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VAGAL GLUCORECEPTORS IN THE SMALL INTESTINE OF THE CAT

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SUMMARY

1. In anaesthetized cats, the unitary activity of seventy-eight sensory vagal neurones was recorded in nodose ganglia by means of extracellular glass microelectrodes.

2. These neurones were stimulated by perfusion of the small intestine (duodenum and first part of jejunum) with glucose or other different carbohydrates at concentrations of 1–20 g/l. (i.e. 55–1100 m-osmole/l.).

3. The neurones were slowly adapting to stimulation and their discharge frequency was always low (1–30 Hz).

4. The activity of these neurones depended on the particular carbohydrate used and on its concentration: the discharge frequency generally increased when the concentration rose.

5. The neurones were of the C type (conduction velocities: 0·8–1·4 m/sec; mean, 1·1 m/sec).

6. In contrast with the known neurones connected to the gastro-intestinal tension receptors, they were not obviously activated by intestinal contractions or distensions.

7. In the same way, the stimuli which produced the response of other known endings, i.e. the mucosal receptors, were not effective; these stimuli included in particular stroking of the mucosa, over-distension of the bowel, intestinal perfusion with alkaline or acid solutions. On the other hand, the use of substances other than glucose (KCl and NaCl of the same osmolarity) showed that the osmotic pressure was not directly related to the receptor activation.

8. Therefore it is proposed to call the endings corresponding to these neurones 'glucoreceptors'.

9. The effect of glycaemia and intestinal motility were also studied. These variables acted presumably by changing the intestinal absorption rate.

10. The functional characteristics of the glucoreceptors (in particular the short latency of their response) strongly suggested that they were located close to the intestinal epithelium.

11. An ultrastructural study was performed in an attempt to identify the histological site of the receptors. Many non-medullated fibres were observed in the villi, especially beneath the epithelial layer. They gave complex branchings with abundant swellings. Some of them, at least, belonged to the vagal sensory component, because they were less numerous after unilateral selective sensory vagotomy. Therefore these complex endings could serve as the vagal glucoreceptors.

12. The roles of vagal intestinal glucoreceptors are discussed. Their functional characteristics as well as the clinical and experimental data suggest that they may be involved in the regulation of different types of alimentary behaviour (hunger, thirst, alliesthesia) and energy balance.

INTRODUCTION

Numerous experimental and clinical observations have suggested the existence of gastro-intestinal chemoreceptors, especially glucoreceptors.

First, satiety, hunger and energy balance depend on internal signals which indicate the nature of nutriments to integrative centres (hypothalamus) (see Anderson, 1972).

Secondly, when introduced into the duodenum, substances such as HCl or glucose are able to modify the gastric motility via the extrinsic innervation (Leek, 1972; Bell & Watson, 1976; Roze, Couturier, Chariot & Debray, 1977).

Thirdly, differences observed during oral and intravenous glucose tolerance tests suggest the existence of nervous or neuro-humoral mechanisms originating from the gastro-intestinal tract (Henderson, Jefferys, Jones & Stanley, 1976).

Fourthly, recent data concerning alliesthesia (modification of alimentary pleasure related to internal state (Cabanac, 1971; Cabanac & Duclaux, 1973) afford evidence for the existence of glucoreceptors.

Unfortunately, there has been no electrophysiological evidence for the existence of gastro-intestinal glucoreceptors. However Iggo (1957), Paintal (1957), Davison (1972), Harding & Leek (1972*a*, *b*), Clarke & Davison (1974), Bitar, Mei & Michelucci (1975), Leek (1977) have described gastro-intestinal receptors located in the mucosa which are activated both by mechanical and chemical stimuli. On the other hand, Sharma & Nasset (1962) obtained multi-unit records from mesenteric nerves and reported that discharges were elicited in these nerves by perfusion of the small intestine with glucose and different amino-acids. Also Niijima (1969*a*) has described glucoreceptors located in the hepatic circulation. Their activity decreased when glycaemia increased.

The morphological properties of the sensory fibres supplying the gastro-intestinal tract may explain the poverty of the electrophysiological data. Actually, the sensory visceral fibres have a small diameter and chiefly belong to the non-medullated population. This is the case for the vagal fibres, seventy per cent of which are non-medullated (Agostoni, Chinnock, De Burgh Daly & Murray, 1957; Mei, 1970*a*).

So far two preliminary notes have pointed out the existence of vagal intestinal glucoreceptors (Mei, 1969; Mei, Boyer & Arlhac, 1973), but the present work provides the first systematic study on this subject. It is based on a technique in which extracellular micro-electrodes are implanted into the sensory ganglia (spinal or cranial ones). This permits easy recording of the unitary discharges carried by all the known fibres, and especially by the small sized ones. We have developed this recording method in our laboratory during the past fifteen years (Mei, 1962), chiefly for the systematic study of the visceral mechanoreceptors belonging to vagal (Mei, 1970*a*, *b*, *c*), splanchnic (Ranieri, Mei & Crousillat, 1973; Ranieri, Crousillat & Mei, 1975) and pelvic (Arlhac, 1972) territories.

METHODS

Electrophysiological experiments

Experiments were performed on fifty-eight male or female adult cats (2·4–5 kg), anaesthetized with chloralose (75 mg/kg I.V.) after halothane induction.

Action potential recording

Single vagal units were recorded from the nodose ganglia by means of external glass micro-electrodes filled with a 3 M-KCl solution. Micro-electrodes were implanted either in the left ganglion (ten experiments) or in the right one (forty-eight experiments). Through a short incision in the lateral laryngeal region, the nodose ganglion was exposed and set on a special metallic support to avoid movement artifacts due to respiratory and cardiac functions. Finally, the connective sheath was carefully dissected under an operating microscope. The electronic apparatus used to record vagal unitary potentials was a classical one which has been previously described (Mei, 1970*a*); an ink-jet recorder Myngograph, a tape-recorder and an impulse-frequency meter were added to facilitate action potential analyses.

Electrical stimulation

Two pairs of stimulating electrodes were set under the ipsilateral vagal nerve, respectively at the inferior cervical level and at the inferior thoracic level (Text-fig. 1) in order to test the vagal C fibre activity and to choose the gastro-intestinal C fibres. Two different surgical preparations were employed for the positioning of the thoracic electrodes according to their type: (1) a large definitive thoracotomy (sternal approach) when using silver hooks or (2) a short temporary thoracotomy (intercostal approach) when using 'chronic' electrodes. In this case, artificial ventilation was stopped as soon as the intrapleural pressure had recovered. The vagal nerve was dissected at the end of the experiment to measure the conduction distance and, hence, the conduction velocity.

The contralateral vagal nerve at its cervical level was placed across another pair of stimulating electrodes in order to produce direct or reflex changes of the intestinal motility.

Chemical stimulation

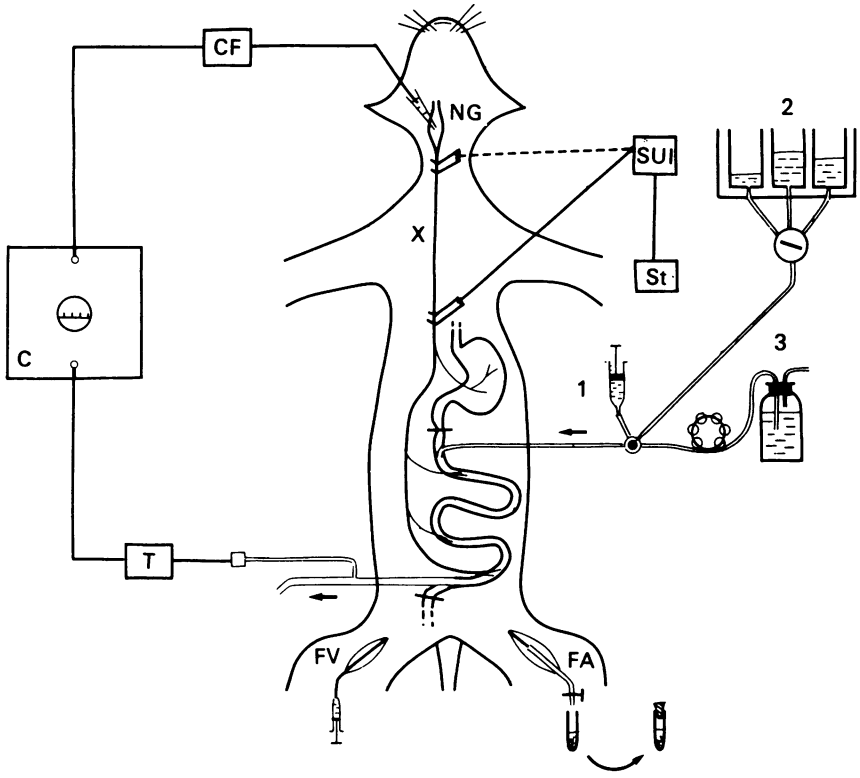
The proximal part of the small intestine, including the duodenum (except the first 2–3 cm corresponding to the intestinal bulb) and the upper part of the jejunum, was isolated and perfused (Text-fig. 1). In addition, the remaining part of jejunum was arranged in the same way (two experiments).

