Br. J. Pharmac. (1983), **78**, 623–628

The role of nicotinic receptors in dog pancreatic exocrine secretion

M.A. Devaux, G.R. Diaz, K. Kubota, D.F. Magee[1] & H. Sarles

Unité de Recherches de Pathologie Digestive, U 31 INSERM, 46 Boulevard de la Gaye, 13009 Marseille, France

1 The effects of pentamethonium, an autonomic ganglion blocker, were studied on the exocrine pancreatic secretion of six conscious dogs given intravenous infusions of urecholine, caerulein or pentagastrin on a background of submaximal doses of secretin.

2 Urecholine-induced protein secretion was not affected but both caerulein- and to a smaller extent, pentagastrin-induced protein secretions were depressed by pentamethonium.

3 These results indicate that intravenous caerulein and pentagastrin, but not urecholine, act at least partially via nicotinic receptors.

4 Volume and bicarbonate output were depressed by pentamethonium when stimulated by intravenous caerulein with a background of secretin, but not when stimulated by pentagastrin on a background of secretin.

5 From these data it is suggested that caerulein and pentagastrin may potentiate secretin-stimulated hydrelatic secretion by different mechanisms.

Introduction

Exocrine pancreatic secretion is regulated by both neural and hormonal mechanisms. The interaction on exocrine pancreatic secretion between cholinergic nerves and gastrointestinal hormones (GI hormones) has been investigated chiefly with atropine, which blocks muscarinic receptors. In spite of extensive studies, there still exists a diversity of opinion about the action of atropine and acetylcholine on exocrine pancreatic secretion (Thomas, 1964; Magee, Fragola & White, 1965; Henriksen, 1969; Konturek, Tasler & Obtulowicz, 1972; Vaysse, Bastié, Pascal, Roux, Martinel, Lacroix & Ribet, 1975). In addition, it is not clear whether or not nicotinic receptors play a part in the exocrine pancreatic secretory response to GI hormones.

The purpose of this study was to examine the effects of an autonomic ganglionic blocker (pentamethonium) on the pancreatic secretion stimulated by urecholine, caerulein or pentagastrin and to elucidate the possible role of the nicotinic receptors on stimulated pancreatic secretion.

[1]Present address: Department of Physiology, Creighton University, School of Medicine, Omaha, Nebraska 68178, U.S.A.

Methods

Six male Beagle dogs, weighing 12–17 kg, were prepared with Thomas cannulae (Thomas, 1941) in both duodenum and stomach after preliminary ligation of the accessory pancreatic duct. Experiments were started no sooner than three weeks after surgery. Dogs were deprived of food but not water for at least 18 h before each test. The gastric cannula was kept open during tests. The dogs were restrained in Pavlov stands.

The exocrine pancreas was stimulated by: (1) urecholine (carbamylmethylcholine chloride, Sigma) 200 μg/h, (2) caerulein (ceruletide, Farmitalia) 35 ng $kg^{-1}h^{-1}$, or (3) pentagastrin (Peptavlon, ICI) 3 μg kg^{-1} h^{-1}. Caerulein was used in preference to cholecystokinin (CCK) because it can be much more readily and inexpensively obtained in pure form. Each stimulant dissolved in saline was infused on a background of GIH secretin 0.5 clinical unit kg^{-1} h^{-1}. Without secretin the volume of juice varies between zero and 1–3 ml/10 min. It is, therefore, impossible to monitor change in both directions. In order to give these stimulants continuously, a Venocath (G-21, Abbott) was placed in a leg vein and a continuous intravenous infusion of isotonic

NaCl solution was maintained by an electric pump (Tubingen) at a rate of 120 ml/h. In addition, during the urecholine and pentagastrin experiments, isotonic saline (60–180 ml/h) was given through a catheter in another leg vein, in order to compensate for the profuse secretion of saliva and gastric juice provoked by urecholine and pentagastrin. This supplementary volume corresponded approximately to the rate of fluid loss. The doses used above were those which in this laboratory have been found to produce comparable and approximately half maximal (Table 1) secretion. The dose of pentamethonium had been used effectively by Tiscornia, Brasca, Hage, Palasciano, Devaux & Sarles (1972).

