Configuration of intestinal slow waves obtained by monopolar recording techniques

ALEX BORTOFF
Department of Physiology, State University of New York,
Upstate Medical Center, Syracuse, New York

BORTOFF, ALEX. Configuration of intestinal slow waves obtained by monopolar recording techniques. Am. J. Physiol. 213 (1): 157-162. 1967.—This paper represents an attempt to analyze the configuration of the intestinal slow wave recorded from cat jejunum with extracellular monopolar electrodes. The “intermediate” form of the monopolar slow wave consists of an abrupt positive deflection followed by a negative notch and a more positive plateau that gradually subsides to the original potential level. It is shown, first, by systematically altering the position of the recording electrode while monitoring interelectrode resistance, and second, by summating monophasic and electronically differentiated forms of the slow potential, that the intermediate form represents a combination of the propagated monophasic slow wave and its associated field potential. Which form predominates depends on the relative magnitude of the shunting resistance, $R_s$, around the tip of the recording electrode. If $R_s$ is large the monophasic form, representing the temporal course of membrane potential, predominates. As $R_s$ decreases, the field potential, representing the temporal course of membrane current, becomes predominant. Unless the time courses, polarities, and magnitudes of each of these components can be determined, the intermediate type of record cannot be interpreted in terms of electrical changes at the membrane level.

small intestine; slow-wave configuration; electrical activity of intestinal smooth muscle; smooth muscle potentials

During the past decade renewed interest has been focused on the electrical slow-wave activity of the small intestine. Most investigators, in studying these potentials, have used extracellular monopolar recording techniques instead of methods employing intracellular electrodes. Although monopolar recording offers certain advantages to the investigation of intestinal slow waves, it usually suffers from the disadvantage of producing a tracing of such complex configuration as to make its interpretation difficult at best. Whereas microelectrode (3, 9) and pressure electrode (4) studies indicate that the slow waves are periodic, almost sinusoidal depolarizations of the intestinal muscle cell membrane, the monopolar slow wave recorded with needle electrodes has been described as consisting of “... an initial rapid positive going deflection followed by a plateau and then a negative going deflection” (7), with a negative notch usually preceding the plateau (13). The initial positive-going deflection has been termed the “b fraction” of the slow-wave complex recorded with intramural needle electrodes (2). Even though there appears to be basic agreement about its configuration, it is essential, in order to give the monopolar slow wave greater physiological meaning, to be able to relate it to membrane phenomena. Does it represent, for example, temporal changes in membrane potential, or membrane current, or both? Can the configuration of the monopolar slow wave be adequately explained in terms of muscle membrane phenomena, or does it include a component contributed by some other intestinal structure, such as the myenteric plexus?

It was in an attempt to answer these questions that the following experiments were performed.

METHODS AND MATERIALS

In vitro preparations consisted of short segments of jejunum removed from adult cats under sodium pentobarbital anesthesia. Each segment was cut open along its mesenteric border and pinned, mucosal side down, to a paraffin block that formed the bottom of a chamber 15 cm long by 10 cm wide. The chamber was filled with 300 ml aerated Tyrode solution maintained at 30-32 C. This temperature was selected over 37 C in order to keep spontaneous spiking to a minimum. The composition of the Tyrode solution is given elsewhere (3).

In vivo recordings were obtained from adult cats initially anesthetized with sodium pentobarbital administered intraperitoneally and maintained with periodic intravenous injections of the same drug. The animal was secured in a supine position to a dissecting board and after exposing the abdominal cavity along the midline the edges of the incision were sutured to an ovai-
shaped aluminum ring measuring approximately 7 x 15 cm. The ring was raised several centimeters and then rigidly clamped to a metal supporting rod, after which the abdominal cavity was filled with Tyrode solution maintained at 37 ± 0.1°C by means of an electric heating pad placed under the animal. Recordings were made from a point on the jejunum that was fixed in position by applying suction through two polyethylene tubes making contact with the intestine several centimeters oral and aboral to the recording point.

Both needle and glass pipette electrodes (pressure electrodes) were used for recording. The needle electrode consisted of 26-gauge silver wire, sharpened at the recording end and then chlorided. All but about 1 mm of the wire was inserted into a glass capillary tube that was tapered at the recording end and sealed at both ends with hard laboratory wax. This served both to support and to insulate the silver wire. The glass pipette electrodes were filled with agar-Tyrode solution and were connected to the Grass polygraph preamplifier by means of chlorided silver wire. Both types of electrode were attached to Grass force displacement transducers and lowered into position by means of mechanical micromanipulators. The indifferent electrode in both cases was a coil of chlorided silver wire which in the in vitro setup was placed in the bath at least 5 cm from the edge of the tissue, and in the in vivo preparation was placed under the skin of the thigh. The resistance between the recording and indifferent electrodes was measured with a Simpson high-sensitivity ohmmeter (model 269) with the electrodes disconnected from the amplifier.

