Insulin and jejunal electrical activity in dogs and sheep

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A COMMON RECURRING PATTERN of electrical activity of the small bowel, comprising a phase of irregular spiking followed by a period of regular spiking and then quiescence has been demonstrated in both dogs (25) and sheep (10). This has been called the migrating myoelectric complex (MMC) because of its propagation along the intestine. The MMC also has a disruptive effect on the bowel (10) and may mediate the postprandial disappearance of MMC in dogs.

MATERIALS AND METHODS

Six adult ewes (40-50 kg) and six male dogs numbered 1-6 (12-15 kg) were housed in individual cages allowing them free movements. Sheep were fed once daily to appetite on a hay-oat diet, 5:1 (wt/wt). Dogs received daily 450 g of Fido food (contents: crude protein 25%, fat 8%, fiber 5%, and moisture 12% (wt/wt); the remainder included carbohydrate, mineral, and vitamin supplements, and inert material). Two milk-fed lambs were also used during a 3-wk weaning period starting at 3 wks of age. They were fed ad libitum and occasionally received 1 liter of milk from a bottle.

Animals were anesthetized with thiopental sodium, 20 mg/kg, iv. Electrodes, made of insulated nichrome wires, 100 μm in diameter, 110 cm in length, were inserted through the serosa and muscular layers using a needle as a trocar (21). Three pairs of electrodes 2 mm apart were thus positioned at intervals of 20 cm in dogs, 40 cm from the pylorus in dogs and 400 cm in sheep. The free ends of the electrodes were brought subcutaneously to the back of the neck, and recordings were started 5-10 days after operation. The electrical activity was amplified through an EEG machine (Reega VIII, Alvar) at a time constant of 0.3 s, without filters. This activity, after eliminating slow waves by a high-pass filter, was continuously plotted at 20-s intervals for uninterrupted periods of 6-8 wk for two sites in a double linear integrator circuit (3) connected to a potentiometric recorder (PM 8010, Philips, 94 Bobigny, France). Wrenching of the electrodes was avoided by using a rotating connection (Air Precision, 92 Le-Plessis-Robinson, France) fixed to the roof of the cages. Right atrial blood (5 ml) was obtained through an indwelling polyethylene catheter (PE-120) at intervals of 10-60 min for estimation of reducing sugar with a Technicon AutoAnalyzer according to the method of Hales and Randle (12), using a human insulin standard.
At intervals of 3 days, starting 10 days after surgery, dogs 1, 2, and 3 received 1 g/kg of d-glucose either orally, in 100 ml of tap water or infused into the jugular vein, in 50 ml of distilled water at a rate of 5 ml/min. All infusions were given in place of food, and equal volumes of 1.8 M NaCl were infused as control. Dogs 4, 5, and 6 received L (+)-leucine or L (+)-arginine (2.4 g in 50 ml of physiological saline, infused at a rate of 5 ml/min). These infusions were repeated; then all subjects were tested for their response to an intravenous injection of 1 IU/kg insulin (Insulin Zinc Mixa Novo lente) and later to 1 mg of glucagon (Novo), given about 2 h before their normal meal time. Dogs 1 and 2 underwent bilateral prediaphragmatic thoracic vagotomy and 10 days later were retested for their responses to insulin. Dogs 3, 4, 5, and 6 received a single intravenous injection of 70 mg/kg alloxan (14%, wt/wt), and 3 days afterward dogs 4, 5, and 6 developed diabetes characterized by a blood glucose level higher than 2.5 g/liter. These same three animals underwent bilateral thoracic vagotomy 1 wk later and were subsequently retested after 10 days for their responses to insulin injection. The effect of feeding was also monitored in all animals.

