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THE EFFECT OF COOLING AND OF 5-HYDROXYTRYPTAMINE ON THE PERISTALTIC REFLEX OF THE ISOLATED GUINEA-PIG ILEUM

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Cooling the guinea-pig ileum to 19 to 26° abolished the emptying phase of the peristaltic reflex. The effects of cooling to 10° were usually reversible, but cooling to 5° for 3 to 8 hr. produced an irreversible decrease in or abolition of the emptying phase. 5-Hydroxytryptamine (5-HT) added in low concentrations to the fluid outside the intestine restored slight peristaltic activity after this activity had been abolished by cooling. If the peristalsis had been depressed but not abolished by cooling, 5-HT in the bath sometimes abolished it. 5-HT introduced into the lumen of the cooled gut regularly restored or increased peristaltic activity. This action was prevented by previous injection of 2-bromolysergic acid diethylamide into the lumen. It is suggested that, when introduced into the lumen, 5-HT may sensitize the sensory receptors in the mucosa of the cooled intestine. When applied outside, 5-HT may facilitate transmission at synapses involved in the peristaltic reflex arc, the excitability of which has been depressed by cooling.

It was found in previous work on the isolated rabbit colon that cooling the preparation had a greater effect on synaptic transmission than on depolarization of the postsynaptic neurone or on conduction in the postganglionic fibres (Varagić and Beleslin, 1957). Bülbring and Lin (1957) suggested that the formation of 5-hydroxytryptamine (5-HT) by the intestinal mucosa may be part of the physiological mechanism of peristalsis; 5-HT is also known to depress or abolish the peristaltic reflex (Kosterlitz and Robinson, 1957; Ginzel, 1957). We have therefore studied the effect on the peristaltic reflex of cooling and of 5-HT on the cooled intestine. It was hoped that these experiments might provide additional information about the physiological rôle of 5-HT in peristalsis.

METHODS

A modification of the method of Trendelenburg (1917) was used. The apparatus was arranged as shown in Fig. 1. The upper end of a piece of guinea-pig ileum (G) was tied to a small glass cylindrical cap with a side-piece on which a rubber membrane was fitted (A). Through this it was possible to introduce the drug into the lumen of the intestine; it could be washed out through another side-piece (B). The glass cap was attached to a lever to record longitudinal muscle contractions. A record of peristaltic activity was made with the volume recorder described by Stephenson (1948). The peristaltic reflex was tested by raising the intraluminal pressure to a constant height (varying from 20 to 35 mm. in different experiments) for 60 sec. at constant intervals.

The gut was cooled by lowering the temperature of the bath. The intraluminal injections were made in 0.2 ml. volume. The intestine was bathed in Tyrode solution through which a gas mixture containing 97% O_2 and 3% CO_2 was bubbled at a constant rate throughout the period of cooling. Unless otherwise stated the volume of the bath was 20 ml.

The drugs used were 5-hydroxytryptamine creatinine sulphate and 2-bromolysergic acid diethylamide (BOL-148).

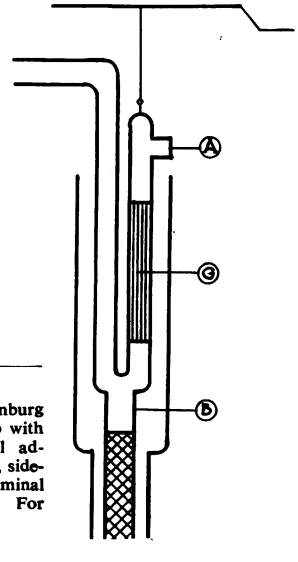

FIG. 1.—Modified Trendelenburg preparation. A, glass cap with side-arm for intraluminal administration of drugs. B, side-arm for removal of intraluminal fluid. G, guinea-pig ileum. For fuller explanation see text.