A system of operational amplifiers (designed and constructed for this purpose by William J. Mueller, Director of the Bioelectronics Laboratory), was used to differentiate electronically the monophasic slow wave and then sum the differentiated form with the original monophasic form. Monophasic slow waves were recorded with the pressure electrode and displayed on one channel of the polygraph. The slow wave, could be varied by means of a potentiometer and then differentiated, summed with the original signal, and then displayed on a second channel of the polygraph. The configuration of the slow wave recorded under

RESULTS AND DISCUSSION

The records of Figs. 1A and 2A are typical of those obtained with either the glass pipette or the needle electrode when the tip of the electrode was in the bath approximately 1 mm above the tissue. The most characteristic configuration of these volume or field potentials consists of a positive-negative-positive deflection, having a peak-to-peak amplitude of about 0.2 mv, followed by an approximately isoelectric period that is usually slightly negative and that terminates in a positive deflection tending to bring the potential back to its original level. Potentials recorded in this manner represent the time course, direction, and density of membrane current associated with membrane-potential changes, assuming that the distribution of potentials surrounding smooth muscle cells in volume is comparable to that of other core conductors under similar conditions (14). A positive deflection indicates that the tissue under the electrode is acting as a source of current, whereas a negative deflection indicates that it acts as a sink. If the membrane-potential change, represented by the monophasic slow wave, propagates past the electrode at a constant velocity the configuration of the field potential may approximate its second time derivative (c.g., Fig. 2A).

When either the needle or the glass pipette recording electrode was brought into contact with the tissue, records such as those of Figs. 1B and 2B were obtained. The configuration of the slow wave recorded under

![Graph](http://ajplegacy.physiology.org/DownloadedFrom/10.2203.30.6/01917)

**Fig. 1.** Slow waves recorded with a chlorided silver needle electrode from a segment of cat jejunum immersed in 300 ml aerated Tyrode solution. In A the tip of the recording electrode was ca. 1 mm above the serosal surface of the tissue. In B the electrode was exerting light pressure on the tissue. In C the tip of the electrode had penetrated the serosal surface of the segment. Indifferent electrode, a coil of chlorided silver wire, was in the bath ca. 5 cm from the edge of the tissue. Resistance between electrodes: A, 1.2 kilohms; B, 1.4 kilohms; C, 2.0 kilohms. Vertical bar: A and B, 0.2 mv; C, 0.5 mv. Horizontal bar: 2 sec. Temp: 32°C. An upward deflection is positive in this and all subsequent figures.

**Fig. 2.** Same recording situation as in Fig. 1, except that the recording electrode was an agar-Tyrode-filled glass capillary tube. In A the tip of the recording electrode was ca. 1 mm above the serosal surface of the tissue. In B it exerted light pressure, and in C, maximal pressure on the tissue. Resistance between electrodes: A, 1.5 kilohms; B, 19 kilohms; C, 30 kilohms. Vertical bar: A, 0.2 mv; B, 0.5 mv; C, 2.0 mv. Horizontal bar: 2 sec. Temp: 32°C.
these conditions resembles that described in the introduction, viz., a rapid positive deflection (the \( b \) fraction of the slow-wave complex), a negative notch, and a prolonged positive phase followed by a gradual return of the potential to its original level. The tracings of Figs. 1C and 2C are typical of those observed after increasing the pressure exerted on the tissue by the recording electrode. In these tracings an obvious difference can be noted between the configurations of the slow waves recorded by the two electrodes. That of the slow waves recorded by the needle electrode remained essentially unchanged, only the amplitude being increased. However, after sufficiently increasing the pressure exerted by the glass pipette electrode the negative notch following the initial positive deflection diminished until the slow wave consisted simply of a positive deflection followed by a gradual return to its initial level (Fig. 2C). (Note also the increase in amplitude.) This configuration is very similar to that of slow waves recorded from longitudinal muscle cells with intracellular electrodes, suggesting that it represents the temporal course of the change in membrane potential associated with the intestinal slow wave.

