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THE ACTION OF PORCINE GLUCAGON ON THE MOTILITY OF THE CANINE DUODENUM AND JEJUNUM

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1 Intravenous bolus doses of porcine glucagon of 0.001–0.05 mg kg^{-1} caused intense stimulation of the duodenum and jejunum of the dog.

2 Intravenous infusion of porcine glucagon at 0.025–0.05 mg kg^{-1} h^{-1} caused similar stimulation. In both cases the stimulation was phasic in nature.

3 Stimulation of the duodenum and jejunum following glucagon was accompanied by a decrease in frequency of the intestinal basic electrical rhythm (BER). No change was seen in the intervals between successive periods of phase III motor activity.

Introduction

Glucagon is a straight chain polypeptide produced by the A cells of the pancreatic islets and is thought to have similar properties to entero-glucagon which has been isolated in the stomach and large intestine (Montgomery & Welbourn, 1975). Whilst having its main action on glucose metabolism it has been shown also to inhibit gastrointestinal smooth muscle, diminishing food or morphine-stimulated activity in the stomach and duodenum of dogs (Necheles, Sporn & Walker, 1966) and the human jejunum (Dotevall & Kock, 1963) and reducing colonic motility in man (Taylor, Duthie, Cumberland & Smallwood, 1975). This action on smooth muscle has been used to clinical advantage during hypotonic duodenography (Chernish & Miller, 1972) and barium enema examination (Gohel, Dalinka & Goren, 1974) and has led to improved visualization during endoscopic retrograde pancreatography (Hradsky, Stockbrugger & Dotevall, 1974).

Wingate and his colleagues (Wingate, Morris & Thomas, 1977; Wingate & Pearce, 1979) studying electrical activity in the duodenum and jejunum of the conscious fasted dog, described an apparent discrepancy between glucagon given as an intravenous bolus and an infusion. Whilst a bolus dose of 1 mg of glucagon caused apparent inhibition of fast spiking electrical activity, an infusion of 0.5, 0.25 and 0.125 mg h^{-1} caused a significant increase in spiking activity.

We, therefore, examined the effect of glucagon on the duodenum and jejunum of the conscious fasted dog using both electrodes and force transducers enabling us to examine simultaneously the activity of both muscle layers.

Methods

In each of 10 greyhound dogs (weight 20–30 kg) three silver/silver chloride bipolar electrodes and three metal foil strain gauges were implanted under general anaesthesia. Two of the electrodes were sutured to the duodenum approximately 10 cm apart and the third to the proximal jejunum 20 cm distal to the ligament of Treitz. The metal foil strain gauges were constructed according to the technique described by Bass & Wiley (1972). Two strain gauges were sutured to the duodenum in a T configuration, one being aligned to record longitudinal, the other transverse muscle activity. The third gauge was sutured transversely to the proximal jejunum. Teflon-coated lead wires from the electrodes and strain gauges led to multi-pin connectors cemented into two stainless steel cannulae sutured into the anterior abdominal wall.

Recording of electrical and mechanical activity

The dogs were trained to stand quietly in a specially constructed cage throughout a recording session and were entirely cooperative even when intravenous procedures were being carried out. A Grass Model 7, eight-channel pen recorder (Grass Instr., Quincy, Mass.) was used for amplification of electrical and mechanical signals. Activity was simultaneously recorded on magnetic tape for subsequent data processing using an analogue seven-channel instrumentation tape recorder (Store 7D, Racal Thermionics, Southampton, England). All data were recorded at a tape speed of 15/16 inch per second, which allowed a frequency response of d.c. to 313 Hz.

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Processing of myoelectrical data

Myoelectrical signals were separated into their fast (spike) activity and slow wave (basic electrical rhythm, BER) components before computer processing. The data were replayed at 8 × real time (tape speed 7.5 inches per second) via a spike processor (Digitimer D139, Welwyn Garden City, Herts) which incorporated a high pass filter (rejecting those frequencies of less than 120 Hz) to give fast spike activity and a low pass filter (rejecting frequencies of greater than 14 Hz) to give slow wave activity. The unprocessed signal (BER and fast activity) and processed high frequency component were monitored simultaneously with an oscilloscope to confirm satisfactory spike discrimination.