RESULTS

The Effect of Cooling on the Peristaltic Reflex.—It is known that, in intestinal preparations which have been stored at 5° for 18 to 24 hr., only contraction of longitudinal muscle (preparatory phase of the peristaltic reflex) can be elicited (Ambache, 1946). We found that lowering the bath temperature to 19 to 26° abolished the emptying phase of the peristaltic reflex, and that cooling the preparation to 5° for 3 to 8 hr. caused irreversible changes in the gut in consequence of which the emptying phase was substantially reduced or abolished.

In 8 out of 20 experiments the emptying phase was abolished at 25°, and in 6 out of 20 at 24°. In 5 experiments in which the bath temperature was lowered to 26 to 27° the peristaltic reflex was only slightly depressed. The rate and amplitude of contraction of the circular muscle were diminished.

In some experiments the effects of cooling to 10° were reversible if sufficient time was allowed for the preparation to recover after rewarming. In other experiments after only moderate cooling (to 24°) the peristaltic reflex was not completely restored after rewarming the gut.

A typical experiment is shown in Fig. 2. Between *a* and *b* the preparation was cooled to 5° for 3 hr. The temperature of the bath was then raised to 37° and a period of 1 hr. was allowed for the preparation to recover. At the end of this period (Fig. 2*b*), raising the intraluminal pressure produced very slight peristaltic activity. The emptying phase was greatly reduced but the preparatory phase was almost unchanged.

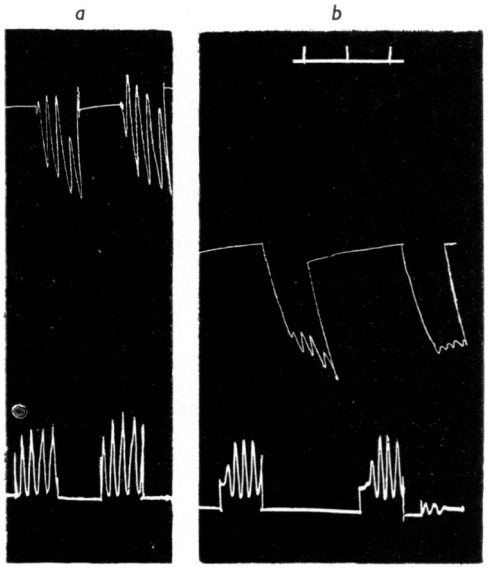

FIG. 2.—Effect of cooling on peristaltic activity of isolated guinea-pig ileum. Upper record, peristalsis. Lower record, contractions of the longitudinal muscle. *a*, normal reflex. Between *a* and *b*, bath temperature 5° for 3 hr. *b*, 1 hr. after rewarming to 37°. Time, 1 min.

The Effect of 5-Hydroxytryptamine (5-HT).—Kosterlitz and Robinson (1957) and Ginzel (1957) reported that 5-HT depressed or abolished the peristaltic reflex of the guinea-pig ileum when added to the bath in concentrations of 10^{-6} to 10^{-5} g./ml.; in these experiments the 5-HT was acting on the outside of the intestine. In the present experiments the addition of 5-HT to the bath in concentrations of 5×10^{-9} to 2×10^{-8} g./ml. caused the reappearance of weak peristaltic activity after this activity had been abolished by cooling. In the experiment illustrated in Fig. 3, lowering the bath temperature to 25° between *a* and *b* abolished the emptying phase of the peristaltic reflex. After the addition between *b* and *c* of 0.4 μg. 5-HT to the bath, making the final concentration 2×10^{-8} g./ml., slight peristaltic activity could be produced. When a similar dose of 5-HT was added to the bath 12 min. later, between *c* and *d*, the peristaltic reflex could not be elicited. When this dose was introduced into the lumen of the intestine, peristalsis appeared again when the pressure was raised, as shown in *e*. The dose of 0.4 μg. of 5-HT was injected in 0.2 ml., and subsequent filling of the intestine produced an internal concentration which lay between 1 and 2×10^{-7}.