The sequence of tracings in Fig. 2 indicates that the intermediate type of configuration represents a superposition of the propagated monophasic slow wave and its associated field potentials. The schematic diagrams of Figs. 3 and 4 illustrate how various recording conditions can give rise to such a sequence of records. \( P_1 \), in both diagrams, represents the grounded indifferent electrode at some distance from the recording electrode. \( P_1 \) Figure 3 depicts the situation where the recording electrode is either very close to or just touching the tissue when the latter is in a volume conductor, either in situ or immersed in a large volume of Tyrode solution. The only potential recorded under these conditions is the field potential, represented by the IR drop across \( R_e \), the resistance between the electrodes. As mentioned above, the time course and polarity of the field potential thus recorded may be regarded as representing the time course and direction of membrane current associated with the intestinal slow wave. Two adjacent “segments” of membrane are represented by their batteries, \( E_m \) and \( E_m' \), and resistances, \( R_m \) and \( R_m' \), respectively. The internal resistance (which may include the effective resistance across intracellular junctions if the segments involve adjacent cells) is represented by \( R_i \).

Figure 4 represents the situation where the recording electrode exerts pressure on the intestinal segment. Since intestinal smooth muscle is depolarized by mechanical deformation, it is quite probable that the tip of the electrode, by deforming the tissue directly beneath it, reduces both the membrane potential and the membrane resistance of the muscle cells comprising this tissue (see also 12). Because of the syncytial properties of intestinal smooth muscle (10), the electrode can effectively make electrical contact with the interiors of muscle cells adjacent to those depolarized. In the diagram of Fig. 4, the reduced membrane resistance is represented by \( R_m' \).

FIG. 3. Equivalent circuit for recording conditions under which field potentials such as those in Figs. 1A and 2A may be obtained. \( P_1 \) represents the indifferent electrode, \( P_1a \), the recording electrode, \( R_e \) is the resistance of the medium between the electrodes (the field potential is recorded as the IR drop across \( R_e \)). \( E_m \) and \( E_m' \) are the batteries of two adjacent segments of membrane, with \( R_m \) and \( R_m' \) their respective resistances. \( R_i \) is the internal resistance and may include the effective resistance across adjacent cell junctions if the membrane segments involve more than one cell.

FIG. 4. Equivalent circuit for recording conditions under which monophasic or intermediate type slow waves are recorded with needle or glass capillary electrodes. \( P_1, R_m, E_m, \) and \( R_i \), as in Fig. 3. \( P_1a \) represents the position of the recording electrode while exerting maximal pressure on the tissue (pressure electrode) or after penetrating the muscle layer (needle electrode). It is assumed that, as a result of applied pressure or penetration of the muscle layer, the cells in immediate contact with the recording electrode are depolarized and that their membrane resistance \( (R_m') \) is reduced. \( R_i \) represents the resistance around the tip of the recording electrode due to indentation or penetration of the tissue beneath it. The membrane potential is recorded monophasically as the IR drop across \( R_e \) and the field potential is recorded as the IR drop across \( R_m \).

No membrane potential is shown, indicating that the electrode is exerting sufficient pressure to depolarize this segment completely. In practice it is assumed (although not directly proven) that this point is reached when further pressure produces no further increase in recorded negativity. With electrodes of the size used in these experiments (tip diam 0.5 mm) an applied pressure of 5 g is usually sufficient to produce maximal depolarization. \( E_m \) represents the membrane potential of the cells adjacent to those depolarized by the electrode, and \( R_m \) represents the membrane resistance of these cells. \( R_e \) is the shunt resistance around the tip of the recording electrode produced by indenting the surface of the tissue when pressure is applied. The circuit is essentially similar to that proposed by Gillespie (12) for recording from apparently single cells with large microelectrodes, except that it includes the field potential component represented by the IR drop across \( R_m \).