Perfusions were performed according to two techniques: (*a*) introduction of 40 ml. in 20 sec with a syringe or (*b*) continuous infusion with perfusion reservoirs or with a peristaltic pump (flow rate 1–5 ml./sec).

The perfusions were carried out with test or rinsing solutions maintained at a constant temperature (36–37 °C). Generally these two types of solutions were held in place for 1, 2 or 5 min.

Test solutions. In most of the experiments (forty-eight), the test solutions consisted of different carbohydrates the list of which is given in Table 1. In these experiments, the carbohydrates were applied at several concentrations (1–20 g/l.) as shown in Table 2*A*, but 10 g/l. was used more often than the other concentrations. In each experiment, glucose was used alone (eleven experiments) or with one, two or three supplementary carbohydrates (thirty-seven experiments). In this last case, there was no preferential order for using the different carbohydrates. In the other experiments (ten), the effect of osmotic pressure was especially investigated. For this purpose, we used solutions of glucose, KCl and NaCl (275, 550 and 1100 m-osmole/l.); these osmotic pressures corresponded to the glucose solutions containing 5, 10 and 20 g/l. (Table 2*B*); in three of these experiments, we tested in addition an acid solution (HCl, pH 2·0, 20 m-osmole/l.) and an alkaline solution (NaOH, pH 9·0, 2×10^{-2} m-osmole/l.).

Rinsing solutions. Between two consecutive tests, the small intestine was washed with NaCl solution (9 g/l.) or water.



Text-fig. 1. Experimental arrangement used for studying the glucoreceptors. On the left: recording of (*a*) the unitary potentials in the nodose ganglion (NG); CF, cathode follower; C, cathode-ray oscillograph; (*b*) the intraluminal pressure; T, pressure transducer. On the right: stimulation of (*c*) the vagal nerve (X) at the thoracic or the cervical level; St, neurostimulator; SUI, isolation unit; (*d*) the small intestine with a syringe (1), a perfusion reservoir (2) or a peristaltic pump (3). FV, femoral vein; FA, femoral artery.

TABLE 1. Different carbohydrates used. Values in parentheses indicate the test number

Carbohydrate class and molecular weight	Name
Pentoses 150.30	D-arabinose (5), D-xylose (4)
Hexoses 180.16	D-glucose (37), D-levulose (18) D-galactose (5), L-sorbose (4), D-mannose (2)
Disaccharides 324.30	Saccharose (11), maltose (10), lactose (11), D-cellobiose (1)
Trisaccharides 594.50	Raffinose (2)

Mechanical stimulation

Different stimulations were employed: distension achieved with the rinsing solution or with a balloon; digital compression of the empty or inflated bowel, phasic or tonic stroking of the mucosa with a soft tip probe, spontaneous contractions of the small intestine or evoked con-

tractions elicited by electrical stimulation of the contralateral vagal nerve. In some experiments, the intraluminal pressure was recorded as shown in Text-fig. 1.

Intravenous injection, dosage of glycaemia

In all the experiments, the femoral vein was cannulated to inject different substances (anaesthetics, glucose, phenyl-diguanide) into the blood flow. In one experiment, the portal vein was bypassed in order to inject glucose into the hepatic circulation. In five experiments, the femoral artery was also cannulated to sample blood and to measure the glycaemia (glucose oxidase method) and the insulinaemia (radioimmunoassay).

TABLE 2. Different solutions used to study the effect of osmotic pressure. *A*, different concentrations of glucose used and equivalent osmotic pressures. *B*, different osmotic pressures used with glucose, NaCl and KCl solutions, and equivalent concentrations (g/l.)

A

Glucose concentration (g/l.)	Osmotic pressure (m-osmole/l.)
1	55
3	163
5	275
10	550
20	1100

B

Osmotic pressure	Concentration (g/l.)		
(m-osmole/l.)	Glucose	NaCl	KCl
275	5	8·05	10·25
550	10	16·10	20·50
1100	20	32·20	40·95

Histological preparations

These involved two additional adult cats anaesthetized with sodium pentobarbitone.

The first was a normal animal (intact vagal nerves). It was perfused *in toto* with: (1) a rinsing solution: tyrode solution containing 0·1 % procaine hydrochloride (0·1 %) and heparin (5000 i.u./l.), (2) a fixation solution: glutaraldehyde, paraformaldehyde, 0·2 M-phosphate buffer, pH 7·2–7·4. Small pieces of mucosa were sampled under an operating microscope. After rinsing (glucose buffer solution) and post-fixation (2 % osmium tetroxide in buffer solution), they were prepared for ultrastructural observations. The mucosa (and more especially the villi) was sectioned either transversally or longitudinally. In three cases, serial longitudinal sections were performed in order to reconstruct the pathway of nerve fibres in a villus.

The second cat was operated upon under aseptic conditions a month before being killed in order to make a selective sensory vagotomy on the right side (ablation of the nodose ganglion according to a method already described; Mei, 1966). Villi were prepared as before.

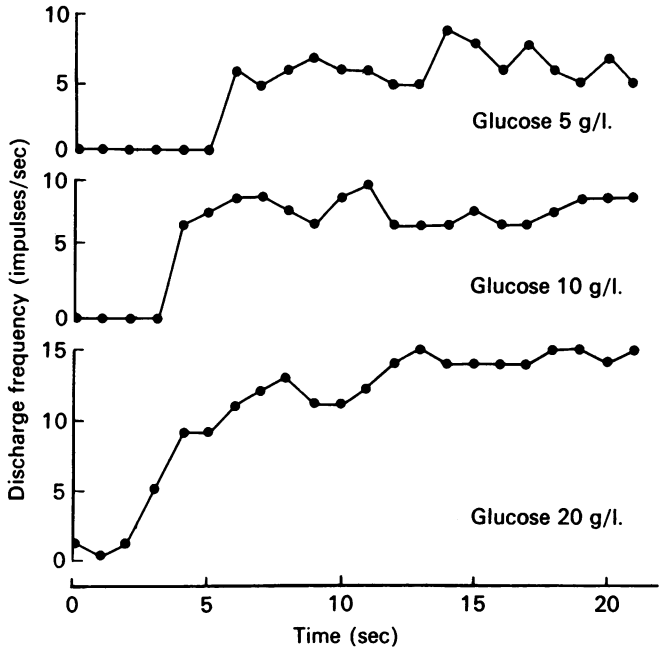
RESULTS

Electrophysiological data

Sixty-eight vagal units were activated by glucose perfusion of the small intestine. Thirty-seven of them were tested in addition with other carbohydrates (see Table 1). In all 140 tests were performed. In the special experiments devoted to the study of the osmotic pressure, ten supplementary receptors were studied.

Receptor response to intestinal glucose perfusion; discharge pattern and adaptation

All the receptors were silent before the first perfusion. However, after one or more tests, a low frequency discharge was visible (Text-fig. 2). They were slowly adapting to stimulation. Actually the discharge generally persisted throughout the perfusion (1–5 min). If the stimulation was maintained longer, the receptor discharge decreased regularly, but it very often persisted 1 hr, as in Text-fig. 3. The discharge often continued after rinsing also, but it was markedly diminished. Sometimes, when using the syringe method, we could observe a transient activation during the rinsing.