Each of the six sheep received 100 ml of a solution (pH 7.1) containing 60 mmol of either acetic acid (C2COOH), n-butyric acid (C4COOH), or a mixture of VFA C4, C3 (propionic acid), C4 in a molar ratio of 2:1:0.5 infused into the jugular vein at a rate of 10 ml/min at intervals of 3 days. All subjects were then tested for their response to an intravenous infusion of insulin (3 IU/kg) and later to an intravenous injection of 1 mg of glucagon (Novo), given about 2 h before their normal meal time. Dogs 1 and 2 underwent bilateral thoracic vagotomy and after 10 days were retested for their response to insulin injection. Dogs 3, 4, 5, and 6 received a single intravenous injection of 70 mg/kg alloxan (14%, wt/wt), and 3 days afterward dogs 3, 4, 5, and 6 developed diabetes characterized by a blood glucose level of about 2 g/liter. The effects of insulin and of VFA were verified in these four sheep 3 days before and 3 and 6 days after bilateral thoracic vagotomy performed under local anesthesia (deep infiltration of Xylocaine, 5 ml, 2%). In the lambs, the effects of milk ingestion on blood insulin level and on the electrical activity pattern were compared before and during weaning. Food was at times withdrawn for 24 h during weaning, and near the end of the weaning period diabetes was induced by alloxan, as described for the ewes.

The slow-wave frequency was determined for each electrode site by counting the number of slow waves in a 10-min period from the daily EEG records (chart speed 2.5 mm/s). The temporal distribution of spiking activity was monitored directly from two continuous integrated records (14). Each MMC is defined as a phase of irregular spiking (phase 1) followed by a phase of regular spiking (phase 2) and separated from the next MMC by quiescence (phase 3) as described for sheep (5) and rats (20). In phase 1, spike potentials are superimposed on the slow waves in a random fashion. In phase 2 each slow wave is superimposed with spike potentials, and in phase 3, none of the slow waves is accompanied by spike potentials. In Figs. 3, 4 and 5, phase 1 is represented by a rectangle, the height of which is a function of the intensity of spiking summed at 20-s intervals, and the phase 2 activity which followed is represented by black columns. Their height corresponds to the full scale of the recorder used, indicating a period of maximal activity. A continuous period of activity similar to phase 1 but not followed by regular spiking activity, is indicated by a shaded area.

Three additional adult diabetic sheep (blood glucose concentration: 2.3 ± 0.5 g/liter) were slaughtered 5 days after alloxan injection (70 mg/kg, iv) together with three control sheep. Sections of duodenum, jejunum, and ileum were rapidly removed at distances of 0.2, 2, and 20 m from the abomasal-duodenal junction, respectively. Tissue was collected and washed in warmed (37°C) incubating medium that was gassed with 100% O2 and contained, in grams per liter, 6.0 NaCl, 0.42 KCl, 0.24 CaCl2, 0.5 NaHCO3, 0.5 glucose, and 0.5 Na acetate, at pH 6.6. Five 2-cm strips of intestine were prepared from each section, and paired strips of control and diabetic tissue were incubated together in the above medium. A strain-gauge recorded the tone, frequency, and amplitude of contractions under isometric conditions, both before and after addition of 0.08 IU/ml insulin.

Results are expressed as means ± standard errors of the mean. Differences between mean values were analyzed for significance by the Student t test for paired observations.

RESULTS

Slow waves and spike potentials were recorded from all electrode sites. Slow-wave frequency averaged 17.6 cycles/min in the dog and 17.2 in the ewe and lamb. The occurrence of spiking activity and its temporal distribution depended on the feeding state in dogs and unwedged lambs. In ewes and weaned lambs, cyclically recurring MMC were evident at an average rate of 19/24 h, regardless of feeding. Feeding and sucking elicited a pattern of activity characterized by a continuous and more or less uniform spiking at all sites, at about half the rate seen during the phases of regular spiking activity in the MMC (Fig. 1A). This pattern lasted about 8 h for the dogs and 6 h for the unwedged lambs and was followed by reappearance of the MMC at an average rate of 10/16 h in dogs and 13/18 h in lambs.