According to the diagram of Fig. 4, the potential \( E_m \) recorded between the two electrodes at positions \( P_1 \) and \( P_1a \) will, at any instant, be equal to \( IR_e + E_m, R_e/R_i \), where \( R_i = R_m + R_i + R_m' \). As noted above, if the slow wave is propagated, the time course of the field potential may approximate the second derivative of the time course of the change in membrane potential \( E_m \).
or $IR_v \propto d^2E_m/dt^2$. As $R_v$ decreases, the configuration of the recorded potential $E$ should approach that of $IR_v$, but as $R_v$ increases the configuration of $E$ should approach that of the change in membrane potential, $E_m$. Thus, according to the diagram, the configuration of the recorded potential $E$ depends on the relative magnitude of the shunt resistance $R_s$, and on the magnitudes of $E_m$ and $IR_v$. Since $E_m$ and $IR_v$ are probably the same when either the pressure capillary electrode or the needle electrode is used for recording, the difference in configuration between the slow waves of Figs. 1C and 2C could be attributed primarily to a difference in $R_s$ for the two recording conditions. This is supported by resistance measurements made in conjunction with the potential recordings of Figs. 1 and 2. The resistance between the recording and indifferent electrodes was found to be 1.2, 1.4, and 2.0 kilohms, during the recording of the slow waves shown in Fig. 1A, B, and C, respectively. Similar resistance values for the slow waves of Fig. 2A, B, and C were 1.3, 1.9, and 3.0 kilohms, respectively. Thus, the value of $R_s$ for the recording situation where the glass capillary electrode exerts sufficient pressure on the tissue to record monophasic slow waves (Fig. 2C) is almost 20 times greater than it is for the needle electrode under similar recording conditions.

In order to determine whether the intermediate form of the slow wave could be solely accounted for on the basis that it represents a combination of the monophasic slow wave with its second derivative form, monophasic slow waves were electronically doubly differentiated, then summed with the original monophasic signal, and the result compared to the intermediate form of the slow wave as recorded in Figs. 1 and 2. The amplitude of the monophasic component could be varied prior to being summed. Decreasing the amplitude of this signal is presumably equivalent to decreasing $R_s$ in the diagram of Fig. 4. Typical results of these experiments are illustrated in Fig. 5. Tracings B, D, F, and H are of the original monophasic slow waves as they are fed into the operational amplifier. Figure 5A illustrates the pure doubly differentiated form of the simultaneously recorded monophasic slow wave shown in B, C, E, and G are tracings of the doubly differentiated form added to the original monophasic slow wave. The relative magnitude of the latter decreases progressively from C to G, being maximum at G. This is equivalent to making $R_s$ of Fig. 4 infinite. The configuration of the recorded slow waves in Fig. 5A, C, and G are similar to those of Fig. 1A, B, and C, respectively, indicating that the intermediate type of configuration can indeed result from a superposition of the monophasic potential with its second derivative form.

Most of the monophasic slow waves recorded with the pressure electrode have been obtained from isolated segments of intestine in vitro, whereas the majority of monopolar recording utilizing needle electrodes has been done in situ. In order to demonstrate that it is the recording method and not the preparation which determines the type of record obtained, it was necessary to repeat the experiments of Figs. 1 and 2 using in situ preparations. The tracings of Fig. 6 are typical of those obtained with needle electrodes (A, B) and glass capillary electrodes (C) from the cat jejunum in situ. (These experiments were done in collaboration with Dr. Robert Davis). Figure 6A and C were recorded with the tip of the recording electrode about a millimeter above the intestine in the Tyrode solution which filled the abdominal cavity. The configurations of both these tracings indicate that the slow waves were propagated (since the approximate $d^2E_m/dt^2$, even though the first positive deflection in C is relatively larger than its counterpart in A). The tracing in B was recorded with the tip of the needle electrode penetrating the muscle layers of the intestinal wall. Figure 6, D and F, were recorded with the
CONFIGURATION OF INTESTINAL SLOW WAVES

The configuration of the intestinal slow waves (Fig. 4) is a superposition of the propagated monophasic potential with its related field potential, representing a combination of the temporal courses of the membrane-potential change and its associated current. One of these components predominates in any given intermediate type of record, and can be no more than qualitatively estimated unless their individual magnitudes and time courses can be determined. Otherwise, such records in themselves provide very little information about events occurring at the membrane level. In the case of intracellular recording, contamination by field potentials can sometimes make interpretation difficult. For example, Niedergerke and Orkand (16) have recently presented convincing evidence that the “dents” or “notches” that frequently distort the rising phase of the intracellularly recorded cardiac action potential are due to external field potentials. Eccles (11) has pointed out that under certain recording conditions involving the spinal cord, the field potentials are large enough to necessitate recording both the intracellular and the extracellular potential changes and then subtracting one from the other in order to obtain the actual membrane-potential change. In most cases of intracellular recording, however, the field potentials are so small in comparison to the transmembrane-potential change that they produce negligible distortion of the latter. This is also true, but to a lesser extent, of pressure electrode recording of intestinal slow waves, the maximal amplitude of the slow waves recorded by this method being some 5 mv whereas the field potentials are of the order of 0.2 mv.

