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The effect of frequency of coaxial electrical stimulation on the peristaltic activity of the guinea-pig isolated ileum

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Low frequency coaxial electrical stimulation (6/min to 5/sec) of the guinea-pig isolated ileum, arranged for recording peristalsis, was found either to stimulate or to maintain the existing peristaltic activity. High frequency stimulation (5/sec to 100/sec) was found to inhibit regularly the existing spontaneous peristaltic activity. The propulsive peristaltic action was also significantly diminished by high frequency stimulation. In a fatigued preparation all the applied frequencies produced the co-ordinated peristaltic contractions which were blocked by hexamethonium and by cooling. The propulsive activity of these contractions was higher after low frequency stimulation. It is suggested that block of peristalsis after high frequency stimulation might be due to activation of inhibitory elements in the gut.

It has been established that morphine can reduce the output of acetylcholine from the guinea-pig isolated ileum both at rest and during coaxial electrical stimulation (Paton, 1957). A fundamental difference between the effects caused by stimulation at low frequencies (less than 1/sec) and those at higher frequencies was observed. For example, the output of acetycholine per shock was found to be higher at low frequencies and could be inhibited by morphine, whereas the output per shock at higher frequencies was less and morphine resistant.

The significance of the intramural nerve plexuses for the regular peristaltic activity has been repeatedly stressed (Trendelenburg, 1917; Bozler, 1949; Evans & Schild, 1953; Kosterlitz, Pirie & Robinson,1956; Bülbring, Lin & Schofield, 1958). These nerve structures can be stimulated either coaxially (Paton, 1957) or by "field stimulation" (Härtfelder, Kuschinsky & Mosler, 1958). The present experiments were made to examine more closely the effect of various frequencies on the peristaltic propulsive activity of the guinea-pig isolated ileum and particularly to investigate the inhibitory action of high frequency stimulation on the peristaltic activity.

Experimental and results

METHODS

The guinea-pig isolated ileum was immersed in a 40 ml organ bath. The bath fluid was Tyrode solution gassed with a mixture of oxygen 95% and carbon dioxide 5%. The coaxial stimulation was arranged as described by Paton (1957). The impulses were delivered by an electronic square wave stimulator. The duration of pulses was generally 1 msec, and the frequency ranged from 6/min to 100/sec.

For recording the peristaltic activity a method was used which was described in detail by Varagić & Kažić (1965). The oral end of the intestine was tied to the inflow of a U-shaped glass tube 3 mm in diameter. The aboral end of the ileum was tied to a glass tube which was connected

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to a lever with a frontal writing point used for recording the longitudinal muscle contractions. This glass tube was also connected by a soft elastic rubber tube to another U-shaped glass tube which had a side connection to a float recorder for recording the circular muscle contractions. The outflow was measured directly by collecting the propelled fluid in a cylinder. The inflow pressure ranged from 15 to 30 mm water.

Stimulation by various frequencies. When the isolated ileum was perfused by Tyrode solution at a constant small positive intraluminal pressure (about 16 to 20 mm water), a regular peristaltic propulsive activity was observed. This type of activity lasts for several hours (Varagić & Kažić, 1965). When the ileum showing this type of activity was stimulated coaxially by various frequencies, a depression of the peristaltic activity was observed after higher frequencies with either no change or stimulation after low frequencies. Hexamethonium blocked the peristaltic response to stimulation at all frequencies. An ascending order of frequencies was followed, except occasionally when the reverse order was adopted. The frequency of 6/min usually caused no change in the existing spontaneous peristaltic activity, but occasionally produced an increase in number of peristaltic waves. On the other hand, the frequency of 100/sec regularly inhibited the existing peristaltic activity. Fig. 1 shows

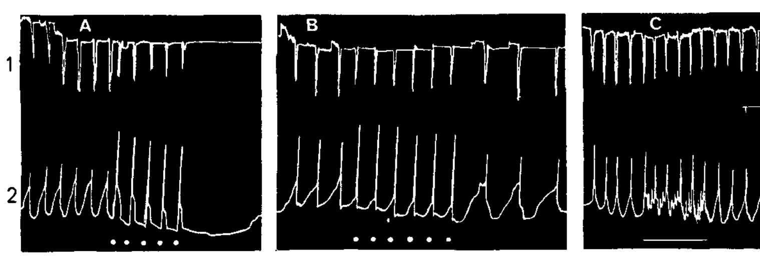

FIG. 1. The effect of electrical stimulation by various frequencies on the peristaltic activity of the guinea-pig isolated ileum. Upper record: contractions of the circular muscle. Lower record: contractions of the longitudinal muscle. At white dots in A, stimulation for 3 sec at 100/sec every 50 sec. At white dots in B, stimulation for 3 sec at 5/sec every 60 sec. At bar in C, stimulation at 6 min continuously for 3·5 min. Time: 1 min.

an experiment in which the ileum was stimulated by various frequencies in descending order. The frequency of stimulation was 100/sec in A and 5/sec in B. Arranging the period of time between stimulations to be very close to the length of time between existing spontaneous peristaltic waves caused a depression of the pre-existing activity after 5/sec frequency (B) and complete block after 100/sec (A). Stimulation with 6/min frequency caused no change in the frequency of existing peristaltic waves, as shown in C. This type of response was obtained in 10 experiments.