Pentamethonium (C_5) (hexamethyl-pentane ammonium dibromide, Delagrange) was used as an autonomic ganglion blocker. The prototype of pentamethonium is hexamethonium (C_6) which acts on the nicotinic receptors of autonomic ganglion cells (Volle & Koelle, 1975). When pancreatic secretion had reached a constant level, 2.0, 1.0 or 0.5 mg/kg C_5 was injected intramuscularly. This occurred usually between 70–90 min after the beginning of stimulant infusion. Sixty minutes after the initial injection, the same dose of C_5 was injected again intramuscularly and the effect was studied for 90 min more. In later experiments with urecholine given in increasing doses, (0, 50, 100 and 200 $\mu g\,kg^{-1}\,h^{-1}$ for 40 min, 0.250 or 0.125 mg $kg^{-1}\,h^{-1}$ of C_5 was given continuously intravenously at each dose level. In this manner the stable blockade needed in this type of experiment was obtained with C_5, a short acting drug. No general side effect was observed after C_5 injections other than conjunctival reddening and relaxation of the nicitating membranes which was seen with every dose used. Doses above 200 mg $kg^{-1}\,h^{-1}$ produced such profuse salivation and evidence of distress that they were not used.

Control studies were performed for each secretory stimulant in the same dogs and in the same manner except that 0.04 ml/kg (equal to the volume of 1 mg/kg of C_5) of saline was substituted for C_5. In every experiment the pH of the duodenal effluent was monitored with pH paper. If the reaction became acid the experiment was abandoned. In only one animal, which is not included, did it fall below four.

Six tests were done on six dogs for each secretory stimulant. Dogs were studied at most twice a week and never on consecutive days. A single experiment was performed in one session.

The volume of pancreatic secretion was measured to 0.1 ml. Bicarbonate concentration was measured by the Van Slyke volumetric method and bicarbonate output was expressed as mEq per 10 min. Protein concentration was estimated by reading at 280 nm against distilled water and using the absorption coefficient $E^{1\%}_{1cm} = 20$. Protein output was expressed as mg per 10 min.

Statistical analysis was as follows: the differences between post C_5 or post-saline control values and the pre-C_5 or pre-saline control plateau values were determined. These were obtained by averaging the last three 10 min periods prior to initial C_5 or saline injection and the 30 min after obvious relaxation of the nictitating membranes (10 min after injection) for each dog. The paired differences between these differences i.e., between those of the saline controls and the C_5 experiments were compared by Student's t test. In Table 3, the means given are those of the amalgamated 30 min after the first and second dose of C_5. $P < 0.05$ was taken as the level of significance. Because pentamethonium consistently decreased the protein in secretin-stimulated juice, the amount of this decrease in each animal was subtracted from its control in calculating the significance of changes in protein secretion. This was unnecessary in the case of water and bicarbonate, neither of which was reduced. For volume see Table 3. Figures for bicarbonate, in mEq/10 min are 3.62 ± 0.62, 2.84 ± 0.71, 3.61 ± 0.66 for 0, 0.125 and 0.250 mg $kg^{-1}\,h^{-1}$ pentolinium respectively.

Table 1 Mean control secretion (± s.e.) for each experiment

Experiment	*Volume* (ml/10 min)	*Protein output* (mg/10 min)	*Bicarbonate output* (mEq/10 min)
Urecholine** 200 $\mu g\,kg^{-1}h^{-1}$	8.3* ± 1.0	41.3* ± 5.7	1.23* ± 0.18
Caerulein** 25 ng $kg^{-1}\,h^{-1}$	10.5* ± 1.4	45.2* ± 7.0	1.45* ± 0.19
Pentagastrin** 3 $\mu g\,kg^{-1}\,h^{-1}$	8.1* ± 0.8	35.2* ± 0.8	1.17* ± 0.12
Secretin** alone	5.6 ± 1.1	10.53 ± 3.1	0.81 ± 0.16

*Significantly greater than secretin alone at least at 95% level.
**Secretin administered throughout 0.5 clinical units $kg^{-1}\,h^{-1}$
$n = 6$

