sodium fluoride (Table 1).

The effects of alloxan on adenylate cyclase were tested under two conditions. First, to avoid alloxonate formation, alloxan was dissolved in ice-cold twice

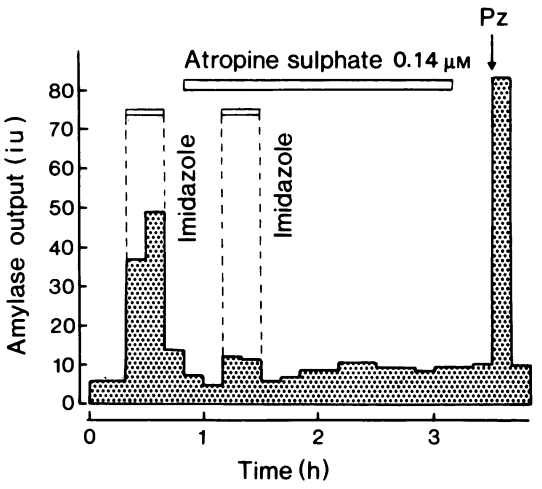

Figure 4 The effect of imidazole on amylase secretion by the pancreas. For the duration of open bars imidazole 20 mM was added to the perfusate, before and after the addition of atropine sulphate (14 μM). At Pz, 0.15 mg of Crick, Harper & Raper (1949) pancreozymin was injected into the arterial line of the perfusion apparatus to demonstrate that the pancreas was capable of responding to an enzyme stimulant. Fluid secretion maximally stimulated throughout with secretin.

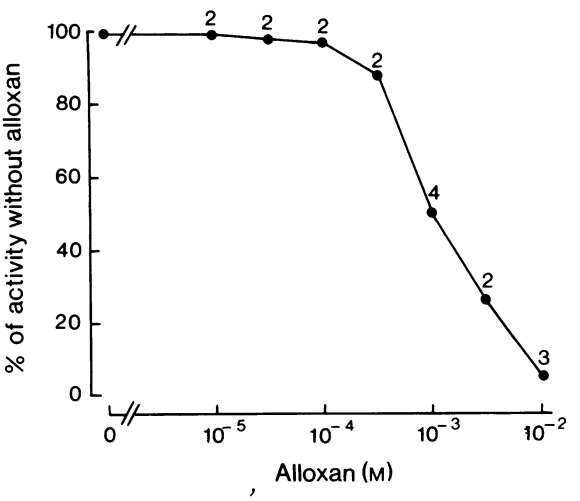

Figure 5 Effect of increasing concentrations of alloxan on cat pancreas adenylate cyclase activity stimulated by secretin 1 μM. Activities are expressed as the percentage of the activity obtained in the absence of alloxan in the same experiment and are given as means; for each concentration the number of experiments is shown. Phosphatidylserine (1 mg/ml) is present in all assays.

distilled water with adjustment to pH 7.4 as late as possible before its addition to the adenylate cyclase medium and the start of incubation. Secondly, to promote alloxanate formation, alloxan was preincubated for 15 min at 37°C before addition to the cyclase medium. Under both conditions exactly the same inhibition of adenylate cyclase activity was observed, which presumably is therefore an effect of alloxanate. At 10 mM alloxanate strongly inhibited basal and secretin-stimulated activities (Table 2). Inhibition of pancreozymin- and NaF-stimulated activity was also observed. The concentration of alloxanate required for 50% inhibition of the secretin-stimulated activity was about 1 mM (Figure 5). Addition of 10 mM cysteine to the incubation buffer completely prevented the inhibitory action of alloxanate on adenylate cyclase activity. Both nicotinic acid and imidazole (10 mM) stimulated basal adenylate cyclase activity, but had little effect on secretin-stimulated activity (Table 2). At 50 mM the stimulatory effect of imidazole was more pronounced (Table 2). When these substances were added to the isolated pancreas in the absence of secretin, there was no stimulation of pancreatic secretion.

Table 1 Properties of cat pancreas adenylate cyclase

Stimulus	*Activity (pmol cyclic AMP formed 10 min^{-1} mg prot^{-1})*
	112 ± 14 (32)
PzO (1 μM)	208 ± 14 (27)
Secretin (1 μM)	396 ± 25 (23)
NaF (10 mM)	655 ± 33 (11)

Values represent means of activities in a 6 min 8000 *g* particulate fraction with s.e.; the number of experiments is given in parentheses. PzO = the C-terminal octapeptide of cholecystokinin-pancreozymin.

Phosphodiesterase. The effects of all three drugs on cat pancreatic phosphodiesterase activity are presented in Table 3. Alloxanate (10 mM), nicotinic acid (10 mM) and imidazole (10 mM and 50 mM) were all without effect when tested in the presence of micromolar concentrations of cyclic AMP. At higher cyclic AMP concentration (2 mM) alloxanate and nicotinic acid were again without effect, whereas imidazole stimulated activity slightly at 10 mM and more markedly at 50 mM.

