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# THE EFFECT OF SUBSTANCE P ON THE PERISTALTIC REFLEX OF THE ISOLATED GUINEA-PIG ILEUM

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The introduction of substance P into the lumen of the isolated guinea-pig ileum caused an increase in the number and amplitude of the peristaltic waves. In preparations in which the peristaltic reflex was abolished, by fatigue, by external or internal application of 5-hydroxytryptamine, or by lowering the temperature of the bath, the introduction of substance P into the lumen of the intestine restored peristalsis. This effect of substance P was absent in preparations in which the mucous membrane was removed. Hexamethonium abolished the effect of substance P on peristalsis. It is concluded that substance P acts on the afferent nervous elements of the peristaltic reflex arc, possibly on the sensory receptors.

Substance P has been found in relatively high amounts in the intestine and brain of various animals, such as the monkey, horse, ox, pig, sheep, dog, cat, rabbit and man (v. Euler and Gaddum, 1931; Douglas, Feldberg, Paton and Schachter, 1951; Ehrenpreis and Pernow, 1952; Pernow, 1953). A factor having, qualitatively and quantitatively, the same properties as substance P has been shown to occur in the intestine of the cod and the dogfish and in cod brain (v. Euler and Östlund, 1956). The retina of the cow has also been found to contain high amounts of substance P (Duner, v. Euler and Pernow, 1954).

The exact physiological role of substance P is still unknown. A central stimulating effect of substance P has been shown by v. Euler and Pernow (1954), and evidence has been presented that substance P stimulates afferent fibres (Lembeck, 1957). In evaluating the action of substance P on the jejunum of the rabbit, Blair and Clark (1956) concluded that the stimulant action of substance P on intestinal motility is mediated through a very labile mechanism which could well have a trophic influence on the amplitude of spontaneous contractions.

It is known that the peristalsis elicited by increased intraluminal pressure is augmented in the presence of substance P (Gernandt, 1942). In the present experiments the effect of substance P on the peristaltic reflex was studied, introducing the substance into the lumen of the guinea-pig ileum.

## MATERIALS AND METHODS

For recording the peristaltic activity a modification of the method of Trendelenburg (1917) was used, which has been described in detail in a previous paper (Beleslin and Varagić, 1958). By this method it was possible to introduce the drug into the lumen of the intestine and to wash it out. The peristaltic activity was recorded by means of Stephenson's float recorder which records volume changes (Stephenson, 1948).

The peristaltic reflex was tested by raising the intraluminal pressure (varying from 30 to 50 mm. in different experiments) for 90 sec. at constant intervals.

Several experiments were done with preparations from which the mucosa had been removed. For this purpose a loop of intestine was turned inside out by slipping it over a glass rod. The mucosa was gently scraped off using a piece of filter paper, and then the loop of intestine was returned to its normal position. As a control for these experiments another piece of ileum was subjected to the same procedure but without scraping off the mucosa.

The intraluminal injections were made in a volume of 0.1 to 0.2 ml. The intestine was kept in Tyrode solution which was oxygenated with a mixture containing 97% $O_2$ and 3% $CO_2$. The volume of the bath was 20 ml.

The drugs used were 5-hydroxytryptamine creatinine sulphate, hexamethonium bromide and nicotine hydrogen tartrate. Two samples of substance P were used, one containing 2.6 units/mg. and the other 3 units/mg.

## RESULTS

*Substance P and Normal Peristalsis.*—It has been shown that substance P may potentiate the peri-

stalsis produced by increased intraluminal pressure. Gernandt (1942) found that, in the presence of substance P, peristaltic activity was produced by an otherwise subliminal pressure and that at higher pressure peristaltic activity was increased. Similar results were obtained in the present experiments. Substance P was found to potentiate peristalsis when introduced into the lumen of the intestine. This potentiation was evident by an increase in the number or amplitude of the peristaltic waves. In a preparation in which fatigue was allowed to develop, substance P produced vigorous peristalsis. A typical experiment is shown in Fig. 1. The normal peristalsis is shown in *a*. In *b* the effects of fatigue and of substance P are shown. The intraluminal pressure was raised and was kept high for some time. It can be seen that fatigue developed after 5 min. and peristaltic activity stopped. At the first arrow 0.2 ml. of Tyrode solution was introduced into the lumen of the gut and it produced two peristaltic waves. At P, 10 units of substance P was introduced into the lumen of the intestine. Substance P caused the appearance of vigorous peristalsis which lasted for 15 min. with no sign of fatigue. Between *b* and *c* the intestine and the bath were washed out, after which the emptying phase of the peristaltic reflex was almost abolished, as shown in *c*.