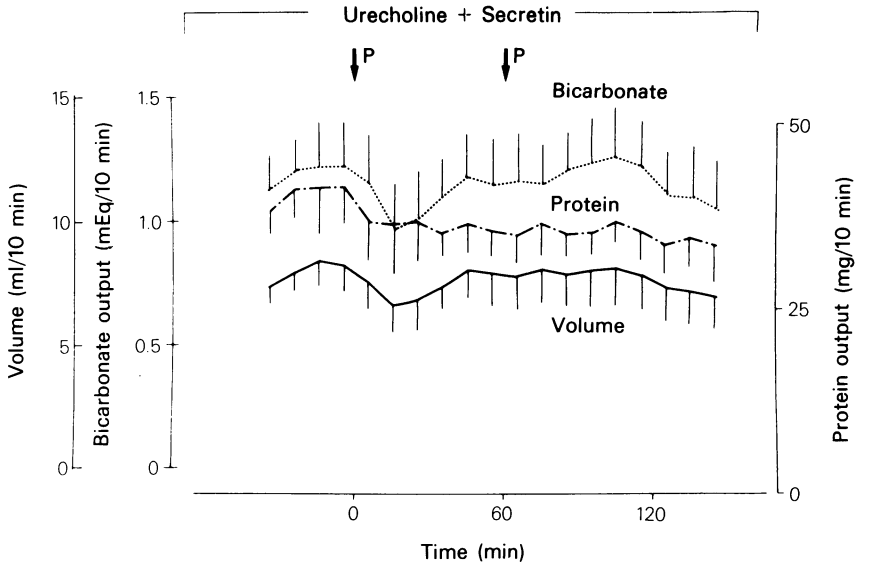

Figure 1 Effects of intramuscular pentamethonium (C_5) (1 mg/kg) given at P on exocrine pancreatic secretion in response to urecholine (200 $\mu g\,kg^{-1}\,h^{-1}$) with background GIH secretin (0.5 clinical units $kg^{-1}\,h^{-1}$). Continuous line: volume output (ml/10 min); dashed and dotted line: protein output (mg/10 min); dotted line: bicarbonate output (mEq/10 min). Each point is a mean of six tests on six dogs; s.e. mean shown by vertical bars. No point shows any significant difference from the values of the control study.

Results

The mean control values in both C_5 and control studies without C_5 are presented in Table 1, showing secretory responses to each stimulant.

Control studies

None of the parameters studied showed any change after the intramuscular injections of saline (0.04 ml/kg). Pancreatic secretions were maintained throughout the tests.

Urecholine

Pancreatic secretion stimulated by urecholine (200 $\mu g\,kg^{-1}\,h^{-1}$) was not influenced by C_5 at any dose whether given continuously intravenously or intramuscularly (Figure 1, Table 2). Bicarbonate and volume output showed a small depression after the

Table 2 Effect of (C_5) by continuous intravenous infusion on urecholine-stimulated pancreatic secretion

	Urecholine ($\mu g\,kg^{-1}\,h^{-1}$, i.v.							
	0	50	100	200	0	50	100	200
C_5 ($mg\,kg^{-1}\,h^{-1}$)	Vol (ml/10 min)				Protein (mg/10 min)			
0	6.3 ±0.7	8.2 ±0.8	9.0** ±0.9	10.0** ±1.0	11.1 ±1.3	23.9** ±3.4	26.6** ±1.6	38.6** ±6.0
0.125	5.2 ±1.2	6.9 ±1.1	7.0** ±1.1	8.5** ±1.2	6.8* ±1.4	13.3** ±1.5	17.8** ±2.5	28.7** ±4.2
0.250	6.4 ±0.7	8.0 ±1.1	9.8** ±1.1	10.5** ±1.2	6.5* ±1.9	13.9** ±0.6	21.7** ±2.8	34.4** ±5.8

Background, secretin 0.5 clinical units $kg^{-1}\,h^{-1}$ throughout.
*Significantly less than zero pentamethonium at least at 95% level.
**Significantly greater than zero urecholine.
In calculating C_5 effect on protein secretion, allowance is made for the effect with zero urecholine.
$n = 6$