Discussion

The results of this study are unfortunately not as clear-cut as we had anticipated when embarking upon the project. The actions of alloxan, nicotinic acid and

imidazole on cat pancreatic adenylate cyclase and phosphodiesterase are not consistently correlated with an action on pancreatic fluid or enzyme secretion. Alloxan, acting probably as alloxanate, and nicotinic acid, both inhibited fluid secretion and enzyme secretion by the perfused pancreas of the cat. During perfusion with either drug there were no changes in the concentrations of sodium or potassium. However, the osmolality of perfusate and juice were equal. The fall in bicarbonate concentration and rise in chloride concentration produced by both drugs simply reflect the redution in secretory rate which in this and other pancreas preparations is known to produce these changes (Case *et al.*, 1968). The gradual decline in bicarbonate concentration with time is a feature of the saline-perfused cat pancreas and has been discussed previously (Case *et al.*, 1968). Alloxan has long been known to cause profound vacuolation of pancreatic ductal epithelium after a single intravenous dose of 50 mg/kg (Goldner & Gomori, 1943), and this necrosis is responsible for a reduced electrolyte secretion by the gland (Grossman & Ivy, 1946; Tiscornia, Janowitz & Drieling, 1968). It seemed to have no permanent deleterious effect on the isolated gland over the time tested in these experiments. No effects of nicotinic acid on pancreatic function have previously been published, though it apparently causes stimulation of gastric acid secretion in humans and dogs with Pavlov pouches (Andersson, Carlson, Orö & Richards, 1971). Alloxanate has a strong inhibitory effect on basal and stimulated pancreatic adenylate cyclase activity. Alloxan is an unstable SH reagent and its effects on adenylate cyclase may depend upon inactivation of SH groups in the cyclase molecule since inhibition could be prevented by cysteine. The inhibitory effect of alloxanate on pancreatic secretion may depend on such inhibition, or by inactivation of SH groups on the serosal membrane of pancreatic cells known to be involved in the action of secretin (Wizeman, Schulz & Simon, 1973).

Nicotinic acid has been claimed to inhibit fat cell adenylate cyclase (Skidmore, Schoenhoefer & Kritchevsky, 1971). It has also been reported to stimulate fat cell phosphodiesterase (Krishna, Weiss, Davies & Hynie, 1966; Schwabe & Ebert, 1969; Nakano, 1970) although the effects were small and have not been confirmed by others (Petersen, Hillman & Ashmore, 1968; Kupieki & Marshall, 1968; Therriault & Winters, 1970; Skidmore *et al.*, 1971; Mengel, Meyer, Ebert & Schwabe, 1972). Nicotinic

Table 2 *Effects of alloxanate, nicotinate and imidazole on basal and secretin-stimulated cat pancreas adenylate cyclase activities

	Basal	*Secretin (1 μM)*
Control	102 ± 15 (8)	366 ± 40 (8)
Control	100%	100%
Alloxanate (10 mM)	14 ± 5.8 (3)	5 ± 1.2 (4)
Nicotinate (10 mM)	179 ± 12 (3)	96 ± 8 (3)
Imidazole (10 mM)	280 ± 40 (4)	106 ± 6 (4)
Imidazole (50 mM)	428 (1)	133 (1)

Control activities are given in the first row as pmol cyclic AMP formed per 10 min per mg protein, and represent means ± s.e. of 8 different cat pancreas preparations.
*For each experiment activities in the presence of these drugs are expressed as a percentage of the control activity. The data in the lower part of the Table represent means ± s.e. of these percentage values, with the number of experiments (each with a separate cat pancreas preparation) given in parentheses.

Table 3 Effects* of alloxanate, nicotinate and imidazole on cat pancreas cyclic AMP phosphodiesterase activities at various substrate concentrations

	1 μM cyclic AMP	*4 μM cyclic AMP*	*2 mM cyclic AMP*
Control	2.4 ± 0.4 (4)	7.4 ± 0.9 (4)	41 ± 6.5 (4)
Control	100%	100%	100%
Alloxanate (10 mM)	108 ± 11 (3)	104 ± 17 (4)	104 ± 6 (4)
Nicotinate (10 mM)	89 ± 18 (3)	101 ± 10 (4)	103 ± 4 (4)
Imidazole (10 mM)	107 ± 4 (4)	100 ± 8 (4)	119 ± 6 (4)
Imidazole (50 mM)	97 ± 9 (4)	101 ± 5 (4)	157 ± 12 (4)

Control activities are given in the first row as nmol cyclic AMP hydrolysed per 15 min per mg protein.
* See legend to Table 2.

acid seemed to have little or no inhibitory effect on either adenylate cyclase or phosphodiesterase from cat pancreas; in fact it seemed to stimulate basal adenylate cyclase activity slightly. It is therefore unlikely that this drug causes inhibition of pancreatic electrolyte or enzyme secretion by lowering intracellular cyclic AMP concentration.

High concentrations of imidazole stimulate phosphodiesterase (Butcher & Sutherland, 1972) and it has been recently confirmed that it is only the high K_m enzyme which is involved (Donelly, 1976). It is likely that this enzyme is not of physiological significance at low cyclic AMP concentrations (Rutten, Schoot, de Pont & Bonting, 1973). Imidazole at a concentration of 50 mM stimulated pancreatic phosphodiesterase. However, the dominant effect was rather a stimulation of adenylate cyclase activity, which was less in the presence of secretin (Table 2). At the same time pancreatic electrolyte secretion was irreversibly inhibited and enzyme secretion stimulated. Enzyme secretion was largely due to release of acetylcholine from nerve terminals within the gland but partly due to a direct effect on the acinar cell. Both these effects (i.e. release of acetylcholine and pancreatic enzymes) are probably due to elevation of intracellular calcium concentration and not to activation of adenylate cyclase. Thus, in cardiac muscle, imidazole is able to evoke contractures (Miller & Chapman, 1972) by release of calcium from an intracellular store, probably the sarcoplasmic reticulum (Chapman, Rutherford & Wallace, 1976). In isolated pancreas of the rat, imidazole will increase calcium efflux from preloaded glands in the absence of extracellular calcium (R.M. Case, unpublished observations). The slight stimulatory effect of imidazole in the presence of atropine could be due to the release of calcium from some intracellular store, but the irreversible effect on electrolyte secretion remains unsolved.

In conclusion it would appear that the effects on pancreatic secretion of nicotinic acid, alloxanate and imidazole, cannot be mediated entirely through their effects on adenylate cyclase or phosphodiesterase.

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