Computer processing of mechanical and electrical data

From the duodenal recording site the BER, fast spike activity, mechanical transverse (MT) and mechanical longitudinal activity (and similarly the electrical components and MT from the jejunal site) were led to the input of the analogue to digital converter of a PDP 11/10 computer (Digital Electronic Corporation, Galway, Ireland). The computer processor was interfaced with two RK05 disc drive units, a Tektronix visual display unit (VDU) with hard copying facility and a Decwriter terminal.

A Fortran programme allowed sampling of all the digital signals at the end of a preset sampling interval. For the work described here we have found that a sampling period of 250 ms ensured accurate recording of all components of the incoming mechanical and electrical signals.

The data from multiple sampling intervals were summated over preset periods which we have termed analysis intervals, usually 10 or 15 s, and presented on the VDU as a series of plots of mechanical and electrical activity against time for the duration of the experiment. Maximum rates of spiking were noted and the total number of spikes were counted. The mechanical activity was quantified in terms of the maximum amplitude of the strain gauge deflection in both the longitudinal and transverse planes and expressed in arbitrary computer units, thus enabling valid comparisons to be made between experiments. Linear correlations were constructed between the mechanical and electrical parameters allowing examination to be made of the interrelationship between mechanical activity in both planes and electrical activity, regression lines and X–Y intercepts were displayed on the VDU and coefficients of variance (F) and correlation coefficients (r) calculated.

Administration of glucagon

Exogenous porcine glucagon (Novo Pharmaceuticals SA) was administered as an intravenous bolus dose or as an infusion by means of a single channel Sage pump (Orion Research Inc., Cambridge, Mass.). Bolus doses of 0.001–0.05 mg kg^{-1} were given and infusions for 30 min at a rate of 0.025–0.05 mg kg^{-1} h^{-1}.

All dogs were fed a standard tinned diet (Pedigree Chum) and then fasted for 18 h before being studied and experiments followed a standard form. Fasting activity was confirmed and observed until the activity front (phase III) of a migrating myoelectrical complex (MMC) (Szurszewski, 1969; Carlson, Bedi & Code, 1972; Marik & Code, 1975) had passed the duodenal recording site. For clarity we use the term 'activity front' to refer to the phase III activity of the MMC only. Ten minutes after the passage of the activity front, the bolus dose of glucagon was given or the infusion of glucagon started. Prior to infusing glucagon, saline (0.9% w/v NaCl solution) was infused for 10 min as a control. In some cases, recordings were extended in order to observe the passage of the following activity front. At least 6 experiments were carried out on each dog (60 experiments in all) and at least 3 days elapsed between each experiment. The first part of each study acted as the control period of fasting activity with which any subsequent activity would be compared. In each animal the order in which experiments were carried out was varied to compensate for changes in strain gauge transducer output, which in practice proved to be insignificant.

Results

Glucagon stimulated the duodenum and jejunum when given either as a bolus or an infusion. Figure 1 illustrates the duodenal response to a 1 mg (0.05 mg kg^{-1}) intravenous bolus of glucagon. The injection was given during the quiescent period (phase I) of interdigestive activity, ten minutes after the passsage of a phase III activity front on the duodenum and before its arrival in the jejunum. Stimulation was seen within 20 s, intense spiking activity being associated with contraction of the circular muscle and reciprocal lengthening of the longitudinal, an increase in length that may be due to a neuronally mediated inhibition or merely artefactual. Figure 2 shows the computer analysed record of this activity, illustrating the quantitative analysis of motility parameters and correlation coefficient of these data produced by the computer. A rapid increase in spiking occurs from quiescence to 80 spikes per min and at the end of the stimulation period, the total number of spikes is presented. Contraction and re-