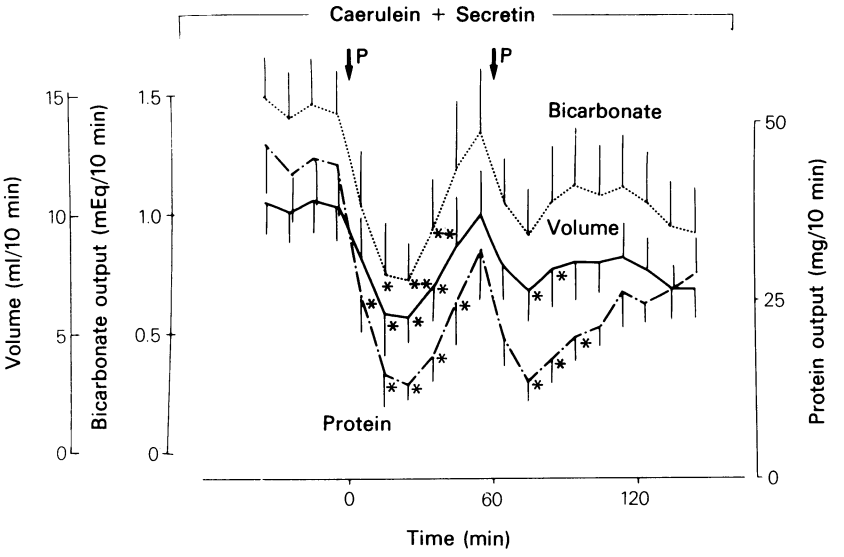

Figure 2 Effects of intramuscular pentamethonium (C_5) (1 mg/kg) given at P on exocrine pancreatic secretion in response to caerulein (25 ng kg^{-1} h^{-1}) with background GIH secretin (0.5 clinical unit kg^{-1} h^{-1}). Key as in Figure 1. Each point is a mean of six tests on six dogs; s.e.mean shown by vertical bars. Significant difference from the values of the control study: $*P < 0.05$; $**P < 0.01$.

initial intramuscular injection of C_5 but this decrease was not significant. In the experiments with intravenous C_5 and increasing doses of urecholine the increments in protein and volume with increasing doses were unchanged. This experiment was done to eliminate the possibility that the failure of C_5 to depress urecholine-stimulated secretion may have depended on the dose of urecholine. This is evidently not so. If allowance is made for the C_5 depression of protein at zero urecholine, there is no significant change in either volume or protein at any dose of C_5 or urecholine.

Caerulein

The administration of C_5 produced a significant decrease of pancreatic secretion stimulated by caerulein (25 ng kg^{-1} h^{-1}). Protein output was inhibited more markedly than either flow rate or bicarbonate output (Figure 2, Table 3). This was seen at all doses of C_5 used. Protein concentration also decreased significantly during the periods corresponding to decrease in protein output from 4.29 ± 0.67 to 2.45 ± 0.40 mg/ml in the experiments with intramuscular C_5. This depression did not outlast the effect of C_5 as judged by the nictitating membrane i.e., 60 min approximately. The concentration of bicarbonate was not significantly influenced.

Pentagastrin

Pentagastrin (3 μg kg^{-1} h^{-1})-induced pancreatic flow and bicarbonate output showed no significant change after C_5 but protein output decreased significantly. The action of C_5 on pentagastrin-stimulated protein output lasted longer than on protein output by caerulein (Figure 3) and indeed it did not recover with recovery of the nictitating membrane. Protein

Table 3 Effect of various doses of pentamethonium (C_5) on the caerulein response

C_5 (mg/kg, i.m.)	*Volume* (ml/10 min)	*HCO_3* (mEq/10 min)	*Protein* (mg/10 min)
0	8.22 ± 0.94	1.09 ± 0.13	41.87 ± 8.54
0.5	6.6 ± 0.70	0.86 ± 0.11	21.59 ± 3.24
1.0	5.4* ± 1.16	0.69* ± 0.17	16.05* ± 3.42
2.0	5.31* ± 0.7	0.68* ± 0.09	15.22* ± 4.18

Background, secretin 0.5 clinical units kg^{-1} h^{-1} throughout.
*Significantly less than zero pentamethonium at least at 95% level.
$n = 6$