In records obtained with the needle electrode in vitro (Fig. 1) the initial hump (or b-fraction) of the slow wave could not be eliminated. This hump is also a prominent feature of slow potentials recorded in vivo by similar type electrodes (Fig. 6B, also refs. 2, 13). In such in vivo recordings, field potentials associated with the intestine are maximal when the reference electrode is situated on the animal at a considerable distance from the recording electrode (e.g., subcutaneously on the inner aspect of one of the thighs). The tip of the recording electrode effectively makes electrical contact with the interiors of many smooth muscle cells, just as the pressure electrode, because of the large area of electrode exposed at the tip, and second, because there is nothing comparable to the glass lip of the pressure electrode that serves to seal its tip and thereby to increase the shunt resistance. Since the shunt resistance (R_s) is comparatively small, the IR drop produced over this resistance is correspondingly small. Since the time course of the IR drop represents the monophasic form of the slow wave, this component of the intermediate form recorded with the needle electrode is smaller than it is in the pressure electrode recordings, whereas its associated field potential is at least as large due to the large distance between the re-

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**Fig. 6.** Slow waves recorded monopolarly from a segment of cat jejunum in situ with chlorided silver needle electrodes (A, B) and glass capillary electrodes (C-E). Animal’s abdominal cavity filled with warm Tyrode solution prior to recording. Tip of the needle electrode was ca. 1 mm above the tissue in A, lodged in muscle layer of intestinal wall in B. Glass capillary electrode ca. 1 mm above tissue in C, exerting slight pressure in D, and maximal pressure in E. Vertical bar: A, 0.5 mv; B, 2.0 mv; C and D, 0.5 mv; E, 5.0 mv. Horizontal bar: 4 sec. Temp: of abdominal cavity, 36 ± 1°C.
cording and indifferent electrodes. Thus, the needle electrode method produces records of comparatively small amplitude (arule as large as 2 mv) with the field potential being a prominent component. The same arguments may also be applied to records obtained with chronically implanted "surface electrodes" (1, 15).

Slow waves recorded with intraluminal electrodes (6) also appear to contain elements of both forms of the potential, but in this case the equivalent circuit is considerably more complicated than it is for the recording methods discussed above. Since the recording electrode presumably does not make contact with intestinal muscle, the monophasic component cannot be accounted for as it is in the case of pressure or needle electrodes. It has recently been shown, however, that monophasic slow waves about 2 mv in amplitude and of configuration similar to those recorded with the pressure electrode, can be obtained in vitro by recording across the combined circular and longitudinal muscle layers of the intestine (5). The explanation that has been offered to account for this phenomenon is that the slow waves generated by longitudinal muscle cells spread electrotonically (and, therefore, decrementally) into the circular muscle layer. It is assumed that the combined muscle layers exhibit syncytial properties, accounting for the fact that during the generation of a given slow wave a potential difference is developed across the muscle layers with the serosal side being more negative than the luminal side. The time course of the change in potential difference across the two muscle layers corresponds to that of the monophasic slow wave. It is very likely that the transmural potential change is responsible for the monophasic component of the intraluminal recording since the electrodes are situated on either side of the intestinal wall, the indifferent electrode being placed on the surface of the animal. The field component may actually be the resultant of field potentials associated with current flow on both the luminal and the serosal sides of the muscle layers.

The analysis of the intermediate type of slow wave discussed above deals only with the case where the slow wave is uniformly propagated, i.e., when the configuration of the field potential approximates dE_m/dt. However, the basic line of reasoning and the schematics of Figs. 3 and 4 can also be applied to those cases where the slow wave is not uniformly propagated, or even where it is not propagated at all; apparently all three situations exist in intestinal muscle both in vivo (13) and in vitro.

In these cases the configuration of the field potential can range anywhere from monophasic (positive or negative), to what is perhaps the special case of d^2E_m/dt^2. In any event it will contribute to the configuration of potentials obtained with monopolar electrodes (and even bipolar electrodes, in some cases) if R_f is small enough, or if IR_x is large compared to the change in membrane potential.

In summary, these experiments indicate that most monopolar recording methods produce records of intestinal slow waves that contain elements of the temporal courses of both membrane potential and membrane current. Such records cannot be interpreted in terms of one or the other of these components unless the time course, polarity, and relative magnitude of each can be determined. If these parameters cannot be resolved, then the records provide very little information about electrical changes at the membrane level.

REFERENCES