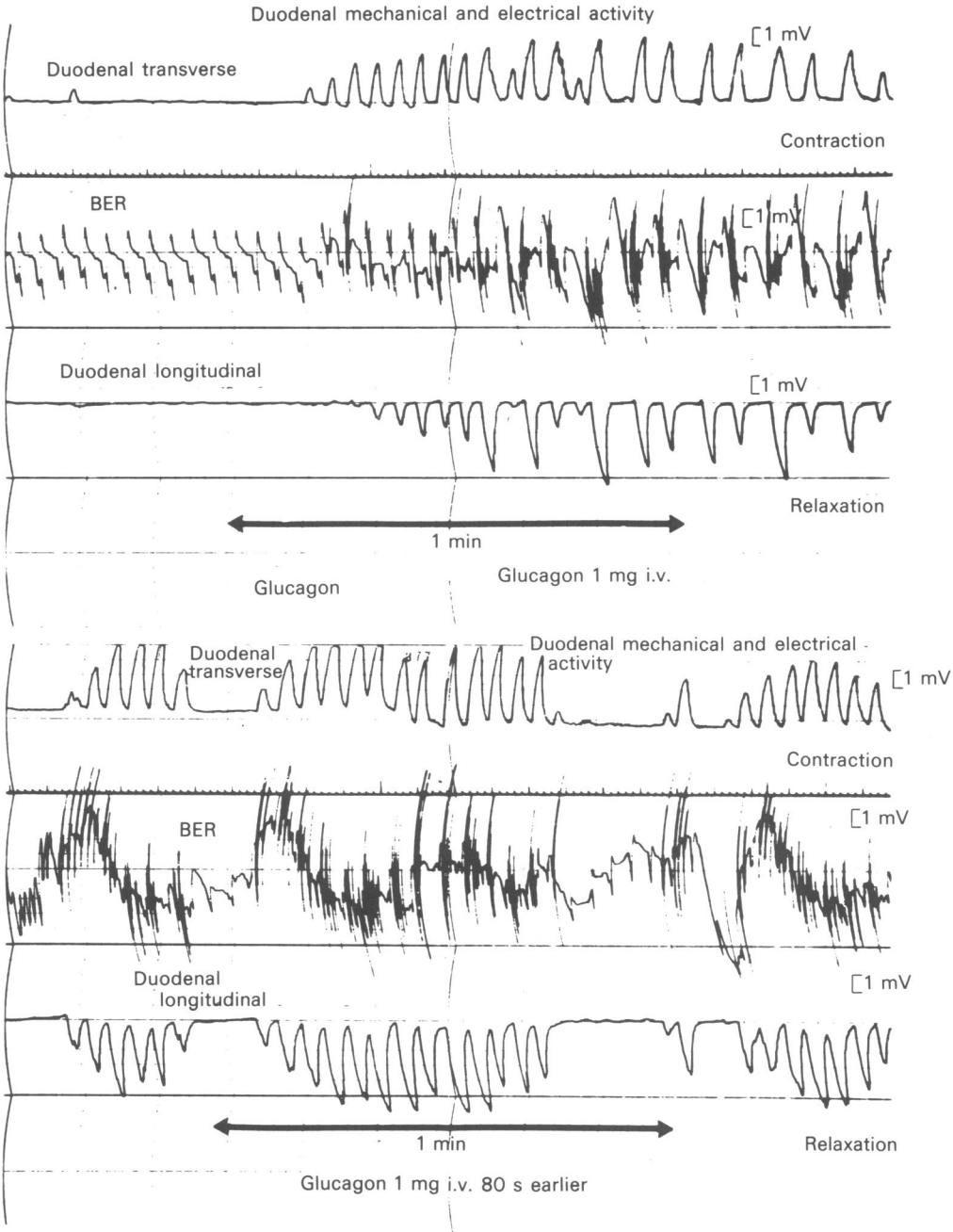

Figure 1 Record showing the effect of a 1 mg dose (i.v.) of glucagon on the electrical and mechanical activity of the canine duodenum. The upper three traces are sequential with the lower three traces. The electrical trace in the lower portion of the figure shows a superimposed rate of discharge of 3 per min.

ciprocal lengthening of the muscle layers is quantified in arbitrary units enabling the results of multiple experiments in the 10 animals to be compared. Reference to Figures 1 and 2 reveals that the glucagon stimulation occurs in a phasic manner, periods of stimulation lasting for about 40 s, alternating with shorter periods of quiescence. This phasic pattern was seen in 60% of all experiments; in the