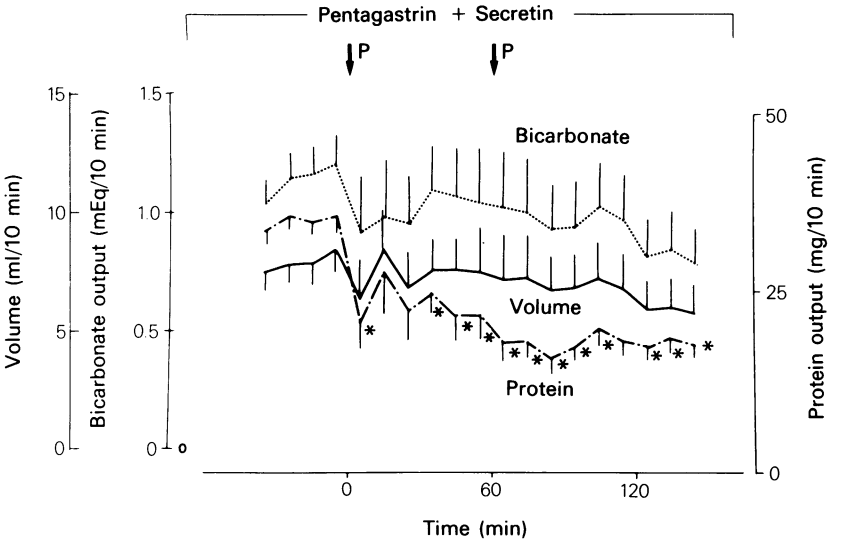

Figure 3 Effects of intramuscular pentamethonium (C_5) (1 mg/kg) given at P on exocrine pancreatic secretion in response to pentagastrin (3 $\mu g\,kg^{-1}\,h^{-1}$) with background GIH secretin (0.5 clinical units $kg^{-1}\,h^{-1}$). Key as in Figure 1. Each point is a mean of six tests on six dogs; s.e. mean shown by vertical bars. Significant difference from the values of the control study: $*P < 0.05$.

concentration was not significantly depressed except for the first 10 min period after the initial injection of C_5 (from 8.0 ± 0.9 to 6.5 ± 1.7 mg/ml during the first 10 min).

Discussion

Views on the effect of atropine on exocrine pancreatic secretion in response to exogenous CCK or caerulein are somewhat conflicting (Henriksen, 1969; Nakamura, English & Magee, 1969; Konturek *et al.*, 1972; Vaysse *et al.*, 1975) and it is still difficult to evaluate precisely the role of muscarinic mechanisms on the hormonally induced secretion. There have been few studies (Thomas, 1964; Hong & Magee, 1970) of the action of the nicotinic receptors on exocrine pancreatic secretion. The present results demonstrate that the action of caerulein or pentagastrin on pancreatic protein secretion is significantly depressed by C_5 (Figures 2 and 3). Therefore, the actions of caerulein and pentagastrin seem, at least partially to depend on a nicotinic receptor, but C_5 has a much more profound and transient effect on caerulein stimulation than on pentagastrin. The depression of pentagastrin far outlasts the obvious paralysis of the nicitating membrane while that on caerulein is in synchrony with it. Urecholine, on the other hand, must act distal to this; but differently since its action on exocrine pancreatic secretion was not significantly affected by C_5 (Figure 1) and because it is indisputably inhibited by atropine (Nakamure *et al.*, 1969).

In vitro studies on gut smooth muscle indicate that gastrin (Bennett, 1965; Lipshutz, Tuch & Cohen, 1971), CCK (Hedner & Rorsman, 1968) and its related peptides (Vizi, Bertaccini, Impicciatore & Knoll, 1972) stimulate motility via a cholinergic mechanism. Hexamethonium, however, did not modify this action of these peptides.

C_5 depressed volume and bicarbonate significantly in the caerulein but not in the pentagastrin or urecholine experiments. Since urecholine, pentagastrin and caerulein all augment secretin stimulation (Table 1) this must indicate involvement of a nicotinic mechanism for this action of caerulein also and possibly for pentagastrin but not for urecholine (Henriksen, 1968; Meyer Spingola & Grossman 1971; Douglas & Duthie, 1971).

In accord with previous work in intact and isolated pancreata, an obvious augmentation in secretin-stimulated fluid and bicarbonate secretion was seen with urecholine, caerulein and pentagastrin (Table 1) and the basal secretion of protein during secretin stimulation was reduced by pentamethonium (Table 2). As argued above, these effects of C_5 on protein and fluid secretion might be due to caerulein-sensitive nicotinic receptors on effector cells or in cholinergic ganglia or to the removal of cholinergic facilitation e.g., vagal tone at the ganglionic level. The above experiments do not distinguish between these alternatives.

The relevance of *in vitro* studies is difficult to assess since most of the work has been done in rat and guinea-pig pancreas in which, unlike the dog and man, secretin increases protein secretion proportionally with dose (Dockray, 1972; Gardner & Jackson, 1977).

It is noteworthy that the action of atropine on the pancreatic action of exogenous CCK or caerulein has always been equivocal and disputed (Thomas, 1941; Magee *et al.*, 1965; Henriksen, 1969; Konturek *et al.*, 1972; Singer, Solomon & Grossman, 1980). The few who have used ganglionic blockers (Thomas, 1964; Hong & Magee, 1970) have found unequivocal depression, as we did. These findings strongly support some form of nicotinic cholinergic mediation of the pancreatic actions of these polypeptides and could have considerable clinical potential. We have not found that increasing doses of C_5 have produced any increase in inhibition, one would suspect because we have used relaxation of the nicitating membrane as an independent sign of blockade. This was seen with every one of our doses and may represent maximal effectiveness.