Slow-wave frequency was not markedly altered by insulin in either dogs or sheep. However, spiking activity was greatly affected. In dogs the pattern of MMC was interrupted for 4-5 h by a pattern of uniform spiking activity resembling that seen after feeding (Fig. 1B). In sheep the MMC were not disrupted by this dose of insulin (1 IU/kg), but a threefold higher dose stimulated irregular spiking activity for 1 h in five of the six animals. In all sheep, the quiescent phase disappeared for 1 or 2 MMC intervals, and the frequency of MMC was increased for 4-5 h. Glucagon (1 mg/animal) did not significantly affect slow-wave or spiking activity in either species.

Effects of insulin in alloxan-diabetic dogs and sheep. Within 3 days of alloxan injection, blood glucose levels had risen to near 2 g/liter in most dogs and sheep. This was accompanied by a significant (P < 0.05) increase
FIG. 1. Spiking activity after feeding and insulin injection in the dog. Original integrated tracings from 2 electrode sites at 20 and 40 cm from ligament of Treitz (LT) after ingestion of 450 g of canned food (A). A similar pattern lasting only 5-6 h was seen after an intravenous injection of insulin (B). Full-scale deflection represents 16 μV.

TABLE 1. Effect of alloxan-diabetes and subsequent insulin injection on slow-wave frequency

<table>
<thead>
<tr>
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<th>Slow-Wave Frequency, cycles/min</th>
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<tr>
<td></td>
<td>Before</td>
</tr>
<tr>
<td>Dogs</td>
<td>17.6 ± 0.3</td>
</tr>
<tr>
<td>Sheep</td>
<td>17.2 ± 0.3</td>
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<tr>
<td></td>
<td>Before</td>
</tr>
<tr>
<td>Dogs</td>
<td>19.2 ± 0.4</td>
</tr>
<tr>
<td>Sheep</td>
<td>19.8 ± 0.3</td>
</tr>
</tbody>
</table>

Values are means ± SE for at least 10 determinations in each of dogs 4, 5, and 6 and sheep 3, 4, 5, and 6. * Significantly different from before injection (P < 0.05). †1 IU/kg in dogs, 3 IU/kg in sheep, given 4 days after alloxan.

(Table 1) in slow-wave frequency which persisted for 2–4 wk. In dog 3, which did not respond to alloxan by a stabilized hyperglycemia, the slow-wave frequency only increased during periods of high blood glucose. A transient but significant decrease in the slow-wave frequency was recorded for nearly 60 min after an injection of insulin in both diabetic dogs and sheep (Table 1). This decrease was restricted to the period of normoglycemia.

In diabetic dogs, the temporal distribution of spiking activity was not markedly altered, although slightly fewer MMC were recorded, and their frequency was very irregular from day to day. As in normal subjects, insulin induced a pattern of uniform spiking activity after which the MMC's recurred regularly for 4–5 h. Spiking activity was consistently altered by diabetes in sheep and lambs with a 30–40% decrease in the number of MMC per day. The phase of irregular spiking activity was lengthened and could last up to 90 min, as shown in Fig. 2. Injection of insulin (3 IU/kg) restored a faster motor profile for several hours (Fig. 2).

Effect of insulin release. In dogs, the ingestion of food regularly increased the IRI level (mean maximal increase 71 ± 11 μU/ml) for 5–7 h, which is almost identical to the duration of the pattern of continuous and more or less uniform spiking activity. The intravenous infusion of glucose greatly elevated plasma IRI for 90 min (mean maximal increase 132 ± 26 μU/ml for 6 experiments) and was accompanied by a small increase in irregular spiking. The same amount of glucose by mouth resulted in both a prolonged elevation of IRI lasting 3–4 h (mean maximal increase 126 ± 17 μU/ml for 6 experiments) and a motor profile similar to that seen after feeding, lasting at least 3 h (Fig. 3). Infusions of L-arginine or L-leucine induced long-lasting increases in the IRI concentration. Spiking activity was continuous during the period of hyperinsulinemia and for about 60 min thereafter. Blood glucose was reduced by leucine but was not significantly affected by arginine (Fig. 4).