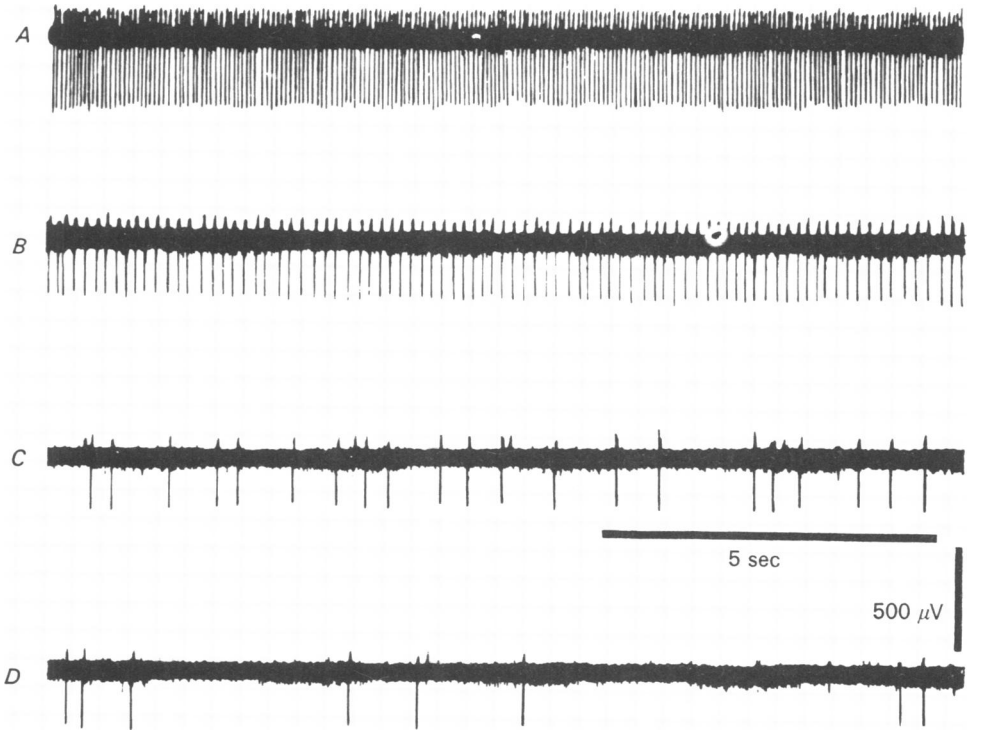
Text-fig. 2. Effect of glucose concentration on the activity of a glucoreceptor. With the three solutions used (5, 10 and 20 g/l.), the discharge frequency increases (mean values, calculated for the first 20 sec, are respectively 6, 8 and 15 impulses/sec). The vertical dotted line shows the beginning of intestinal perfusions. Note the persistence of a slight activity before application of the 20 g/l. solution.

The frequency of discharge was always low, whatever the experimental conditions (see the following data) since it never exceeded 30 Hz. The rhythm was regular or irregular, continuous or discontinuous. Usually continuous and regular rhythms concerned higher discharges whereas discontinuous and irregular rhythms characterized the lower discharges (Text-fig. 3).

Effect of glucose concentration

For sixty receptors, the activity appeared with the most frequently used glucose concentration, i.e. 10 g/l. For the last eight receptors, the concentration threshold was determined, starting with lower values (1, 3 and 5 g/l.). The response was elicited with concentrations of 1 g/l. (one receptor), 3 g/l. (two receptors) and 5 g/l. (five receptors) respectively. If we considered the results as a whole (Table 3),

the discharge frequency depended on the glucose concentration: the mean rose when the concentration increased, but the values lay in a wide range (see S.E.). If we considered the results separately, the relation between concentration and impulse frequency was often clear, as in Text-fig. 2. In other cases, it was less obvious; for instance, the maximal discharge could occur with glucose concentration of 10 g/l.



Text-fig. 3. Adaptation of a glucoreceptor discharge after administration of glucose solution (10 g/l.) in the small intestine (syringe method, the solution was held in place). Response: *A*, immediately after the end of injection; *B*, after 15 min; *C*, after 30 min; *D*, after 1 hr. Note that the discharge frequency decreases in *B*, *C* and *D* and that the activity is discontinuous in *C* and particularly in *D*.

TABLE 3. Effect of glucose concentration on the discharge frequency and on the latency of receptors. *n* indicates the test number

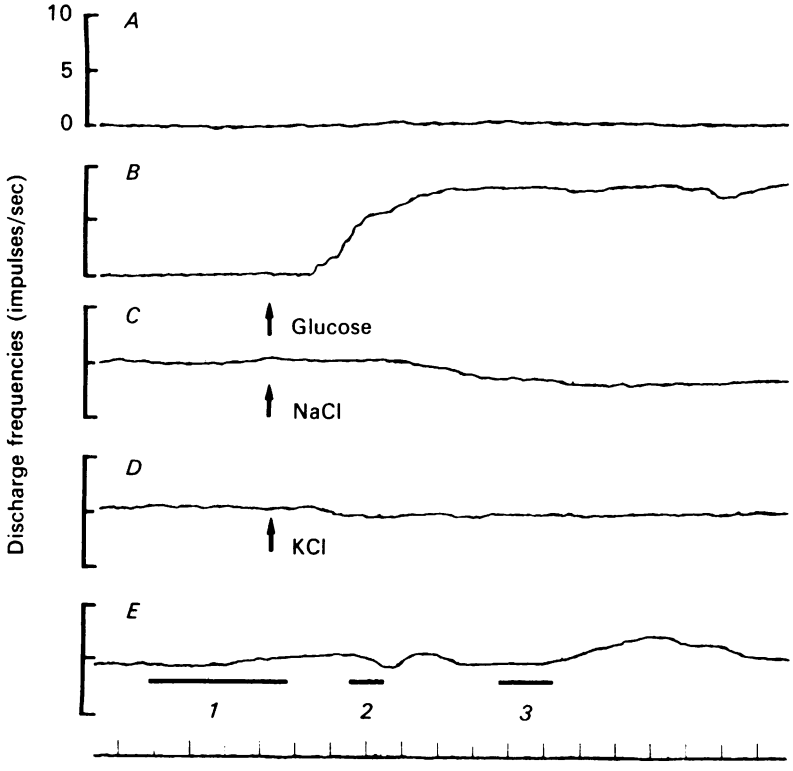
Glucose concentration (g/l.)	Mean discharge frequency ± S.E. (Hz)	Mean latency ± S.E. (sec)
5	5 ± 4 (n = 10)	5·5 ± 4·5 (n = 8)
10	13 ± 12 (n = 24)	4·3 ± 3·9 (n = 23)
20	19 ± 8 (n = 9)	3·8 ± 3·6 (n = 9)

Response latency

The response latency of the receptors was very variable. It depended on several parameters:

(1) the receptor itself; between 0·2 and 15 sec with the glucose solution of concentration 10 g/l.

(2) the glucose concentration: the mean time was shorter using a concentration of 20 g/l. than 10 g/l. (Table 3) but, as before, the discharge frequency values were very scattered.

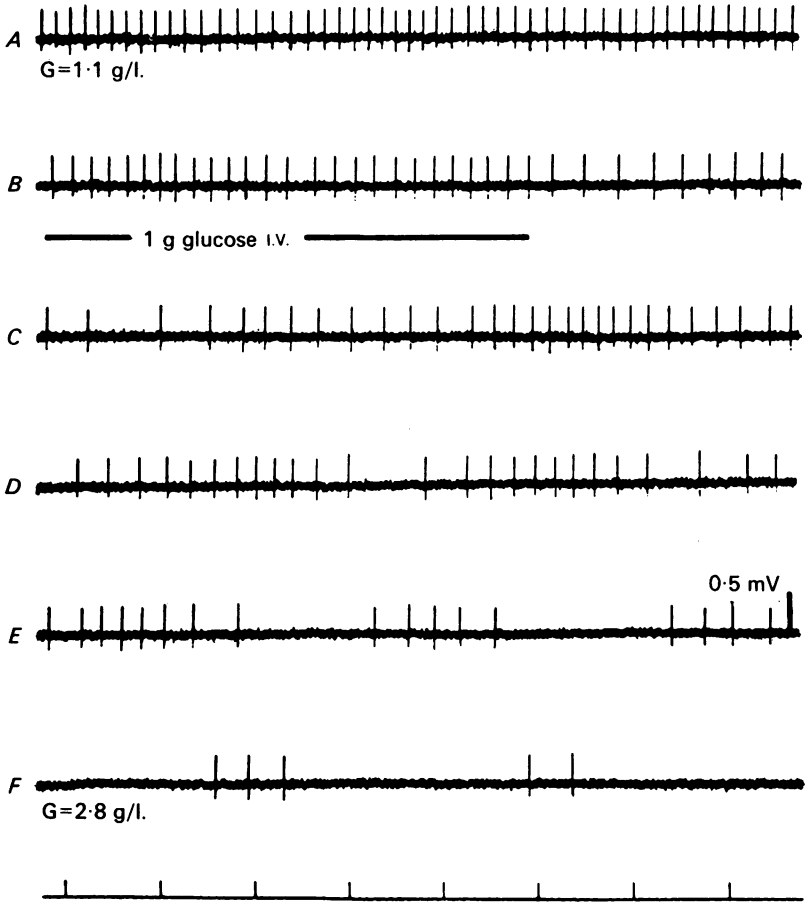


Text-fig. 4. Effect of osmotic pressure and of mechanical stimulation on a gluroceceptor (ratemeter recording) *A*, control; *B*, response to intestinal perfusion with a glucose solution (550 m-osmole/l., 10 g/l.). Arrows indicate the beginning of perfusion which is maintained for 5 min. *C*, response to isotonic NaCl solution 10 min after rinsing. Note the persistence of a remaining activity before the perfusion and the decrease of receptor activity during the perfusion. *D*, response to isotonic KCl solution 10 min after rinsing. As in *C*, there is a discrete fall in frequency. *E*, response to stroking of the mucosa (*1*, *2* and *3*) 5 min after rinsing. The variations of the receptor activity are not related to the three stimulations, but at the end of the recording, the discharge frequency is slightly enhanced. Time mark: 1 sec.