The propulsive activity. Generally, the stimulation with frequencies up to 5/sec was found not to affect significantly the peristaltic propulsive activity, whereas higher frequencies depressed it. Fig. 2 shows an experiment in which the propulsive activity was measured during 6 existing spontaneous peristaltic contractions and during the same number of peristaltic contractions produced by stimulation with various frequencies. Again, the period of time between stimulations was so arranged as to be close to the length of time between the existing peristaltic contractions. It can be observed that stimulation with 5/sec (A) caused no change in the amount of propelled fluid for 6 peristaltic contractions. Stimulation with 100/sec (C) produced reductions in the amount of propelled fluid of 85% (5 experiments).

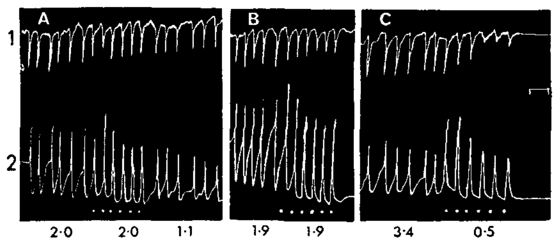

FIG. 2. The effect of frequency of electrical stimulation on the peristaltic propulsive activity of the guinea-pig isolated ileum. The numbers below the tracing indicate the amount of propelled fluid for 6 peristaltic contractions. Upper and lower records as in Fig. 1. At white dots in A, stimulation for 3 sec at 5/sec every 30 sec. At dots in B, stimulation for 3 sec at 30/sec every 30 sec. At dots in C, stimulation for 3 sec at 100/sec every 50 sec. Time: 1 min.

Stimulation during existing peristaltic activity. The depression of the existing spontaneous peristaltic activity produced by stimulation with high frequencies depends on the number of stimulations applied. Fig. 3 shows

FIG. 3. The effect of high frequency stimulation on the spontaneous peristalic activity of the guinea-pig isolated ileum. Upper and lower records as in Fig. 1. At white dots, stimulation for 3 sec at 100/sec every 50 to 60 sec. Time: 1 min.

the effect of coaxial stimulation at 100/sec on the existing spontaneous peristaltic activity. Two stimulations were applied first (at white dots), then four, and finally eight and it was found that the duration of post-stimulatory inhibition was longest after 8 and shortest after 2 stimulations.

Repeating the same number of stimulations with 100/sec caused the post-stimulatory inhibition after the second stimulation to be significantly longer than after the first.

On transferring the bath fluid after high frequency stimulation of the ileum to a bath containing another segment of guinea-pig ileum, arranged for recording peristalsis, no inhibition of peristalsis could be detected.

Stimulation of the fatigued preparation. In a preparation in which a high intraluminal pressure (25–30 mm H_2O) was maintained for a long time, the existing spontaneous peristaltic activity ceased. Stimulation of this fatigued preparation coaxially with low frequencies caused peristaltic propulsive waves to be produced again. (Every single pulse need not be

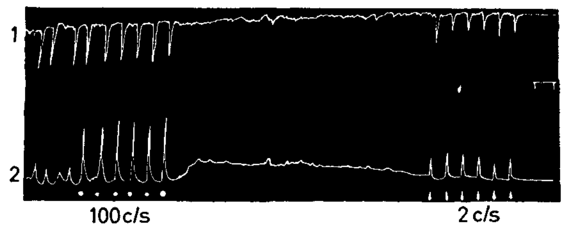

FIG. 4. The effect of a low frequency stimulation on the post-stimulatory inhibition of the peristaltic activity of the guinea-pig isolated ileum. Upper and lower record, as in Fig. 1. At white dots, stimulation for 3 sec at 100/sec every 55 sec. At the arrows, stimulation for 3 sec at 2/sec every 55 sec. Time: 1 min.

followed by a complete peristaltic propulsive contraction.) Meanwhile, this type of stimulation usually initiates a series of spontaneous peristaltic contractions. High frequency stimulation (5–100/sec) for 3 sec at 30 sec intervals also produced the co-ordinated peristaltic contraction in the fatigued preparation. But the propulsive activity was smaller after high frequency than after low frequency stimulation.

The stimulatory effect could also be produced when low frequency stimulation was applied during the post-stimulatory inhibition of peristalsis caused by high frequency stimulation. In Fig. 4, the existing spontaneous peristaltic activity was inhibited by previous stimulation with 100/sec frequency (at white dots); stimulation with 2/sec frequency (at the arrows) restored the peristaltic contractions.