The mean maximal increase in IRI was the same for leucine (37 ± 7 μU/ml for 6 experiments) and arginine (33 ± 9 μU/ml for 6 experiments). In diabetic dogs (4, 5, and 6), feeding also increased motility but only for 2–3 h, and the intensity was reduced by 40–60% when compared with normal subjects, as judged by the integrated activity. The mean maximal increase in IRI level was only half that seen before the injection of alloxan (Table 2). Control injections of isoosmotic saline had no effect on IRI level, or the electrical spiking activity.

In sheep, the IRI level was unchanged or only slightly increased (24 ± 3 to 37 ± 6 μU/ml; P > 0.05) during a main feeding period spread over 3 h. An intravenous infusion of 60 mmol of a mixture of VFA markedly increased the IRI value for 2–3 h (Table 3). In this case,
TABLE 2. Postprandial changes of immunoreactive insulin level in normal and alloxan-diabetic dogs

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>After feeding</th>
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<tbody>
<tr>
<td></td>
<td>Plasma Insulin, μU/ml</td>
<td>1 h</td>
</tr>
<tr>
<td>Normal</td>
<td>Normal</td>
<td>21 ± 4</td>
</tr>
<tr>
<td>Diabetic</td>
<td>Diabetic</td>
<td>12 ± 5</td>
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Values are means ± SE for nine values: three measurements in each of the dogs 4, 5, and 6. * Significantly different from normal dogs (P < 0.01).

TABLE 3. Insulin release stimulated by infusion of VFA, acetic, and n-butyric acids in normal and alloxan-diabetic sheep

<table>
<thead>
<tr>
<th></th>
<th>Plasma Insulin, μU/ml</th>
<th>After infusion</th>
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</thead>
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<tr>
<td></td>
<td>Control</td>
<td>40 min</td>
</tr>
<tr>
<td>Normal</td>
<td>Normal</td>
<td>24 ± 7</td>
</tr>
<tr>
<td>Acetic acid</td>
<td>Acetic acid</td>
<td>23 ± 6</td>
</tr>
<tr>
<td>n-butyric acid</td>
<td>n-butyric acid</td>
<td>24 ± 9</td>
</tr>
<tr>
<td>VFA</td>
<td>VFA</td>
<td>24 ± 6</td>
</tr>
<tr>
<td>Acetic acid</td>
<td>Acetic acid</td>
<td>9 ± 4</td>
</tr>
<tr>
<td>n-butyric acid</td>
<td>n-butyric acid</td>
<td>9 ± 4</td>
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Values are means ± SE for four sheep. * Significantly different from normal sheep (P < 0.01).

FIG. 3. Effects of an acute glucose load given orally or intravenously on plasma immunoreactive insulin (IRI) and glucose levels in a dog fasted for 24 h. Oral glucose was given 3 days before intravenous infusion. When glucose was given orally, high insulin level was prolonged beyond 3 h of hyperglycemia, and motor profile displayed continuous spiking activity (shaded area). In contrast, insulin level increased transiently, and phases of regular spiking (black columns) were undisrupted by same amount of glucose given intravenously.

FIG. 4. Blood glucose and insulin (IRI) levels with a diagrammatic representation of jejunal electrical activity in a fasted dog after infusions of leucine and arginine. Both amino acids produced a continuous spiking pattern (shaded area) concomitant with insulin release, regardless of blood glucose level.

FIG. 5. Blood glucose and insulin (IRI) levels with a diagrammatic representation of jejunal electrical activity in a fasted dog after infusions of leucine and arginine. Both amino acids produced a continuous spiking pattern (shaded area) concomitant with insulin release, regardless of blood glucose level.

The MMC also disappeared for 2–3 h, being replaced by a continuous pattern of activity (Fig. 5). An intense insulin release was also induced by the intravenous infusion of n-butyric acid. Among the changes in the pattern of motility, a common feature was a continuous pattern of electrical activity lasting 5–6 h, either preceded or followed by two or three phases of regular spiking occurring at short intervals of 10–15 min. Acetic acid was less insulinotropic than either butyrate or the VFA mixture and hyperinsulinemia, at half the level seen after VFA, was only recorded about 4 h after the infusion. At this time, a continuous period of jejunal activity was observed. In the diabetic sheep, the IRI level was reduced, and no changes in its level or in the pattern of motility were observed after infusion of VFA, acetic, or butyric acids.