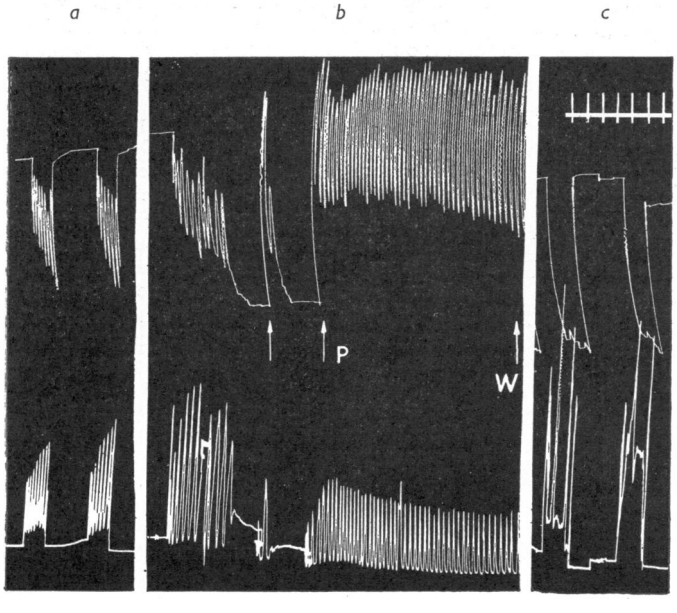


FIG. 1.—Effect of substance P on fatigued guinea-pig ileum. Upper record: peristalsis. Lower record: contractions of longitudinal muscle. *a*, two normal peristaltic responses to a raised intraluminal pressure. *b*, at first arrow, 0.2 ml. Tyrode solution injected intraluminally (i.l.); at P, 10 units substance P i.l.; at W, bath and intestine washed out. *c*, 110 min. after washing. Time, 1 min.

*Effect of Lowering the Bath Temperature.*—It has been shown that cooling the guinea-pig ileum to temperatures between 19° and 26° abolished the emptying phase of the peristaltic reflex (Kosterlitz and Robinson, 1957; Beleslin and Varagić, 1958). The addition of 5-hydroxytryptamine (5-HT) in low concentrations to the fluid outside the intestine restored slight peristaltic activity after this activity had been abolished by cooling. The introduction of 5-HT into the lumen of the cooled gut regularly restored or increased peristaltic activity (Beleslin and Varagić, 1958). We have now found that the introduction of substance P into the lumen of the cooled intestine also caused the reappearance of peristalsis. A typical experiment is shown in Fig. 2. The normal peristaltic reflex at 37° is shown in *a*. Between *a* and *b* the temperature of the bath was lowered to 24°. At this temperature the peristaltic reflex was abolished. At P in *b*, 10 units of substance P was introduced into the lumen of the gut. The immediate effect of the substance P was a slight rise in the tone of both the circular and longitudinal muscles. After a latent period of 13 min., vigorous peristalsis occurred. This activity lasted for 10 min. with no sign of fatigue. The bath and the intestine were then washed out at W. Twenty min. later, raising the intraluminal pressure caused no peristalsis.

*Substance P and 5-HT.*—5-HT added to the bath at 37° in concentrations from $10^{-6}$ to $10^{-5}$ g./ml. will depress or abolish the peristaltic reflex (Kosterlitz and Robinson, 1957; Ginzel, 1957). It is now shown that substance P may antagonize this effect of 5-HT. At H in Fig 3*a*, 1 mg. of 5-HT was put into the bath, making the final concentration $5 \times 10^{-5}$ g./ml. This drug was allowed to act for 1 min. and then the intraluminal pressure was raised. This caused only one peristaltic wave followed by a complete inhibition of the peristaltic reflex. At P, 10 units of substance P was introduced into the lumen of the gut. After a latent period of 3 min. this dose of substance P caused vigorous peristalsis. Between Figs. 3*a* and *b*, the bath and the intestine were washed out. Twenty-five min. after washing out 5-HT and substance P, the peristaltic reflex was still abolished, but it had recovered 140 min. after washing (Fig. 3*b*).