These effects were observed in 11 of 13 experiments in which the gut was cooled to 24 to 27°. In the other two experiments the cooling caused only a decrease in the number and amplitude of the peristaltic waves. The addition of 5-HT to the bath caused an initial slight increase and a subsequent abolition of the peristaltic reflex. The preparation, whose activity was recorded in Fig. 4, was cooled between *a* and *b*. The effect of 5-HT, 4×10^{-8} g./ml., is shown in *c*. Between *c* and *d*, the 5-HT was allowed to act for 20 min., then washed out, and the preparation was left for 2 hr. The peristaltic reflex was still abolished (Fig. 3*d*). However, after the introduction of a similar dose of 5-HT into the lumen of the intestine between *d* and *e* the peristaltic reflex was restored.

In another series of 6 experiments the bath was cooled to 20 to 25°; this caused complete abolition of the peristaltic reflex. The addition of 5-HT to the bath in concentrations of 5×10^{-9} to 5×10^{-8} g./ml. did not cause the reappearance of peristalsis in these experiments.

The introduction of 5-HT into the lumen of the guinea-pig ileum in doses of 10 to 400 ng., after peristalsis had been abolished by cooling, regularly caused the reappearance of peristalsis. This was in accordance with the findings of Bülbring and Lin (1957), and was observed in 10 experiments. In 2 experiments, 5 ng. of 5-HT introduced into the lumen was found to be insufficient to produce peristalsis.

Effect of 2-Bromolysergic Acid Diethylamide (BOL-148).—Fig. 5 illustrates the abolition of the effect of 5-HT by BOL-148. Lowering the bath temperature to 24° abolished the peristaltic reflex (Fig. 5*b*, 1). The addition of 0.1 μg. 5-HT to the bath, making the concentration 10^{-8} g./ml., did not produce any visible change (2). After the introduction of 5 ng. 5-HT into the lumen, raising the pressure produced three peristaltic waves (3). Between *b* and *c*, BOL-148 was left in the lumen for 5 min. The introduction of 5-HT into the lumen while BOL-148 was present caused no reappearance of peristalsis (Fig. 5*c*).

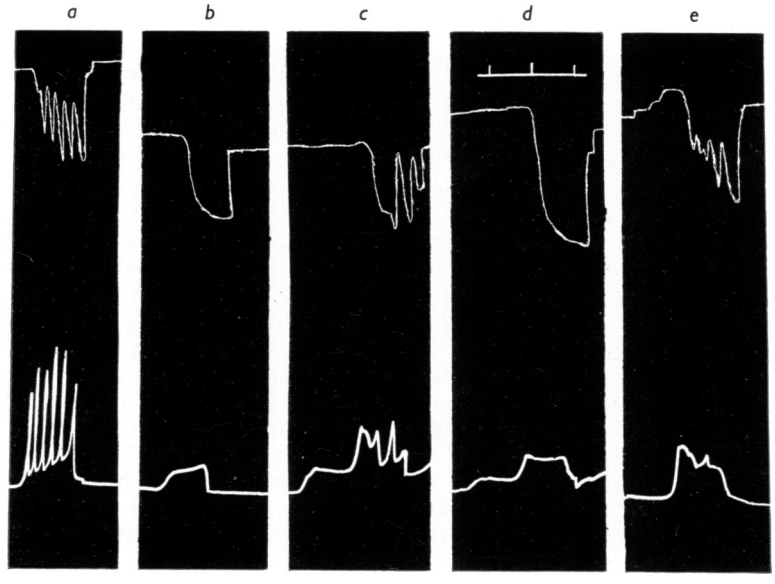

FIG. 3.—Effect of 5-HT on cooled guinea-pig ileum. Upper record, peristalsis. Lower record, contractions of longitudinal muscle. *a*, normal reflex at 37°. Temperature after this point, 25°. *b*, reflex at 25°. *c*, 0.4 μg. 5-HT added to bath. *d*, similar dose 12 min. later. *e*, similar dose introduced into lumen of gut. Time, 1 min.