In lambs, ingestion of milk before or after weaning was followed by a pattern of continuous activity which was of longer duration during weaning if the animal was fasted (Fig. 6). In both animals a good correlation was found between the IRI level (mean maximal increase 98 ± 17 μU/ml) and the duration of the pattern of continuous activity (4–5 h) before weaning. Toward the end of the weaning period, the IRI increase was halved (mean maximal increase 50 ± 13 μU/ml), and the continuous pattern of activity lasted only about 3 h. The mean maximum IRI level after milk increased to 70 ± 20 μU/ml after a 24-h fast, and the continuous activity lasted 1 or 2 h longer. As in the adult sheep, injection of alloxan was followed by hyperglycemia (2 g/liter) within 48 h in the two lambs. After alloxan, an increase in the
intensity of the irregular spiking phases was the only response to sucking (Fig. 6), and the mean maximal IRI level was only $30 \pm 9 \mu U/ml$.

**Effects of vagotomy.** In dogs 1 and 2, the slow-wave frequency was unchanged after vagotomy, and the recurrence of MMC's was slightly increased. Their phases were irregular, especially the phase 3 of inactivity which was increased at the expense of a shorter phase 1. Feeding resulted in an irregular pattern of intense activity lasting less than 8 h. The spiking activity of the fed pattern was more intense after vagotomy, but was interspersed with phases of inactivity (Fig. 7). The change in IRI level after feeding was moderate (mean maximal increase $45 \pm 12 \mu U/ml$), but the control level was relatively high ($30 \pm 7 \mu U/ml$), above that seen before vagotomy ($21 \pm 4 \mu U/ml$). The effects of insulin injection were similar to those seen before vagotomy, although responses tended to be less marked. In alloxan-diabetic dogs, the changes due to feeding declined further after vagotomy. Before feeding, these dogs exhibited a continuous pattern of activity resembling that of the normal sheep, except that the phases of quiescence were prolonged. After feeding, the intervals between the next two or three MMC's were increased for 2–3 h, and the irregular spiking phases were more intense for 4–5 h (Fig. 7). No significant change in the plasma IRI occurred after feeding in vagotomized al-
loxoan-diabetic dogs, but the effects of insulin injection on electrical activity were still observed.

In sheep, vagotomy had little effect on the pattern of motility, except for a reduction in the irregular spiking activity. The IRI level and the pattern of activity following infusion of \( n \)-butyric acid decline by 20% when compared to normal subjects. In alloxan-diabetic sheep, no major changes occurred following vagotomy except that the effect of insulin injection on the frequency of MMC was less prolonged.

**Motility of sheep intestine in vitro.** Insulin (0.08 IU/ml) had only slight effects on the motility of normal sheep intestine in vitro (Fig. 8). The tone of contraction of duodenal strips was somewhat increased, but the amplitude and frequency of contractions were unaffected and ileal strips did not respond to insulin at all. The tone of jejunal contractions was moderately stimulated with no change in frequency (Fig. 8). The intestine of diabetic sheep showed a much greater responsiveness to added insulin. The tone of jejunal contractions was greatly increased, and slight increases in amplitude and frequency were also observed (Fig. 8). The duodenum of diabetic sheep also showed definite increases in tone and amplitude of contractions in the presence of insulin. Even in diabetic sheep, the ileum was largely unresponsive to insulin except for a small increase in amplitude of contractions in two animals.