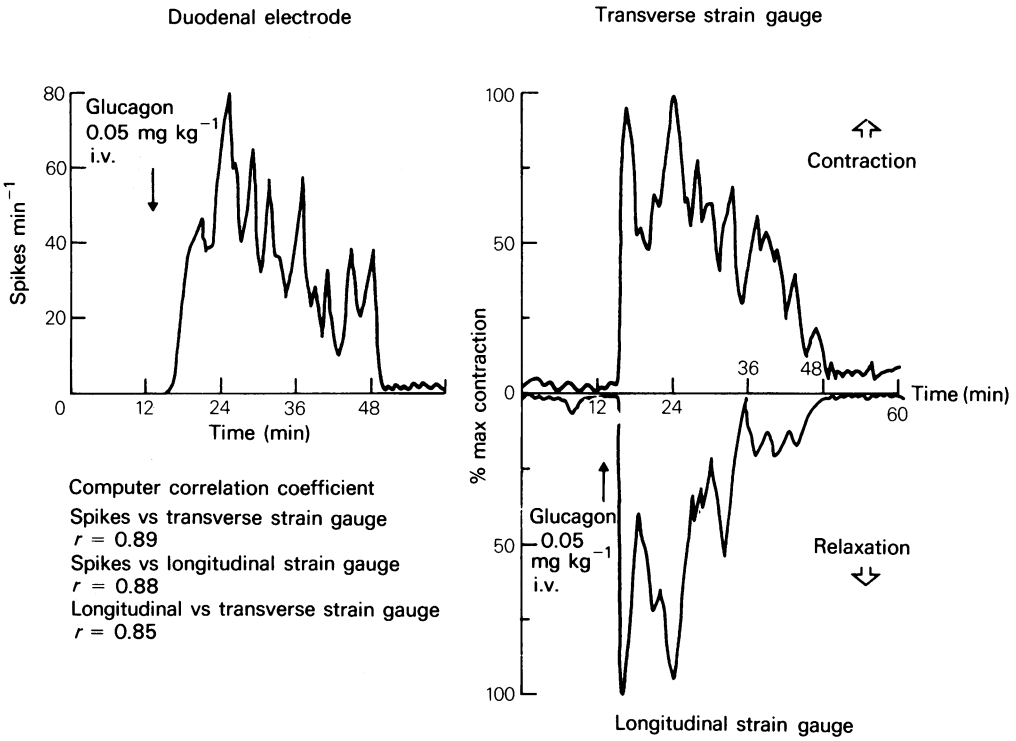

Figure 2 Computer analysis of activity following the bolus dose of glucagon seen in Figure 1. Glucagon given at arrow. Computed correlation coefficients for each plot are also included.

others the glucagon stimulation was continuous.

The activity seen after a 0.05 mg kg^{-1} bolus of glucagon is comparable to that seen during an activity front (phase III) and is of a similar magnitude. Stimulation of the jejunum occurred simultaneously and to the same degree (see below).

Dose-response relationship to glucagon

Bolus intravenous injections of glucagon caused stimulation of the duodenum in doses as low as 0.025 mg (1 μg kg^{-1}), a dose of 0.01 mg (0.4 μg kg^{-1}) having no effect. Increasing doses of glucagon were given in two experiments in each of 4 dogs and the degree of stimulation analysed. The results are illustrated in Figure 3.

Intravenous infusion of glucagon

Intravenous infusion of glucagon caused profound mechanical and electrical stimulation of the duodenum. A dose of 0.05 mg kg^{-1} h^{-1} caused stimulation to start within 30 s (Figure 4) which continued in a phasic manner throughout the period of the infusion; 40 to 60 s bursts of activity alternated with quiescent periods. Activity during glucagon infusion was quantified as before, enabling the results of 3 infusion experiments in each dog to be compared; each experiment was performed on a different day. Stimulation occurred at infusion rates as low as 0.0125 mg kg^{-1} h^{-1}, infusion rates lower than this having no effect.

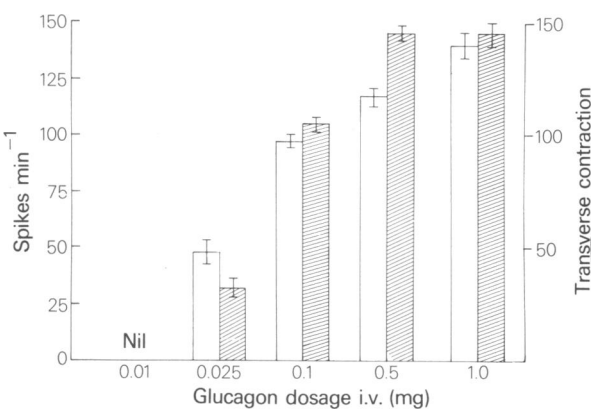

Figure 3 Dose-response relationship of electrical and mechanical activity following glucagon injection in dog. Open column = spikes; hatched columns = contractions. Vertical lines indicate s.d.