Effect of osmotic pressure

In the special experiments (see Methods and Table 2), we studied the effect of NaCl and KCl solutions on ten receptors which were previously activated by a glucose solution (275 m-osmole/l., 5 g/l. or 550 m-osmole/l., 10 g/l.). The NaCl and

KCl solutions used at the same or a higher osmotic pressure (1100 m-osmole/l.), did not produce any significant activation (Text-fig. 4). Actually, we observed: (*a*) no change in five cases; (*b*) a discrete decrease in two cases (Text-fig. 4*C* and *D*); and (*c*) a slight increase of 1–2 impulses/sec in three cases. These changes were comparable to those observed during the rinsing. In contrast we noted, after KCl or NaCl perfusion, a clear activation for three receptors which were not sensitive to glucose. In any case the HCl solution (three tests) and the NaOH solution (one test) were able to modify the receptor activity.

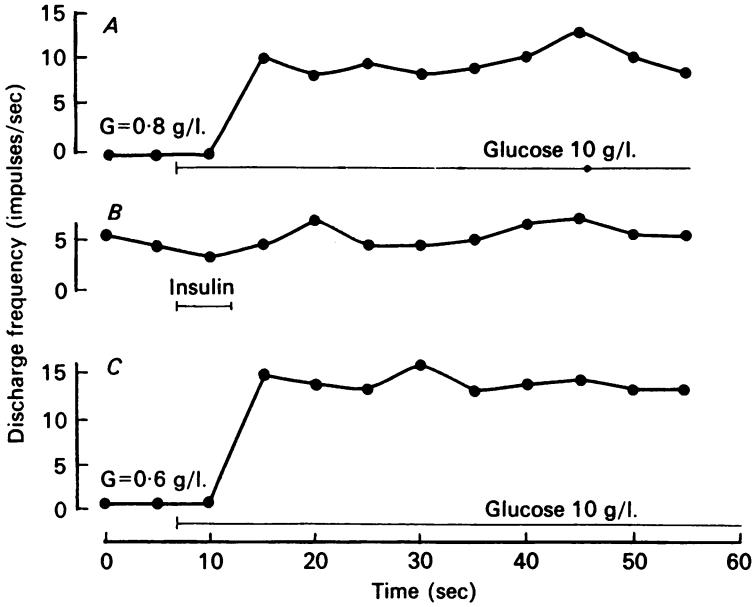


Text-fig. 5. Effect of I.V. injection of glucose on the activity of a glucoreceptor. *A*, *B*, *C*, *D*, *E* and *F* are continuous recordings. *A*, control discharge produced by intestinal perfusion with glucose solution (10 g/l.). The glycaemia (G) is equal to 1·1 g/l. *B*, after injection of I.V. load of glucose, the discharge decreases. In *D*, *E*, *F*, the receptor discharge continues to decrease. In *F*, the receptor activity consists only of two short bursts, glycaemia then reaches 2·8 g/l. Time mark: 1 sec.

Glycaemia effect on the receptors

I.V. injection of 5–10 ml. of glucose solution (100 g/l.) into the systemic or portal circulation never produced any discharge of the receptors. On the contrary, this procedure modified the receptor discharge elicited by intestinal glucose perfusion

in the following way: (1) at first, a transient and slight increase of the discharge (2–10 sec after the end of injection), this phenomenon being noted in only two out of ten cases; (2) later, a lasting and marked decrease of activity (ten out of ten cases) as easily seen in Text-fig. 5. When glycaemia was diminished by I.V. injection of 20–50 ml. isotonic NaCl solution or of 10–20 i.u. of insulin, the reverse effect was observed, i.e. an increase of receptor activity (Text-fig. 6).

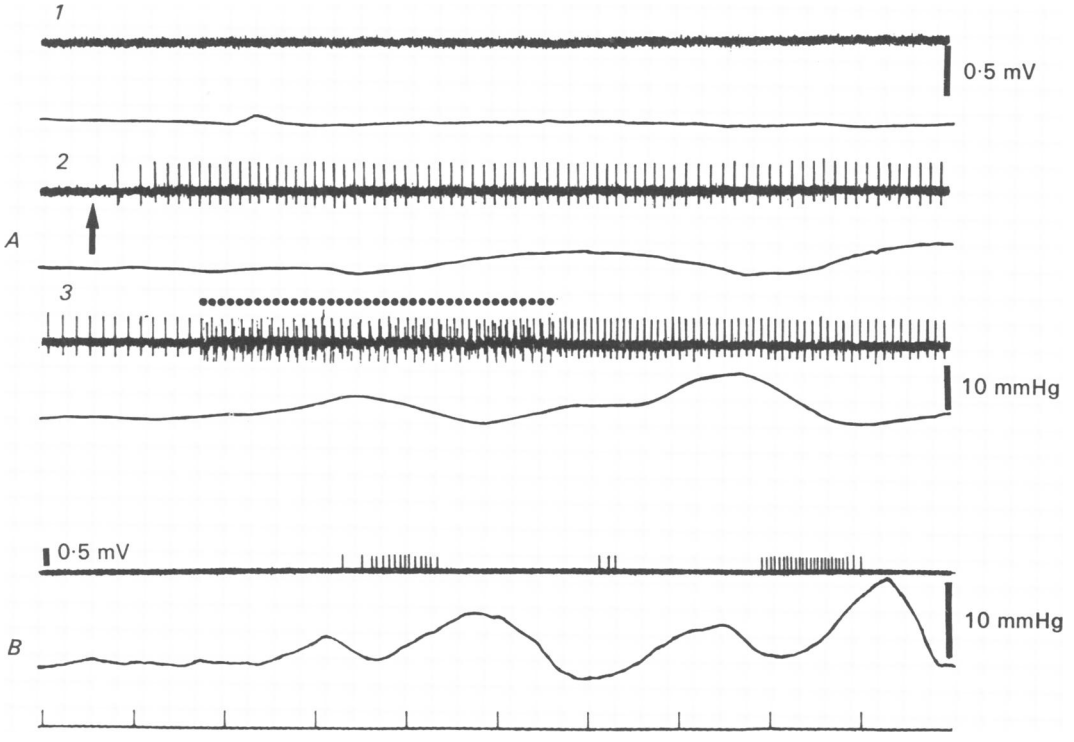


Text-fig. 6. Effect of I.V. injection of insulin on the activity of a glucoreceptor. *A*, control discharge produced by intestinal perfusion with glucose solution (10 g/l.). *B*, after 10 min, 20 i.u. insulin are injected I.V. The remaining discharge is not obviously affected. *C*, one hour later, the receptor is silent and the glycaemia is lower. The perfusion is more effective than the control (mean discharge frequency: 15 instead of 10 impulses/sec).

Effect of mechanical activity of the gut

During the rinsing we never observed obvious receptor stimulation. The intestinal contractions also did not elicit any response (Text-fig. 7*A*, *2* and *3*). Therefore, these receptors contrast with the intestinal mechanoreceptors which were obviously activated by the contractions of the gut (Text-fig. 7*B*). In eight experiments we firmly and systematically compressed all parts of the perfused small intestine between finger and thumb, after identification of receptors. In three of these cases, the perfusion was stopped and a small balloon was introduced through the inferior intestinal cannula for systematic strong distensions. Digital compressions were also achieved on the inflated intestinal area. None of the eight receptors tested was activated in these different ways. In three other experiments, the mucosa was stroked tonically or phasically with a soft tipped probe. In no case could we obtain a receptor response clearly related to the mechanical stimulation. However the pattern of the discharge due to glucose perfusion was modified in such conditions (Text-fig. 4*E*).