**DISCUSSION**

These experiments have demonstrated that injection of insulin has a considerable effect on the pattern of jejunal electrical activity in normal dogs and sheep. In dogs, the increased activity seen after the injection of large doses of insulin mimicked the response seen after feeding, whereas in sheep insulin injection increased the irregular spiking activity and shortened the intervals between MMC's. The reduction in the jejunal response to feeding or to insulin-releasing factors in alloxan-diabetic subjects suggests that endogenous insulin release is responsible for these changes. The altered slow-wave frequency in alloxan-diabetic dogs and sheep and the MMC frequency in sheep were restored to normal by insulin injection. Indirect effect of insulin may be involved in vivo, e.g., increased gastric secretion or release of glucagon. Gastrin injections were only marginally effective in sheep (23) and in dogs (26). Since a high exogenous dose of glucagon did not appreciably affect electrical activity in normal subjects, insulin probably does not act by altering glucagon levels. Glucagon may, however, oppose the action of insulin, since its injection would be expected to release insulin. The large doses of insulin required in sheep correspond to the well-known resistance of this species to both insulin and pancreatectomy (18). Intestinal motility in the sheep is, however, directly sensitive to insulin as demonstrated in vitro. Although high levels of insulin were required, the increased responsiveness of alloxan-diabetic sheep intestine suggests that insulin may have a permissive role in regulating the basal pattern of motility, perhaps by provision of glucose (1). Prolonged insulin therapy in undernourished humans was found to induce hypermotility in vivo, and a direct stimulatory effect of insulin on intestinal muscle tone was described as a possible mechanism (17). The decreased MMC frequency in alloxan-diabetic dogs and sheep also suggests that insulin may regulate both basal activity and the disruption of that activity after feeding in dogs or VFA infusion in sheep.

When insulin release was stimulated in dogs by feeding or by intravenous injections of glucose or amino acids (9), a continuous pattern of activity was always registered, and this was seemingly correlated with the increase in plasma insulin level. Moreover, infusion of amino acids also revealed that the blood glucose level is not by itself the causal factor in regulating the pattern of electrical activity.

In sheep a pattern of activity similar to that seen after feeding in unweaned lambs or dogs could be induced by intravenous infusion of VFA or \( n \)-butyric acid, both potent insulin-releasing stimuli (15, 24). Although the response was not uniform, the increase in the plasma IRI level concomitant with the changes in the pattern of activity indicates that insulin may have mediated the disruption in MMC activity. Alloxan diabetes suppresses any insulin release in sheep, and no major changes in the pattern of activity then occur after VFA infusion. In diabetic dogs, complete suppression of insulin release is not obtained, and a higher sensitivity to even small amounts of insulin may be responsible for the persisting response to feeding. Other factors such as serotonin (7), gastrin (26), or gastric inhibitory polypeptide or vasoactive intestinal peptide (11) may also be implicated in the jejunal response to feeding. Release of these hormones may explain the greater efficiency of oral glucose compared with intravenous injection, by stimulation of additional insulin release (19) perhaps coupled with a direct effect on the jejunum (11). Release of these hormones or other hormonal changes may also be responsible for the greater disruptive effect of amino
acids than of oral glucose, despite the greater hyperinsulinemia in the latter case.

Division of the vagus nerves in the thorax disrupts the extrinsic parasympathetic nerve supply to the intestine. In dogs the MMC still occurs after vagotomy (16) with a tendency for an increased number of MMC per day, a change that was paralleled by the increased plasma IRI level. Vagotomy further decreased the persisting partial response to feeding in alloxan-diabetic dogs, but the explanation of this is uncertain. Vagus nerve interruption reduced the persisting insulin release in diabetic dogs. A deficiency in the release of gastrin may also occur after vagotomy (27). A simple mechanism would be that vagotomy induces gastric stasis and therefore results in a slower passage of food constituents and thus of their insulin releasing effect on the remaining pancreatic islets, a possibility that requires further study.

In conclusion, the MMC appears to be more a basic pattern of activity of the small intestine than a phenomenon linked to fasting. One might suggest that the term “interdigestive” is less appropriate for MMC than the term “postprandial” for the pattern of continuous activity seen in dogs (8, 10), lambs, or rats (20) after feeding. The normal stability of the MMC’s in sheep may be related to the negligible variations of plasma insulin in response to the amount of VFA normally reaching the pancreas after feeding in ruminants (24). In dogs rendered diabetic by alloxan, the reduced disruption of the fasting MMC pattern after feeding indicates the major part played by insulin release in the conversion of the “fasted” pattern of activity to the “fed” state in normal subjects.

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REFERENCES