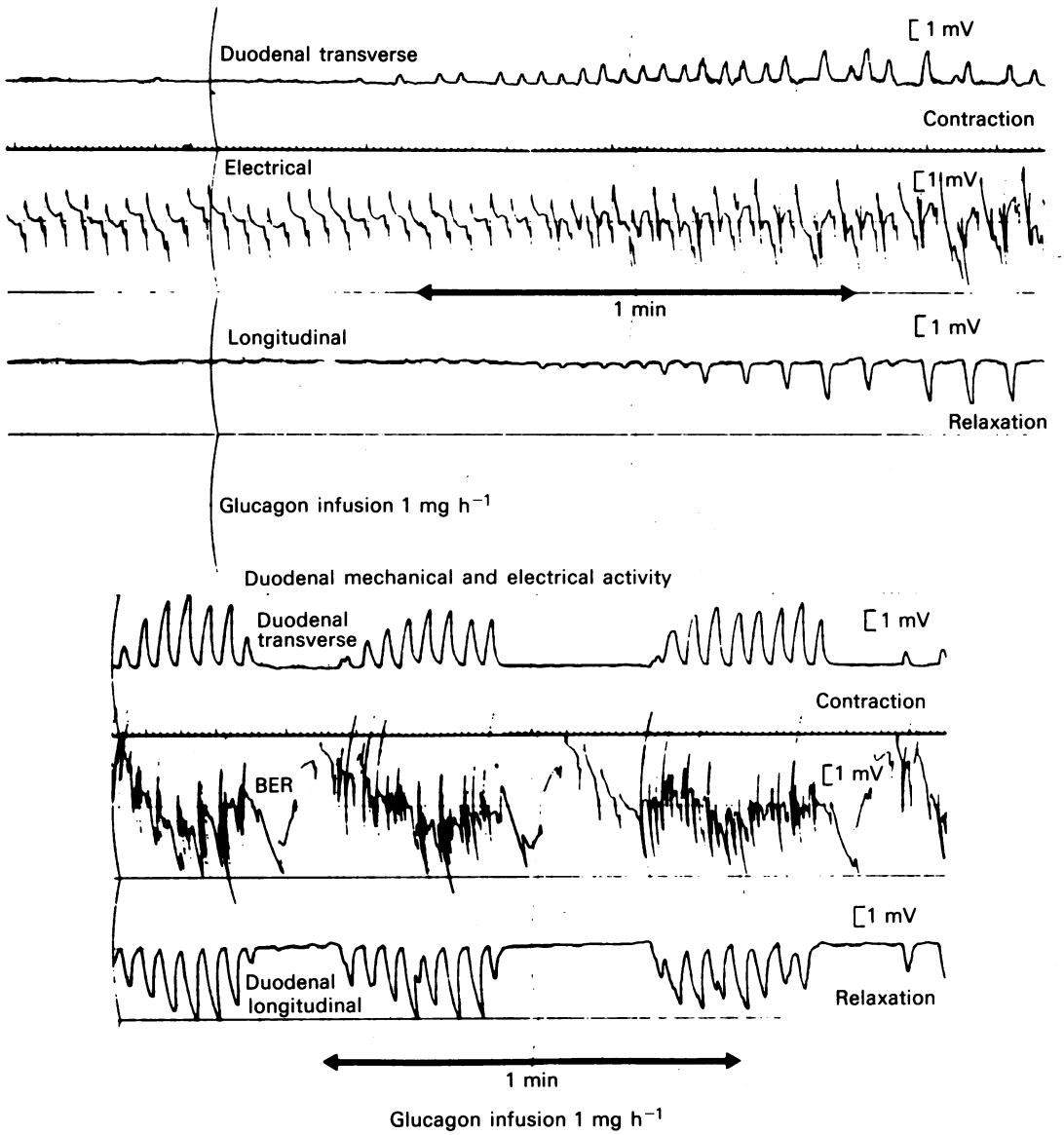

Figure 4 Recording of duodenal mechanical and electrical activity during infusion of glucagon at 1 mg h^{-1}. Infusion started at arrow shown in upper three traces, the lower traces are sequential.

Table 2 summarizes the results of the glucagon bolus and glucagon infusion experiments on numbers of spikes and spiking rates and on contraction and lengthening of the muscle layers.

Administration of glucagon and the interdigestive complex

The interval between successive activity fronts (phase III of the complex) was examined both before and after a 0.05 mg kg^{-1} bolus dose of glucagon given at a standard time in the cycle. No change was seen after a bolus dose of glucagon (Table 1), the next activity front occurring when expected and propagating at the normal rate between duodenal and jejunal transducers.