After the saturation of the tryptamine receptors by the introduction of 5-HT into the lumen of the gut, a similar observation was made. Fig. 4 shows the effect of 5-HT and substance P on a fatigued guinea-pig ileum. The intraluminal injection of 400 ng. of 5-HT caused the reappearance of peristalsis which lasted for 120 sec., as shown at the first H. The second intraluminal injection of the same dose of 5-HT produced only three peristaltic waves. The third intraluminal injection of the same dose of 5-HT had no effect, as shown at the third H. The introduction of 20 units of substance P into the lumen of the gut now caused vigorous peristalsis lasting 5 min., as shown at P.

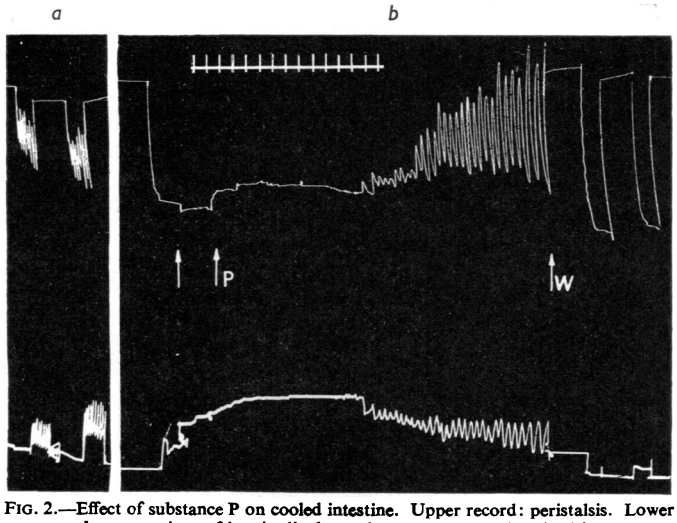


FIG. 2.—Effect of substance P on cooled intestine. Upper record: peristalsis. Lower record: contractions of longitudinal muscle. *a*, two normal peristaltic responses at 37° to raised intraluminal pressure. Between *a* and *b* the temperature of the bath was lowered to 24°. *b*, at first arrow, 0·2 ml. Tyrode solution i.l.; at P, 10 units substance P i.l.; at W, bath and intestine washed out. Time, 1 min.

*Effect of Hexamethonium.*—The contractions of the longitudinal muscle of the guinea-pig ileum caused by substance P are not blocked by ganglion-blocking agents. It was therefore concluded that the action of substance P is probably due to a direct action on the smooth muscle fibres (Pernow, 1953). In the present experiments, it is shown that the effect of substance P on peristalsis can be abolished by hexamethonium. The effect of 10 units of substance P, introduced intraluminally, on the fatigued intestine is shown in Fig. 5*a*. At $C_6$ 0.4 mg. ($2 \times 10^{-7}$ g./ml.) hexamethonium was added to the bath and produced a complete abolition of the effect of substance P.