Mechanical variations presumably acted by changing the perfusion efficiency, because the modulation of receptor discharge was also noted when the pressure and flow of rinsing perfusion were modified or when the intestinal motility was spontaneously high or enhanced by vagal stimulation. In this last case, the discharge was increased during and after the intestinal contractions, as seen in Text-fig. 7*A*, *3*.



Text-fig. 7. Effect of intestinal motility on activity of a glucoreceptor. Comparison with a tension intestinal mechanoreceptor. Lower trace in each record is a recording of intraluminal pressure. *A*, glucoreceptor. *1*, control: there is no action potential. *2*, discharge of the glucoreceptor elicited by intestinal perfusion with glucose solution (10 g/l.); the arrow indicates the beginning of the perfusion; the pressure variations do not affect the neuronal activity. *3*, repetitive central stimulation of the contralateral vagal nerve (frequency, 10 Hz) produces an enhancement of the sustained discharge (dots indicate the stimulation artifacts). As in *2*, the discharge is not modulated by mechanical variations (pressure) produced by the vagal stimulation. *B*, intestinal tension mechanoreceptor. Contrary to the glucoreceptor, the mechanoreceptor is spontaneously active during the pressure variations. The coincidence is not perfect, because the pressure recording involves the whole perfused intestine. Time mark: 1 sec.

Response of receptors to other carbohydrates and comparison with glucose

The results are presented in Table 4. There were few negative tests (only fifteen out of 110). They are not restricted to a peculiar carbohydrate or class of carbohydrates (pentose, hexose, di- or trisaccharide). Nevertheless, the hexose group gave more positive responses (89 %) than pentose (66 %) or disaccharide (81 %); the trisaccharide (raffinose) is not considered because the test number is too small. On the other hand, there are differences within each group. For instance, for the

hexose group, the number of positive responses is clearly lower for the levulose (66·6 %) than for the glucose (94·5 %). If we compare the responses of receptors to glucose and to other carbohydrates, we find also quantitative differences for the same concentration used (10 g/l.). Actually the mean value of discharge frequency was $10 \pm 7{\cdot}5$ Hz for other carbohydrates instead of 13 ± 12 for glucose.

With the different concentrations of saccharose solutions used in one experiment, the maximal frequency increased with the concentration as in the case of glucose (3, 7 and 12 Hz for solutions containing 5, 10 and 20 g/l. respectively).

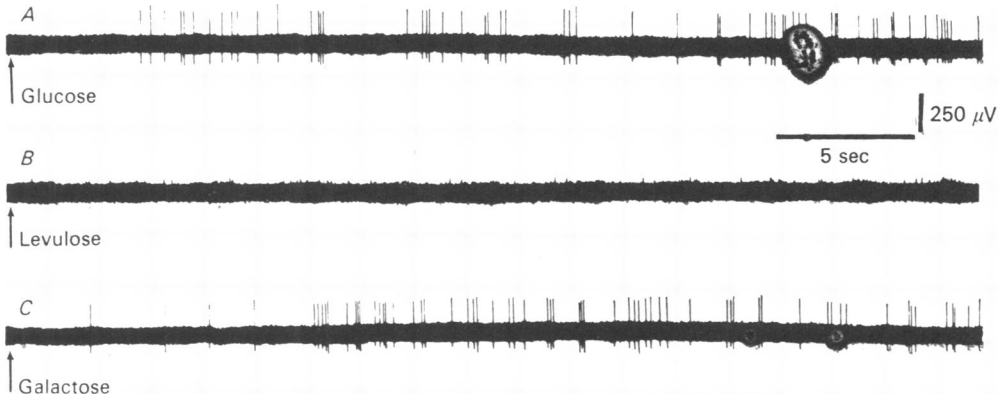
TABLE 4. Effect of the different carbohydrates tried on the receptors. For each of the thirty-seven receptors studied, it is indicated whether there was an effect (+) or not (0)

	Carbohydrates											
Receptor	D-arabinose	D-xylose	D-glucose	D-levulose	D-galactose	L-sorbose	D-mannose	Saccharose	Maltose	Lactose	D-cellobiose	Raffinose
1			+	0		+						
2	+		+	0	+				0			
3			+	+					+			
4			+	+					+			
5			+	0				+				
6			+					+				
7			+	+								
8			+	+	+					+		
9	+	+	+									
10			+				+					
11			+					+		+	+	
12			0	+								+
13			+					+	+			
14			+	+	+			+				
15			+	+					0	+		
16			+							+		
17			+			+				+		
18			+			+			0			
19		0	+	+						+		
20		+	+	0						0		
21	0		+					+		0		
22			0	+								+
23			+							+		
24	+		+	0	+				0			
25			+	+					+			
26			+	0		+						
27			+	+								
28			+				+					
29	+	0	+									
30			+					+				
31			+	+				+				
32			+					+				
33			+					+				
34			+		+					+		
35			+							+		
36			+					+	+			
37			+						+			

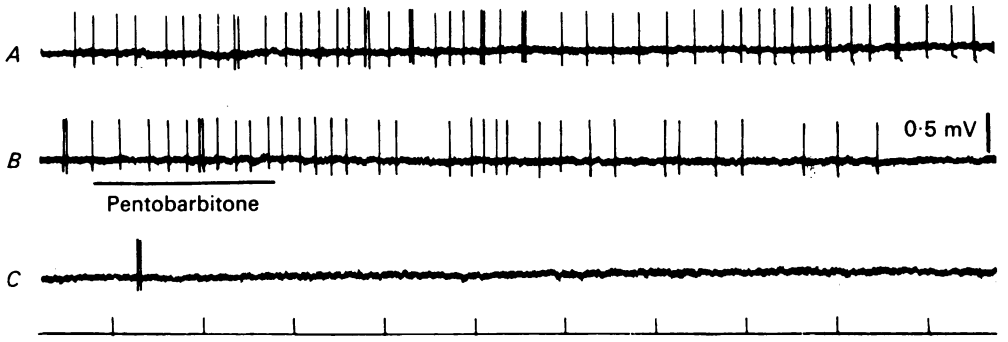
Effect of barbiturate anaesthesia

The inhibitory effect of barbiturates on the gastro-intestinal motility is very well known. For this reason, the chloralose anaesthesia was chosen in this study.

Small doses of sodium pentobarbitone (5–10 mg) given intravenously were able to diminish and sometimes to suppress completely the receptor discharge (Text-fig. 9). This effect persisted for several minutes.



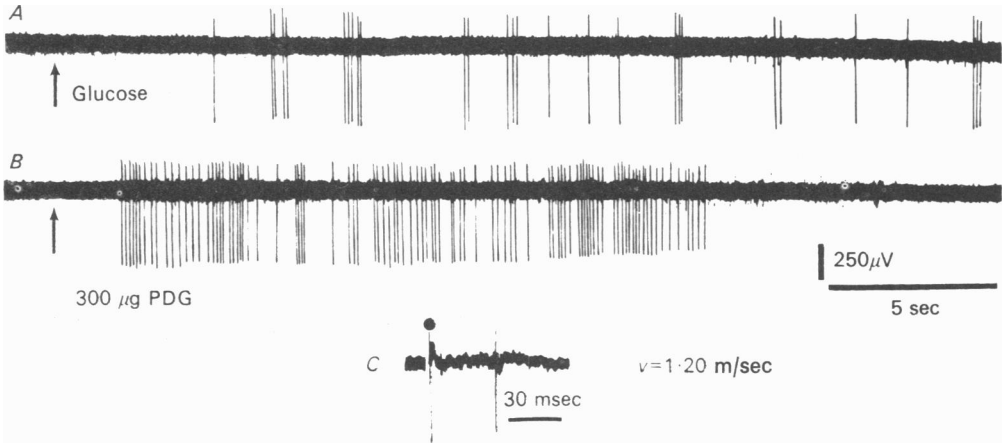
Text-fig. 8. Response of a glucoreceptor to glucose, levulose and galactose (10 g/l. concentration). Arrows indicate the start of continuous intestinal perfusion. This receptor is activated by glucose (*A*) and galactose (*C*) but not by levulose (*B*).