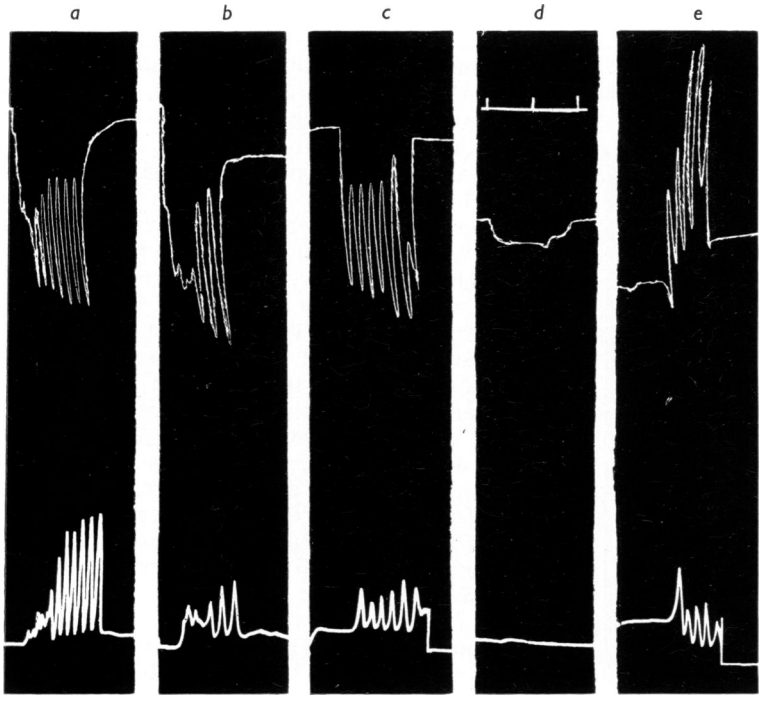

FIG. 4.—Effect of 5-HT on cooled guinea-pig ileum. Upper record, peristalsis. Lower record, contractions of longitudinal muscle. *a*, reflex at 37°. Temperature after this point, 27°. *b*, reflex at 27°. *c*, after adding 0.4 μg. 5-HT to the bath. Between *c* and *d*, 5-HT was allowed to act for 20 min. and then washed out. *d*, reflex 2 hr. after washing. *e*, effect of 0.4 μg. 5-HT inside the lumen. Time, 1 min.

DISCUSSION

In the present experiments, cooling the guinea-pig ileum to temperatures between 19° and 26° abolished the emptying phase of the peristaltic reflex. In the majority of experiments this abolition took place at 24 to 25°. Feldberg and Lin (1949) observed incomplete inhibition of the emptying phase at 32.5°, while Kosterlitz and Robinson (1957) observed