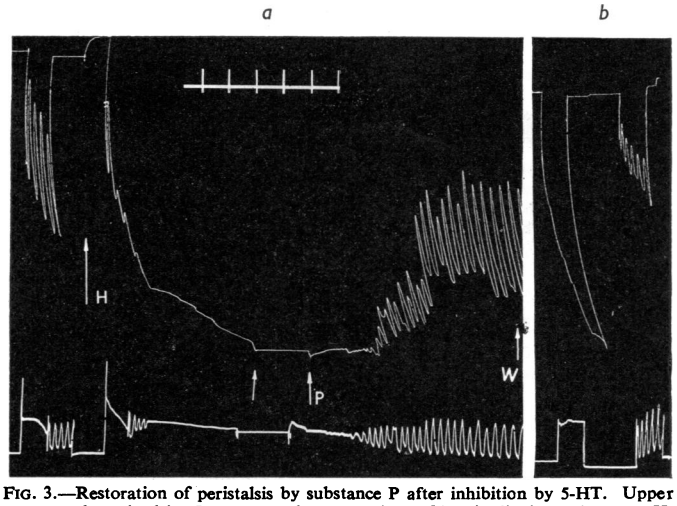


FIG. 3.—Restoration of peristalsis by substance P after inhibition by 5-HT. Upper record: peristalsis. Lower record: contractions of longitudinal muscle. *a*, at H, 1 mg. 5-HT added to bath; at arrow, 0·2 ml. Tyrode solution i.l.; at P, 10 units substance P i.l.; at W, bath and intestine washed out. *b*, responses obtained 25 and 140 min. after washing out. Time, 1 min.

*Effect of Removing the Mucous Membrane.*—In a separate series of 9 experiments substance P was tested on pieces of intestine from which the mucous membrane had been removed. The peristaltic reflex was abolished in all 9 preparations. This is in accordance with the finding of Bülbring, Lin and Schofield (1958). The introduction of 10 units of substance P into the lumen of an intestine from which the mucous membrane had been removed did not cause peristalsis to return. This was the case in all 9 experiments. In 7, nicotine caused a contraction of the gut, showing that a ganglionic response could be obtained although substance P failed to produce peristalsis. The effect of substance P in a fatigued preparation is shown in Fig. 6*a*. The effect of substance P on a preparation from which the mucous membrane had been removed is shown in Fig. 6*b*. Although substance P failed to produce peristalsis, nicotine (at G) caused a contraction of the longitudinal muscle.

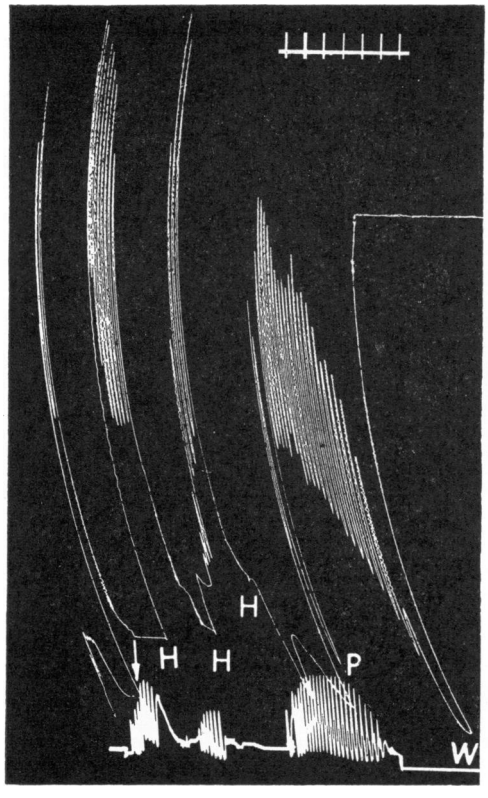


FIG. 4.—Effect of 5-HT and substance P on peristalsis. Upper record: peristalsis. Lower record: contractions of longitudinal muscle. At arrow, 0·2 ml. Tyrode solution i.l.; at H, 400 ng. 5-HT i.l.; at P, 20 units substance P i.l. Time, 1 min.