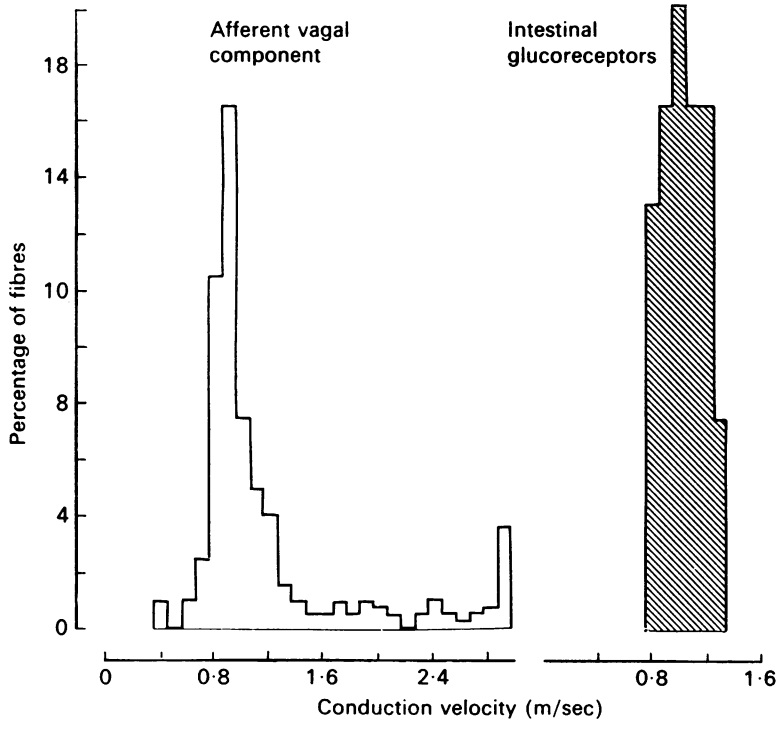
Text-fig. 9. Effect of I.V. administration of sodium pentobarbitone on a glucoreceptor response. *A*, *B* and *C* are continuous recordings. *A*, control response produced by glucose solution (10 g/l.); *B*, after I.V. injection of a small dose of sodium pentobarbitone (6 mg in 2 ml. saline solution), the discharge frequency decreases; *C*, the discharge disappears completely. Time mark: 1 sec.

Effect of intravenous injection of phenyl-diguanide (PDG)

Fifteen receptors were tested with PDG (100 μg/kg), I.V. In every case, receptor activity rose. This activation appeared quickly (3–10 sec; mean value, 5 sec after the end of injection), lasted 5–30 sec (mean, 15 sec) and was generally slight (discharge frequency less than 10 Hz in thirteen cases, and greater than 20 Hz in two cases; Text-fig. 10).



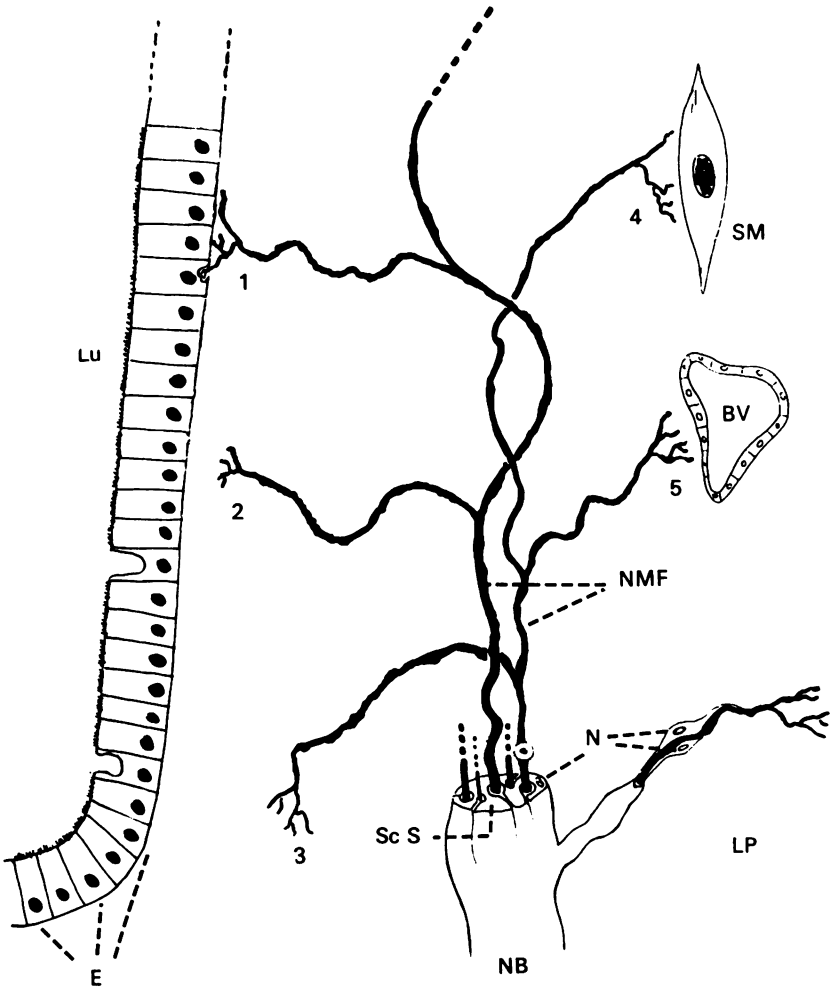
Text-fig. 10. Effect of phenyl-diguanide (PDG) on a vagal glucoreceptor. *A*, receptor discharge elicited by intestinal perfusion with glucose solution (5 g/l.). The arrow indicates the start of stimulation. *B*, response produced by PDG (100 μg/kg I.V.); the arrow indicates the end of the injection. Note the rapid appearance of the discharge (about 2 sec after the end of injection) so that its duration is short (about 15 sec). *C*, response to single electrical stimulation of the ipsilateral nerve. Conduction velocity: 1·20 m/sec.



Text-fig. 11. Conduction velocities of the vagal fibres coming from the intestinal glucoreceptors. Comparison with the undifferentiated afferent vagal component (from Mei, 1970*a*). The fibres connected to the vagal glucoreceptors have conduction velocities ranging from 0·8 to 1·4 m/sec. Therefore they belong to the C peak of the sensory vagal component.

Conduction velocity of corresponding vagal fibres

Conduction velocities were calculated for twenty-nine fibres (Text-fig. 11). They ranged between 0·8 and 1·4 m/sec (mean $1·1 \pm 0·2$). For the other fibres, it was difficult to calculate exactly the conduction velocity because the electrical stimulation evoked several action potentials or a compound potential. But in this case it is possible to estimate approximately the conduction velocity of these complex responses (1 m/sec in mean).



Text-fig. 12. Reconstruction of the innervation of a villus. This scheme was constructed from serial longitudinal sections of a villus. NB: nervous bundle; Sc S: Schwann sheath; NMF: non-medullated fibres; N: nuclei of Schwann cells; BV: blood vessel; SM: smooth muscular fibre of the villus; E: enterocytes; LP: lamina propria; Lu: intestinal lumen. 1 and 2: external endings situated more or less close to the epithelium. 3, 4 and 5: internal endings; 4 and 5 are located near a muscular fibre and a blood vessel respectively.

Histological data

In the normal cat, the ultrastructural study on transverse or longitudinal sections of intestinal villi showed the presence of numerous small nerve bundles which were only composed of non-medullated fibres. The numbers (five–thirty) and size (0·2–2 μm) of these fibres were quite variable. The bundles were observed all over the villi: in the middle (Pl. 1*A*, *B* and *C*), near the smooth muscle cells (Pl. 1*D*) and the blood vessels or beneath the epithelial layer (Pl. 1*E* and *F*). The Schwann sheath did not envelop each nerve fibre (Pl. 1*B* and *C*). Sometimes the fibres were completely free, especially near the epithelium (Pl. 1*F*). At this level, nerve fibres often possessed mitochondria and dense and clear vesicles. As shown in Pl. 1*E* and *F*, axons ran very close to the cytoplasmic membrane of enterocytes and sometimes (*F*), tunnelled in cytoplasmic grooves. No typical synaptic junction was found. The total number of fibres was obtained for three transverse sections of villi to be 140, 295 and 490 (mean, 308). For the operated cat (ablation of one nodose ganglion) the corresponding number of fibres was lower being 110, 150 and 165 (mean, 140).