After an intravenous infusion of glucagon (0.05 mg kg^{-1} h^{-1}) causing phasic stimulation for approximately the duration of the infusion, the onset of the next phase III activity front was delayed by approximately the time of the infusion (30 min) (Table 1) but again propagated at the normal rate.

Table 1 The effect of glucagon on phase III intervals in the dog

	Bolus		*Infusion*	
	Control period	After 0.05 mg kg^{-1}	Control period	After infusion 0.05 mg kg^{-1} h^{-1}
Duodenum	100 ± 3.14	98.6 ± 7.3	103 ± 2.1*	132 ± 5.6*
Jejunum	105 ± 2.9	101 ± 3.8	108 ± 4.6**	143 ± 7.3**

Values are mean ± s.e.mean.
*$t = 10.31$, $P < 0.001$; **$t = 9.56$, $P < 0.001$.

Glucagon and basic electrical rhythm frequency

Frequency analysis of recordings of BER after a bolus injection of 0.04 mg kg^{-1} glucagon showed a significant slowing of the intrinsic duodenal electrical rhythm from 18.2 to 16 cycles min^{-1} ($t = 4.5$, $P < 0.005$). The entire rhythm is also modulated by a slower component of 3 cycles min^{-1}, accounting for the increase in amplitude of the slow wave in Figure 1 after the administration of glucagon.

Action of glucagon on the jejunum

Jejunal electrodes and transversely orientated strain gauges were implanted in 4 dogs in addition to sensors on the duodenum. At least 2 bolus and infusion doses of glucagon were given to each; in these experiments the glucagon was administered during phase II of interdigestive activity prior to the arrival of the activity front (phase III). Glucagon stimulated the jejunum in a similar manner to that described in detail for the duodenum, the results are summarized in Table 2. Stimulation was simultaneous to that seen on the duodenum and did not propagate from one to the other.

Correlation between mechanical and electrical activity

The correlation programme allows analysis to be made of the interactions between spiking and longitudinal and transverse mechanical activity and of the 2 mechanical parameters together. Table 3 shows the mean computed correlations for glucagon stimulated activity both after bolus and infusion and for the period of the activity front (phase III). All correlation

Table 2 Mechanical and electrical stimulation of the duodenum and jejunum in the dog after intravenous bolus or infusion of glucagon

Glucagon dosage	*Total number of spikes*	*Spikes/min*	*Longitudinal strain gauge*	*Transverse strain gauge*	*Significant change from control period*
Duodenum					
Bolus 0.05 mg kg^{-1} $n = 30$	432 ± 37	140 ± 9.2 *	105 ± 6.9	144 ± 9.01 **	*$t = 14.2$ $P < 0.001$ **$t = 14.06$ $P < 0.001$
Infusion 0.05 mg kg^{-1} h^{-1} $n = 30$	1,366 ± 45	116 ± 13 *	90.4 ± 4	113 ± 7.06 **	*$t = 8.9$ $P < 0.001$ **$t = 16.00$ $P < 0.001$
Jejunum					
Bolus 0.05 mg kg^{-1} $n = 8$	158 ± 29	76 ± 7 *	141 ± 17.01 **	---	*$t = 19$ $P < 0.001$ **$t = 14$ $P < 0.001$
Infusion 0.05 mg kg^{-1} h^{-1} $n = 8$	1,480 ± 53	84 ± 5 *	132 ± 8.3 **	---	*$t = 29$ $P < 0.001$ **$t = 27$ $P < 0.001$

Values are mean ± s.e.mean.

Table 3 Correlation between spiking rates and activity of circular and longitudinal muscle of duodenum after glucagon bolus, glucagon infusion and during an activity front (Phase III)

	Transverse		*Longitudinal*		*Longitudinal and transverse*	
Glucagon bolus $0.05\,mg\,kg^{-1}$	*F* 494	*r* 0.89	*F* 224	*r* 0.80	*F* 320.7	*r* 0.85
Glucagon infusion $0.05\,mg\,kg^{-1}\,h^{-1}$	886	0.93	842	0.88	1,131	0.91
Phase III activity front	117	0.68	187	0.69	187	0.68

r = Correlation coefficient; *F* = coefficient of variance.
All *r* values significantly different from zero <0.001.

coefficients of variance (*F*) are significant but closer correlation occurred after glucagon than during an activity front (phase III).