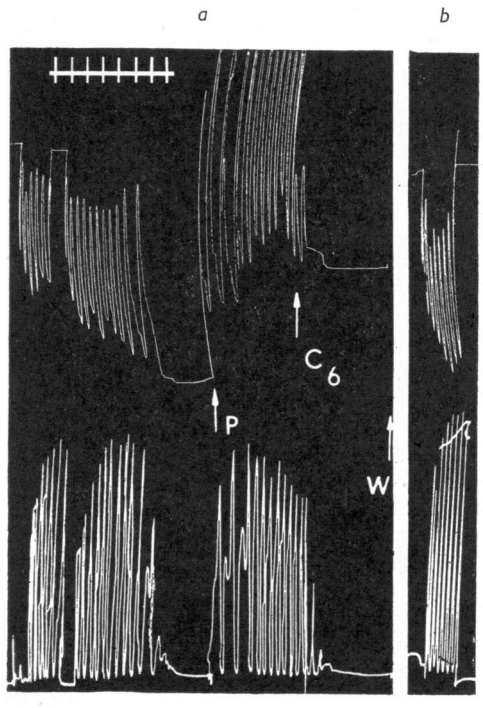


FIG. 5.—Effect of hexamethonium ($C_6$) on peristalsis produced by substance P. Upper record: peristalsis. Lower record: contractions of longitudinal muscle. *a*, at P, 10 units substance P i.l.; at $C_6$, 0·4 mg. $C_6$ added to bath; at W, bath and intestine washed out. *b*, 45 min. after washing. Time, 1 min.

## DISCUSSION

Substance P potentiates peristalsis which occurs in response to increased intraluminal pressure. Local and intravenous application of small amounts of substance P may increase the tone and rhythmic spontaneous activity of the small intestine *in situ* (Gernandt, 1942). The results of the present experiments are in accordance with these findings. In addition, it was found that the introduction of substance P into the lumen of the fatigued intestine gave rise to vigorous peristalsis. In some experiments the period of increased peristaltic activity was followed by a partial or almost complete abolition of the emptying phase of the peristaltic reflex.

The present experiments show that substance P may produce peristalsis when the tryptamine receptors have been saturated by a large dose of 5-HT added to the bath or introduced into the lumen of the gut. The receptors for substance P may therefore differ from those for tryptamine.

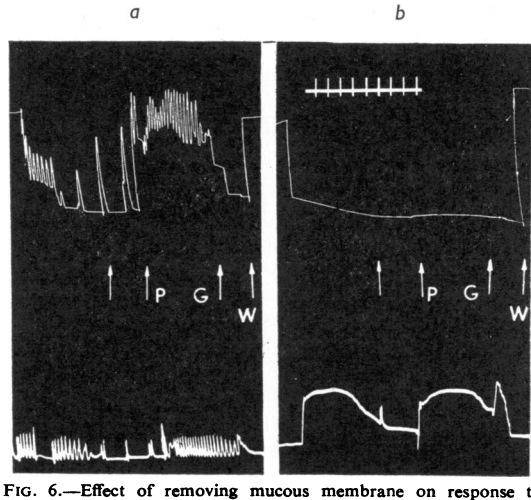


FIG. 6.—Effect of removing mucous membrane on response to substance P. Upper record: peristalsis. Lower record: contractions of longitudinal muscle. *a*, control preparation. *b*, preparation from which mucous membrane had been removed. At first arrow, 0·2 ml. Tyrode solution i.l.; at P, 10 units substance P i.l.; at G, 0·75 mg. nicotine into bath; at W, bath and intestine washed out. Time, 1 min.

The experiments with hexamethonium show that substance P acts at two different sites. A direct effect of substance P on the smooth muscle fibres has been postulated by Pernow (1953). The present experiments show that substance P may also act on the preganglionic nervous elements of the reflex arc. It was possible in some experiments to differentiate between these two actions of substance P. Thus the addition of hexamethonium to the bath stopped the contractions of the circular muscle and left the contractions of the longitudinal muscle intact. Substance P has been found to stimulate the afferent nervous fibres in the rabbit ear (Lembeck, 1957). The present experiments suggest that substance P stimulates the afferent fibres of the peristaltic reflex arc. The experiments with the intestine from which the mucous membrane was removed seem to support this view. On the other hand these experiments also indicate that substance P may act on the sensory receptors in the mucosa. Whether the action of substance P is part of a physiological process required for peristalsis is not proved by these experiments. Medaković and Radmanović (personal communication) have found that during peristalsis a substance (or substances) is liberated into the lumen of the guinea-pig ileum which contracts the guinea-pig ileum and fundus of the rat stomach. The effect of this substance cannot be abolished by atropine or antazoline (Antistin) and only occasionally can it be abolished by 2-bromo-lysergic acid diethylamide.

In some experiments the effect of substance P was only seen after a latent period. This period was found to be considerably longer when the substance P was introduced into the lumen of the cooled intestine. It is possible therefore that substance P may act through another active substance which it liberates, and that the process of liberation is affected by changes in temperature.

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