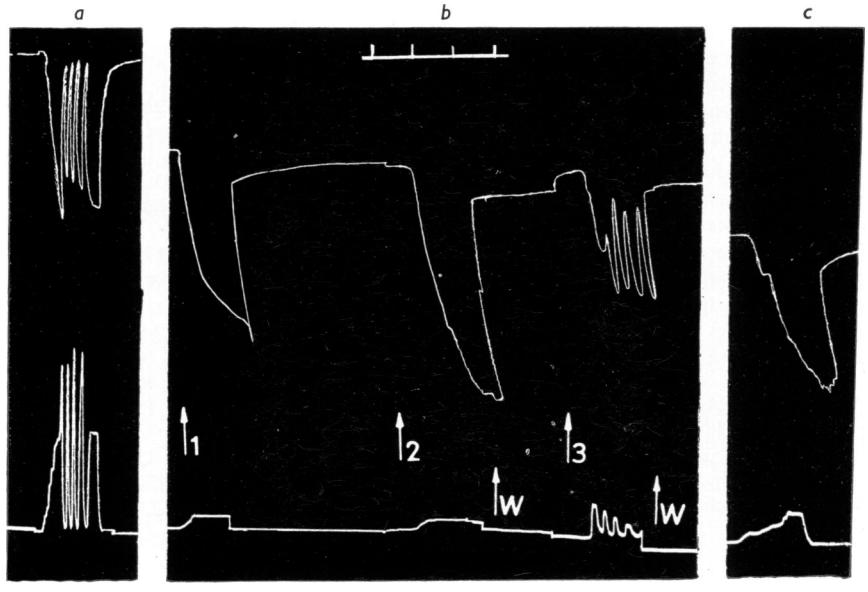

FIG. 5.—Effects of 5-HT and BOL-148 on cooled guinea-pig ileum. *a*, reflex at 37°. Temperature after this point, 24°. *b*, no addition to bath. 1, reflex. 2, 0.1 μg. 5-HT added to bath. 3, 5 ng. 5-HT intraluminally. Between *b* and *c* 0.2 μg. BOL-148 was left in the lumen for 5 min. *c*, 5 ng. 5-HT intraluminally in the presence of BOL-148. Time, 1 min.

that the co-ordinated contractions of the circular muscle layer ceased at 21°. In the present experiments it was found that the effects of cooling to temperatures between 27° and 10° were usually reversible. On the other hand cooling to 5° even for short periods (3 to 8 hr.) caused irreversible changes, and reduced or abolished the emptying phase of the peristaltic reflex. The cooling seldom affected the contractions of the longitudinal muscle.

5-HT added to the bath at 37° in concentrations from 10^{-6} to 10^{-5} g./ml. depresses or abolishes the peristaltic reflex (Kosterlitz and Robinson, 1957; Ginzel, 1957). The present experiments show that 5-HT added to the bath in concentrations from 5×10^{-9} to 2×10^{-8} g./ml. may cause reappearance of slight peristaltic activity in preparations which have been cooled to 24 to 25°. This was evident when the peristaltic reflex had been abolished and also when it had only been depressed by the cooling. It was particularly easy to restore peristalsis in the cooled guinea-pig ileum with 5-HT by introducing the drug into the lumen in doses of 10 to 400 ng.

There are at least three possible explanations of the effect of 5-HT on the cooled guinea-pig ileum: (*a*) it may act by sensitizing the sensory receptors in the mucosa which trigger the reflex, as suggested by Bülbring and Lin (1957); (*b*) it may facilitate the transmission of impulses from afferent to efferent parts of the reflex arc; or (*c*) it may act in both ways, according to the site of application. In favour of the second hypothesis (*b*) is the finding of Trendelenburg (1956) that 5-HT potentiates the response of the nictitating membrane to submaximal preganglionic stimulation of the cervical sympathetic chain. It has also been shown that synaptic transmission in the isolated rabbit colon is more easily blocked by cooling than is depolarization of the postsynaptic neurone or conduction in the postganglionic fibres (Varagić and Beleslin, 1957). Morphological investigations (Bülbring, Lin, and Schofield, 1958) suggest that there is at least one synapse in the peristaltic reflex arc. This is also supported by the fact that this reflex may be abolished by large doses of nicotine (Feldberg and Lin, 1949) and by methonium compounds (Paton and Zaimis, 1949). It is therefore possible that 5-HT may act by facilitating transmission at a synapse of the peristaltic reflex arc. Thus, while 5-HT at normal temperature prevents the peristaltic reflex, it appears to be able to exert the opposite effect when the excitability is depressed under the special conditions of cooling.

In favour of the first hypothesis is the finding that 5-HT is most effective in restoring the peristaltic reflex after cooling when it is introduced into the lumen. In addition, we found that, both in the cooled guinea-pig ileum (Fig. 4) and in the ileum at 37°, the intraluminal injection of 5-HT sometimes restores slight peristaltic activity when

this has been prevented by a large dose of 5-HT acting on the outside of the intestine. In these conditions, the stimulation of the sensory receptors in the mucosa by intraluminal 5-HT may increase the impulses going to the effector cells and thereby overcome the block.

It is concluded that applications of 5-HT to the outside may have a directly facilitating action on ganglionic transmission in the cooled intestine, which is not seen in normal conditions. The intraluminal application of 5-HT, on the other hand, may relieve ganglionic block in an indirect way, by augmenting sensory impulses arising from mucosal receptors.

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