D. F. M. is a Fogarty International Fellow. We gratefully acknowledge the excellent technical assistance of Robert Grimaud.

References

BENNETT, A. (1965). Effect of gastrin on isolated smooth muscle preparations. *Nature*, **208**, 170–173.

DOCKRAY, G.J. (1972). The action of secretin, CCK and caerulein on pancreatic secretion in the rat. *J. Physiol*, **225**, 679–692.

DOUGLAS, M. & DUTHIE, H.L. (1971). Pancreozymin and secretin in the control of pancreatic exocrine secretion. *Gut*, **12**, 862–869.

GARDNER, J.D. & JACKSON, M.J. (1977). Regulation of amylase release from dispersed pancreatic acinar cells. *J. Physiol.* **270**, 439–454.

HEDNER, P. & RORSMAN, G. (1968). Structures essential for the effect of cholecystokinin on the guinea pig small intestine in vitro. *Acta physiol. scand.*, **74**, 58–68.

HENRIKSEN, F.W. (1968). Effect of pancreozymin on the canine pancreatic secretion of fluid and bicarbonate. *Scand. J. Gastroenterol.*, **3**, 637–640.

HENRIKSEN, F.W. (1969). Effect of vagotomy or atropine on the canine pancreatic response to secretin and pancreozymin. *Scand. J. Gastroenterol.*, **4**, 137–144.

HONG, S.S. & MAGEE, D.F. (1970). Pharmacological studies on the regulation of pancreatic secretion in pigs. *Ann. Surg.*, **172**, 41–48.

KONTUREK, S.J., TASLER, J. & OBTULOWICZ, W. (1972). Effect of atropine on pancreatic responses to endogenous and exogenous cholecystokinin. *Am. J. Dig. Dis.*, **17**, 911–917.

LIPSHUTZ, W., TUCH, A.F. & COHEN, S. (1971). A comparison of the site of action of gastrin 1 on lower esophageal sphincter and antral circular smooth muscle. *Gastroenterology*, **61**, 454–460.

MAGEE, D.F., FRAGOLA, L.A. & WHITE, T.T. (1965). Influence of parasympathetic innervation on the volume of pancreatic juice. *Ann. Surg.*, **161**, 15–20.

MEYER, J.H., SPINGOLA, L.J. & GROSSMAN, M.I. (1971). Endogenous cholecystokinin potentiates exogenous secretin on pancreas of dog. *Am. J. Physiol.*, **221**, 742–747.

NAKAMURA, M., ENGLISH, G. & MAGEE, D.F. (1969). Relationship between pancreozymin and cholinergic stimulation of pancreatic secretion. *Can. J. Physiol. Pharmac.*, **47**, 33–38.

SINGER, M.V., SOLOMON, T.E. & GROSSMAN, M.I. (1980). Effect of atropine on secretion from intact and transplanted pancreas in dog. *Am. J. Physiol.*, **238**, G18–G22.

THOMAS, J.E. (1941). An improved cannula for gastric and intestinal fistula. *Proc. Soc. exp. Biol. Med.*, **46**, 260–261.

THOMAS, J.E. (1964). Mechanism of action of pancreatic stimuli studied by means of atropine-like drugs. *Am. J. Physiol.*, **206**, 124–128.

TISCORNIA, O., BRASCA, A.F., HAGE, G., PALASCIANO, G., DEVAUX, M.A. & SARLES, H. (1972). Les effets de l'atropine, du penthonium, de la vagotomie et du fluothane sur la secretion pancreatique du chien. *Biol. Gastro-Enterol.*, **5**, 249–256.

VAYSSE, N., BASTIÉ, M.J., PASCAL, J.P., ROUX, P., MARTINEL C.H., LACROIX, A. & RIBET, A. (1975). Role of cholinergic mechanisms in the response to secretin of isolated canine pancreas. *Gastroenterology*, **69**, 1269–1277.

VIZI, E.S., BERTACCINI, G., IMPICCIATORE, M. & KNOLL, J. (1972). Acethylcholine-releasing effect of gastrin and related polypeptides. *Eur. J. Pharmac.*, **17**, 175–178.

VOLLE, R.L. & KOELLE, G.B. (1975). Ganglionic stimulating and blocking agents. In *The Pharmacological Basis of Therapeutics*, ed. Goodman, L.S. & Gilman, A. pp. 565–574. New York: Macmillan Publishing Company, Inc.

(Received July 29, 1982. Revised September 14, 1982.)