From serial sections, the pathway of three bundles was partly reconstructed. It is clear that the non-medullated fibres had a path with numerous windings and swellings. Each fibre gave many complex branchings, especially near the epithelium. Text-fig. 12 is a scheme which attempts to represent the complex pathway of the fibres of a bundle, inside the villus.

DISCUSSION

The development of the micro-electrode technique (Mei, 1962) enabled general data about visceral sensory innervation to be obtained. Thus much electrophysiological information has been gathered on visceral mechanoreceptors belonging to vagal (Mei, 1970*b*, *c*), splanchnic (Ranieri *et al.* 1973) and pelvic (Arlhac, 1972) regions or on corresponding neurones (Mei, 1970*a*; Ranieri *et al.* 1975; Duclaux, Mei & Ranieri, 1976). These various results strikingly demonstrate the usefulness of this method for studying the sensory fibres and especially the small sized ones. The advantages of this method (ease of use, numerous recordings from single neurones, good signal to noise ratio, no necessity to cut the nerves) are particularly attractive for the study of the visceral afferents. As a result, the first systematic investigation of a new type of intestinal receptor has been realized in the present work.

Differences between the receptors and previously known gastro-intestinal receptors

(1) *'In series' tension mechanoreceptors*

These receptors have been described in the stomach or in the small intestine of different species. They are connected with vagal (Paintal, 1954; Iggo, 1955; Leek, 1969; Mei, 1970*c*) or splanchnic fibres (Andrews & Andrews, 1971; Morrison, 1973; Ranieri *et al.* 1973; Floyd & Morrison, 1974; Floyd, Hick & Morrison, 1976); see also the reviews of Leek (1972) and Paintal (1973). Contrary to the above receptors, those studied here are not obviously activated by mechanical stimuli such as distension, contraction and compression. Additionally, the tension receptors are not

sensitive to glucose (N. Mei, personal observations). On the other hand, the vagal tension receptors are situated in the muscular layers whereas our receptors are certainly distributed in the mucosa (see below).

(2) *Mucosal receptors*

The existence of mucosal receptors in the gastro-intestinal tract was reported by several authors, in particular Paintal (1957), Iggo (1957), Davison (1972), Harding & Leek (1972*a*, *b*), and Clarke & Davison (1974). These receptors responded to spontaneous contractions of the muscularis mucosae, tactile stimulation of the mucosa (firm stretching, stroking), coarse stimulations of the whole viscera (over-distension for example). The discharges elicited by such mechanical manoeuvres were irregular and rather rapidly adapting. However mucosal receptors did not respond only to mechanical stimulations but presented a complex chemical sensitivity. Some were excited by either alkaline or acid solutions (Iggo, 1957; Davison 1972,; Clarke & Davison, 1974); others were sensitive to different substances such as hypertonic NaCl, organic and inorganic acids (Harding & Leek, 1972*a*, *b*; Clarke & Davison, 1974). Therefore it is probable that the mucosal receptors include different types of endings, i.e. mechanoreceptors (the muscularis mucosae receptors of Paintal, 1957), specific chemoreceptors (the alkali- and acid-sensitive receptors described by Iggo (1957) and Davison (1972)) and non-specific chemoreceptors (for example the reticulo-epithelial and abomasal receptors studied by Harding & Leek, 1972*a*, *b*).

The main functional properties of the receptors we have investigated permit them to be distinguished from the mucosal receptors since (1) the perfusion of the intestine with hexose solutions always excited them, (2) they were not obviously stimulated by any of above mechanical manoeuvres, (3) they always gave a very slowly adapting discharge, (4) changes of acidity/alkalinity or osmotic pressure clearly did not affect their activity, and (5) the effect of I.V. administration of PDG seemed different for the two types of receptors, the activation lasting longer in our receptors than in the mucosal ones (see Paintal, 1957).

Thus the receptors studied in this work are neither mechano-receptors nor osmoreceptors. On the other hand, they are different from the already known chemoreceptors (alkali and acid sensitive receptors). Therefore they must be considered as true glucoreceptors. The term of glycosidoreceptors, more generic, might be chosen because many carbohydrates tested were efficient. But we have preferred the previous one, because it was already used in the literature and because glucose was one of the most effective carbohydrates.

Differences between vagal glucoreceptors and other osmoreceptors or glucoreceptors

The glucoreceptors described here seem to be functionally different from hepatic glucoreceptors already known (Niijima, 1969*a*). Actually the hepatic glucoreceptors have an opposite behaviour, since their activity decreases when glucose concentration increases. Also vagal glucoreceptors are different from hepatic osmoreceptors, the existence of which was recently reported (Niijima, 1969*b*; Adachi, Niijima & Jacobs, 1976). The main reasons are that (i) they are not sensitive to I.V. injection of glucose, (ii) unlike the hepatic osmoreceptors, they have no spontaneous activity,

(iii) they are always stimulated by carbohydrates whereas the discharge of the hepatic osmoreceptors can be diminished, (iv) they are not sensitive to osmotic pressure. On the contrary, vagal glucoreceptors seem to be similar to the mesenteric receptors studied by Sharma & Nasset (1962); these endings were activated by perfusion of the gut with glucose solution (5·4 g/l.). In addition, the discharge pattern and persistence of the activation characterize both mesenteric and vagal glucoreceptors. However, the mesenteric receptors had a spontaneous rhythm, but this might be due to the fact that the records were not obtained from single units (multifibre recordings).

Discharge pattern of vagal glucoreceptors

The vagal glucoreceptors belong to the slowly adapting type as do many other enteroceptors (in particular the tension mechanoreceptors), but their rate of adaptation appears slower since they continue to discharge for over one hr after the perfusion breaks off.

The discharge frequency is always low and varies according to several variables (see below).

The irregular pattern of discharge, particularly marked for the slightly excited receptors, seems to be a general characteristic of chemoreceptors. This was already mentioned, for example by Paintal & Riley (1966) for the arterial chemoreceptors.

Exact stimulus of vagal glucoreceptors

The results of the present work allow us to affirm that the adequate stimulus of vagal glucoreceptors is directly related to the presence of carbohydrates (glucose in particular) at the receptor site. All the variables that are able to modify the intestinal absorption of carbohydrates can also change the glucoreceptor activity. These variables include:

(*a*) *Carbohydrate concentration in the intestine.* We have noted that the threshold varied between 1 and 10 g/l. and that the mean discharge frequency increased with the intestinal carbohydrate concentration. This relationship is not always evident for each receptor, probably because the other variables interact to a greater or lesser extent.

(*b*) *Carbohydrate type.* At the same concentration (10 g/l.), the different carbohydrates tried did not give identical responses (Table 4 and Text-fig. 8). These differences could be attributed in part to the rate of absorption which is greater for hexoses than for pentoses and greater for galactose and glucose than for levulose. It might certainly be possible to stimulate the receptors in any case, with higher concentrations. Another possible explanation is that each receptor would be activated by a well defined range of carbohydrates. In this regard, the vagal glucoreceptors would resemble the olfactory and gustatory chemoreceptors.

(*c*) *Glycaemia.* We reported that the glucoreceptor activity decreased when the glycaemia rose and vice versa. This result must be related to the fact that absorption partly depends on differences in the glucose concentration between the blood stream and the intestinal lumen.

(*d*) *Osmotic pressure.* The fact that the different hexoses tried did not elicit the same receptor activation suggested that the osmotic pressure was not the stimulus

for the vagal glucoreceptors. The result was completely confirmed by experiments in which glucose was replaced by saline solutions with an identical or a superior osmotic pressure. However this variable can sometimes modify slightly the receptor response (see Text-fig. 4).

(*e*) *Intestinal motility.* Movements of the gut (peristaltic or segmentary ones) must activate the motility of the villi and, therefore, facilitate the glucose absorption. This explains why the glucoreceptor response is enhanced during the phases of activity of the small intestine.

Fibre type connected with the vagal glucoreceptors

All the fibres originating from the vagal glucoreceptors belong to the non-medullated population of vagal sensory component (conduction velocity = 0·8–1·4 m/sec) and especially to the peak corresponding to 1 m/sec (Text-fig. 12). So these fibres presumably have a diameter of 0·7–2 μm (Mei, Boyer & Condamin, 1971).

Situation of vagal glucoreceptors

It seems that the vagal glucoreceptors cannot be situated outside the small intestine for the following reasons: (1) the latency of response is short (sometimes less than 1 sec). During this short time, it is unlikely that glucose can reach and activate the hepatic receptors. Also, the functional characteristics of hepatic glucoreceptors and vagal glucoreceptors are different, as already mentioned.