Stimulation at low bath temperature. Lowering the temperature of the bath fluid to 27° decreased significantly the propulsive response of the

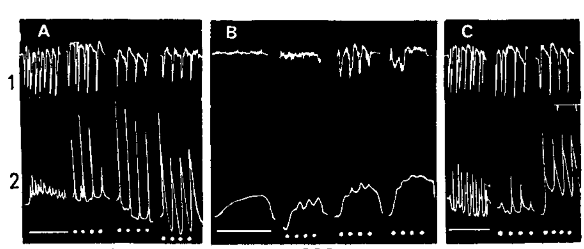

FIG. 5. The effect of cooling on the peristaltic response to various frequency stimulations of the fatigued guinea-pig ileum. Upper and lower record, as in Fig. 1. In A and B, stimulation with 6/min (continuous stimulation), 2/sec, 10/sec, 100/sec. In C, 6/min, 2/sec and 100/sec. Between A and B, temperature of the bath lowered to 20°. Between B and C, temperature raised to 36°. Time: 1 min.

ileum to coaxial stimulation with various frequencies, but its peristaltic contractions were still present. Lowering the temperature of the bath to 23–20° usually blocked the effect of stimulation on the peristaltic propulsive activity. In some experiments the response to low frequency stimulation on these temperatures was more readily blocked than the effect of high frequency stimulation (Fig. 5).

Discussion

The results of the present experiments show a distinct difference between the effects of low and high frequencies of stimulation on the existing spontaneous peristaltic activity of the guinea-pig isolated ileum. The low frequencies (from 6/min to 5/sec) were found either to stimulate or to maintain the existing spontaneous peristaltic activity due to increased intraluminal pressure. Of these various low frequencies only 6/min was applied continuously, whereas higher frequencies had to be applied intermittently, at various time intervals depending on the rhythm of spontaneous peristaltic activity. Adjustment of the stimulation with low frequencies to keep pace with the rhythm of existing spontaneous peristaltic activity, caused no significant change in the co-ordinated peristaltic contractions of longitudinal and circular muscles; nor does the coaxial stimulation with various frequencies produce any change in the peristaltic activity of gut stimulated at shorter time intervals than the periods of time between the existing peristaltic contractions. This is to be expected when the intraluminal pressure is kept low. It seems therefore that the co-ordinated peristalic activity can be produced by coaxial stimulation only if time is allowed for the gut to be filled and if the intraluminal pressure is high enough. Paton (1955) has already shown that an emptying reaction can be seen in response to single shocks in an intestine distended by raising the intraluminal pressure. This type of response resembles the peristaltic reflex and is inhibited by ganglion blocking agents. Similar responses were obtained after coaxial stimulation by Harry (1962). These responses consisted of contractions both of the longitudinal and circular muscle layers and were related to the amount of acetylcholine found in the bath fluid in which the ileum was suspended.

In fatigued preparations under constant intraluminal pressure, peristaltic activity is absent, but all the applied frequencies produced the co-ordinated propulsive contractions of the longitudinal and circular muscle. However, the propulsion activity was less after high than after low frequency stimulation. The peristaltic response to all these stimulations could be blocked by hexamethonium. Sometimes, particularly after low frequencies of stimulation, the spontaneous peristaltic activity was triggered off and continued for some time even after stopping the stimulation.

The responses of the fatigued preparation to the various stimulation frequencies could be reversibly inhibited by lowering the temperature of the bath fluid to 20°. Lowering the temperature of the bath fluid to 27° only depressed the peristaltic propulsive activity, but the peristaltic contractions were still produced by stimulation. The sensitivity of the nervous elements and synaptic transmission in the gut to cooling has already been investigated (Ambache, 1946; Kosterlitz & Robinson, 1957; Varagić & Beleslin, 1958). Paton (1957) concluded from the character of the strength-duration curve and from the pharmacological responses that during coaxial stimulation the nervous structures are stimulated. Our experiments with the cooled preparation support this conclusion.

An inhibition of the existing spontaneous peristaltic activity was regularly observed after stimulation with higher frequencies (from 5 to 100/sec). This inhibition was found to be dependent on the number of stimulations applied. Stimulation with frequencies from 10 to 25/sec produced a short-lasting inhibition, whereas stimulation with 30 to 100/sec caused an inhibition of longer duration. Our experiments do not solve the problem of the origin of this inhibition. Garry & Gillespie (1955) and Varagić (1956) have shown that the optimal frequencies for stimulation of the parasympathetic colonic nerves of the rabbit are about 5/sec, whereas the optimal frequencies for stimulation of lumbar colonic sympathetic nerves are about 50/sec. On the other hand, Rand & Ridehalgh (1965) found that guinea-pig colon responded to stimulation of either nerve at 50/sec with maximal responses. It is still possible that stimulation with higher frequencies specifically affects the adrenergic, or some other inhibitory nervous elements in the isolated ileum leading to cessation of existing spontaneous peristaltic activity. It has also been shown that in preparations where adrenaline causes relaxation and loss of tone, this effect is associated with hyperpolarisation of the smooth muscle membrane and reduction or cessation of spike activity (Bülbring, 1954; 1957). Our experiments do not exclude the possibility of an unspecific hyperpolarisation produced by high frequency stimulation. On the other hand, we were unable to show the presence of an inhibitory substance in the bath fluid after high frequency stimulation.

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