Discussion

Glucagon intensely stimulated the canine duodenum and jejunum when given either as an intravenous bolus or as an infusion. In all experiments the glucagon was administered at a similar time in the interdigestive cycle, phase I in the duodenum and phase II in the jejunum. There was no evidence that glucagon acted differently during the two phases, a similar degree of stimulation being consistently noted at each recording site. After a bolus dose, the next phase III activity front occurred when expected in the cycle; after an infusion, the front was delayed by approximately the length of the infusion thereby suggesting that a disruption of the complex had taken place. Our results differ from those of Wingate and his colleagues (1977, 1979) who, whilst recording stimulation during infusions as low as $0.6\,mg\,h^{-1}$, saw an apparent inhibition of spiking activity after a bolus, the next activity front being attenuated. It is not clear if these workers gave the glucagon bolus at the same point in the interdigestive cycle in every experiment and high speed recording techniques were used which would have made it difficult to identify a short period of stimulation on subsequent replay.

We noted duodenal and jejunal stimulation to occur after bolus glucagon doses as low as $1\,\mu g\,kg^{-1}$ and during infusions of $0.0125\,mg\,h^{-1}$. This is some 100 times greater than the infusion level of $180\,ng\,kg^{-1}\,h^{-1}$ noted by Sacca, Sherwin & Felig (1978) to cause a rise in plasma glucagon in the dog to $200\,pg\,ml^{-1}$, representing peak physiological plasma concentrations. It is of interest to note that Patel, Whalen, Soergel, Wil & Meade (1979) recorded inhibition of jejunal motility in human volunteers only when glucagon infusion rates gave plasma concentrations in excess of $800\,pg\,ml^{-1}$, which is approximately four times the peak physiological concentration.

The myoelectrical and mechanical stimulation after either a bolus or during a constant infusion of glucagon at times occurred in a phasic manner (Figures 1 and 4). Phasic electrical changes have not been reported after glucagon administration but increases in gastric mucosal potential difference after glucagon have been a constant finding (Tarnawski, Ivery, McGuigan & England, 1978). We found glucagon lowered significantly the duodenal BER frequency. It is of interest that pentagastrin has been shown to have the opposite effect, increasing the BER frequency (Wingate, Pearce, Hutton, Dand, Thompson & Winsch, 1978). In contrast, glucagon and pentagastrin both cause an increase in small intestinal fast spiking activity but have opposite effects on mucosal PD and BER reates.

In addition to decreasing the intrinsic BER frequency of the duodenum, glucagon caused superimposition of a slower rate of 3 cycles per min (Figure 1), a phenomenon that has been described in the fasting animal (Waterfall, Duthie & Brown, 1973). This slower rate may be the gastric antral rate superimposed on both canine and human duodenal BER recordings. An antral rate of 3 per min would represent a slowing of the normal canine antral BER. To clarify this point, direct antral recordings would be of interest.

Bueno & Ruckebusch (1976) found insulin caused an increase in electrical fast spiking activity, simulating feeding in dogs and sheep. Inexplicably, they found no response to 1 mg injections of glucagon in their animals. We do not think the stimulation recorded by us was due to glucagon-stimulated endogenous insulin release for the following reasons. The spiking activity reported after insulin was less intense than that seen during activity fronts (phase III) and lasted 4–5 h, in contrast to the 10–15 min of intense stimulation seen after glucagon. After a bolus injection of glucagon or infusion Wingate and co-

workers (1977, 1979) noted a moderate, rapid rise in insulin levels more marked after the infusion than after the bolus.

The method of digital analysis used allows for comparison to be made between patterns of movement of the intestine in the tranverse and longitudinal planes and their association with fast spiking activity. However, it must be noted that the longitudinal lengthening recorded by the transducer may be either an entirely passive elongation as a consequence of circular muscle contraction, i.e. artefactual, or real, due to a neuronally mediated inhibition of the longitudinal muscle fibres. Wood & Perkins (1976) have shown that longitudinal elongation of the intestine takes place even when the longitudinal muscle has been removed, whilst others (Gonella, 1972) suggest relaxation to be an active property of the muscle layer. The longitudinal lengthening noted by us was an exact inverse of the transverse change suggesting an artefactual lengthening merely due to a difference in alignment of the transducers. However, there is no doubt that spiking was more closely correlated with mechanical activity after glucagon than when an activity front was recorded by us (Table 3) or others (Bass & Wiley, 1965).