(2) I.V. injection of glucose did not induce responses in the vagal glucoreceptors.

(3) the vagal glucoreceptors seem to be numerous. This argues for an intestinal location. Classical histological and clinical observations show that the small intestine, with the stomach, receives the major part of the sensory vagal innervation.

Thus it can be assumed that the vagal glucoreceptors are located in the small intestine. Our experimental protocol based on the perfusion of the whole isolated gut, does not allow us to decide on the distribution of receptors along the viscera. Nevertheless, the two experiments performed with two perfused parts of the gut seem to show that the glucoreceptors are less difficult to find in the first part of the perfused gut (duodenum and proximal part of jejunum) than in the second part (end of jejunum). This has been confirmed by recent electrophysiological data (T. El Ouazzani & N. Mei, unpublished results) which demonstrate the existence of many glucoreceptors in the upper part of the intestine (intestinal bulb).

The functional characteristics of glucoreceptors (response with a short latency and depending on glucose absorption) imply a superficial mucosal locus. The complex branchings of nerve fibres described in the villus could be the corresponding endings because (1) they are non-medullated, (2) they are very numerous, especially beneath the epithelial layer and close to the blood vessels and (3) a proportion of them, at least, belong to the sensory vagal population, since after unilateral sensory vagotomy the number of fibres is less important.

Putative roles of vagal intestinal glucoreceptors

In spite of numerous clinical and experimental data implicating the existence of preabsorptive glycosensitivity, there has been no electrophysiological evidence of intestinal glucoreceptors, except for the results on mesenteric multi-fibre preparations obtained by Sharma & Nasset (1962). This fact is due to a double technical difficulty: first the recording of single units from the abdominal fibres which always have a small diameter and are often non-medullated and secondly the control of many variables, acting simultaneously in the glucoreceptor activation.

With the micro-electrode technique, it is now possible to investigate the gastrointestinal chemosensitivity in good conditions. The present work already gives information about the functional characteristics of vagal intestinal glucoreceptors in physiological conditions. Actually the stimulations used (1–20 g/glucose per l. in the intestinal lumen) are similar to the normal intestinal glucose concentrations in the cat. From data reported by Scott (1966), one can estimate the carbohydrate proportion of the total dry meal (150 g) taken once daily to be about 16%. The dilution due to gastric and intestinal juices or to casual immediate drinking (not frequent with dry food) must diminish this value, but it remains comparable to our experimental ones.

From our electrophysiological data, from the previous considerations and from the clinical experimental observations, it is possible to assume several roles for the vagal intestinal glucoreceptors.

1. *Glycaemia regulation.* Authors such as La Barre (1927) and more recently Frohman, Ezdenli & Javid (1967), reported that the stimulation of the afferent vagal nerves induced an enhancement of insulinaemia. It is probable that intestinal vagal glucoreceptors are responsible for this modification. Actually, the selective stimulation of the receptors induces an increase of insulin (Mei, N., Arlhac, A. & Boyer, A., in preparation).

2. *Gastric motility.* The decrease of gastric motility produced by glucose perfusion of the duodenum may be explained by a nervous reflex originating from intestinal glucoreceptors.

3. Finally one can ascribe to the intestinal glucoreceptors a part in the complex neuro-humoral regulation of alimentary behaviour (hunger, thirst) and of energy balance. From this point of view, one may suppose that vagal intestinal glucoreceptors are involved in negative alliesthesia (Cabanac, 1971; Cabanac & Duclaux, 1973).

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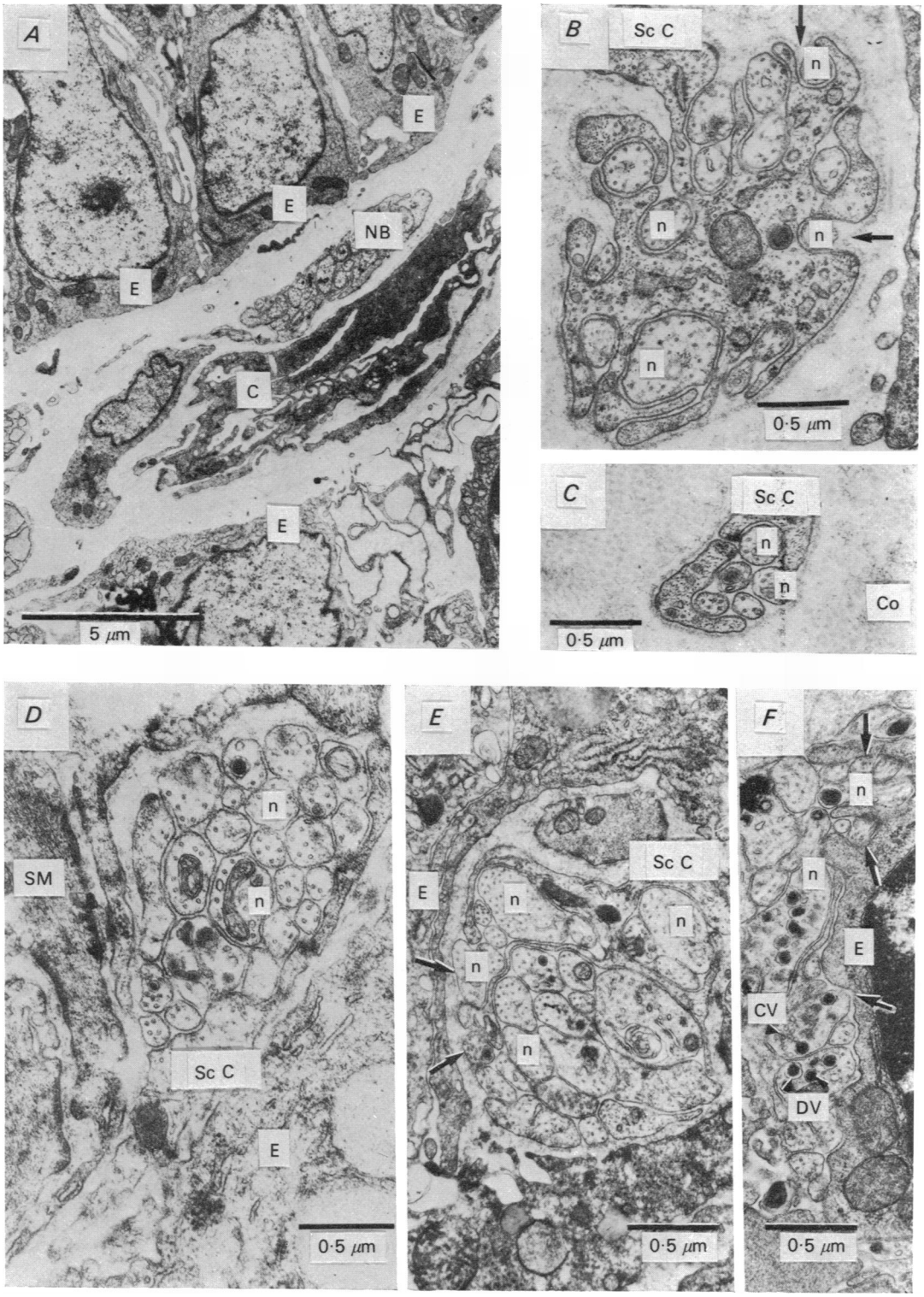
EXPLANATION OF PLATE

Ultrastructural study of the villus innervation. *A*, nerve bundle (NB) of non-medullated fibres situated deeply in the lamina propria, between enterocytes (E) and close to a capillary (C). *B*, small nerve bundle situated deeply in the lamina propria. The Schwann cell (Sc C) does not completely envelop the non-medullated fibres (n); some of them come in contact with the lamina propria (arrows). *C*, another small nerve bundle of the lamina propria. The non-medullated fibres (n) are partly surrounded by the Schwann cell (Sc C); Co, collagen fibrils. *D*, non-medullated fibres (n) located near a smooth muscle cell (SM). The Schwann cell (Sc C), as before, does not provide a complete sheath to fibres. E: enterocyte. *E*, non-medullated fibres (n) run close to an enterocyte (E). Some fibres (arrows) are situated at proximity to the enterocyte; they have no Schwann sheath. *F*, non-medullated fibres (n) located very close to an enterocyte (E). Some of them are situated in cytoplasmic grooves (arrows). Note the presence of dense cored vesicles (DV) and clear vesicles (CV).



N. MEI

(*Facing p.* 506)