The canine duodenum responds differently from the duodenum of man to pharmacological doses of glucagon and the findings described in this paper suggest caution in extrapolating results obtained from the study of gastrointestinal hormones in the dog to the human situation.

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References

BASS, P. & WILEY, J.N. (1972). Contractile force transducer for recording muscle activity in unanaesthetised animals. *J. appl. Physiol.*, **32,** 567–570.

BASS & WILEY, J.N. (1965). Electrical and extraluminal contractile force activity of the duodenum of the dog. *Am. J. dig. Dis.*, **10,** 183–200.

BUENO, L. & RUCKEBUSCH, I. (1976). Insulin and jejunal electrical activity in dogs and sheep. *Am. J. Physiol.*, **230,** 1538–1544.

CARLSON, E.M., BEDI, B.S. & CODE, C.F. (1972). Mechanisms of propagation of intestinal interdigestive myoelectrical complex. *Am. J. Physiol.*, **222,** 1027–1030.

CHERNISH, S.M. & MILLER, R.E. (1972). Hypotonic duodenography with the use of glucagon. *Gastroenterology*, **63,** 309.

DOTEVALL, G. & KOCK, N.G. (1963). The effect of glucagon on intestinal motility in man. *Gastroenterology*, **45,** 364–367.

GOHEL, V.K., DALINKA, M. & GOREN, G.S. (1974). Hypotonic examination of the colon with glucagon. *Radiology*, **115,** 1–4.

GONELLA, J. (1972). Modifications of electrical activity of the rabbit duodenal longitudinal muscle after contractions of the circular muscle. *Am. J. dig. Dis.*, **17,** 327–336.

HRADSKY, M., STOCKBRUGGER, R. & DOTEVALL, R. (1974). The use of glucagon during upper gastrointestinal endoscopy. *Gastrointest. Endosc.*, **20,** 162.

MARIK, J.G. & CODE, C.F. (1975). Control on interdigestive myoelectrical activity in dogs by the vagus nerves and pentagastrin. *Gastroenterology*, **69,** 387–395.

MONTGOMERY, D.A.D. & WELBOURN, R.B. (1975). *Medical and Surgical Endocrinology*. pp. 401–403. London: Edward Arnold.

NECHELES, H., SPORN, J. & WALKER, L. (1966). Effect of glucagon on gastrointestinal motility. *Am. J. Gastroenterology*, **45,** 34–39.

PATEL, G.K., WHALEN, G.E., SOERGEL, K.H., WIL, W.C. & MEADE, R.C. (1979). Glucagon effects on the human small intestine. *Digestive Diseases and Illnesses*, **24,** 501–508.

SACCA, L., SHERWIN, R. & FELIG, P. (1978). Effect of sequential infusions of glucagon and epinephrine on glucose turnover in the dog. *Am. J. Physiol.*, **235,** E287–290.

SZURSZEWSKI, J.H. (1969). A migrating electric complex of canine small intestine. *Am. J. Physiol.*, **127,** 1757–1763.

TARNAWSKI, A., IVERY, K.J., McGUIGAN, J.E. & ENGLAND, J. (1978). Effect of glucagon and pentagastrin on gastric mucosal potential difference in man. *Gut*, **19,** 1116–1121.

TAYLOR, I., DUTHIE, H.L., CUMBERLAND, D.C. & SMALLWOOD, R. (1975). Glucagon and the colon. *Gut*, **16,** 973–978.

WATERFALL, W., DUTHIE, H.L. & BROWN, E. (1973). The electrical and motor action of gastrointestinal hormones on the duodenum in man. *Gut*, **14,** 689–696.

WINGATE, D., MORRIS, D. & THOMAS, P. (1977). Glucagon stimulates intestinal motor activity. *Gut*, **18,** A966.

WINGATE, D.L., PEARCE, E.A., HUTTON, M., DAND, A., THOMPSON, H.H. & WINSCH, E. (1978). Quantitative comparison of the effects of cholecystokinin, secretin and pentagastrin on gastrointestinal myoelectric activity in the conscious fasted dog. *Gut*, **19,** 593–601.

WINGATE, D.L. & PEARCE, E. (1979). The physiological role of glucagon in the gastrointestinal tract. In *Glucagon in Gastroenterology* ed. Picazo, J. pp. 19–38. Lancaster: M.T.P. Press.

WOOD, J.D. & PERKINS, W.E. (1976). Mechanical interaction between longitudinal and transverse axes of the small intestine. *Am. J. Physiol.*, **218,** 762–768.

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