

THE EFFECT OF SUBSTANCE P ON THE PERISTALTIC REFLEX WHEN ACTING ON THE OUTSIDE OF THE ISOLATED GUINEA PIG ILEUM

BY D. BELESLIN AND V. VARAGIĆ

From the Department of Pharmacology, Medical Faculty, Belgrade, Yugoslavia.

Received August 1, 1958

Substance P (30–100 units) when acting on the outside of the isolated guinea pig ileum abolished the peristaltic reflex. Small doses (4 units) occasionally potentiated peristalsis. The block produced by high doses could not be overcome by intraluminal injections of 5-HT or substance P itself. The effect of nicotine was reduced or abolished in the presence of high doses of substance P. The high doses can also block the response of the gut to the small doses of the same substance. It is concluded that the blocking effect of substance P takes place between the synapse in the peristaltic reflex arc and the effector cell in the plain muscle.

PREVIOUSLY¹ we reported that 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. It also restored peristalsis in preparations in which the peristaltic reflex was abolished by fatigue, by 5-HT, intraluminally or in the bath, or by lowering the temperature of the bath.

Small amounts of substance P applied externally to the small intestine *in situ* also increases the tone and the rhythmic spontaneous activity².

This work reports the effect of large doses of substance P in the bath on the peristaltic reflex of the guinea pig ileum.

MATERIALS AND METHODS

The peristaltic activity was recorded as described previously¹. The reflex was tested by raising the intraluminal pressure (varying from 30 to 60 mm. in different experiments) for 90 seconds at 10 minute intervals. The ileum was kept in Tyrode's solution at 37° in a bath volume of 30 ml. and oxygenated with a mixture containing 97 per cent O₂ and 3 per cent CO₂. Intraluminal injections were made in a volume of 0.1 to 0.2 ml.

The drugs used were 5-hydroxytryptamine creatinine sulphate, nicotine hydrogen tartrate and a sample of substance P containing 1 unit/mg.

RESULTS

The Effect of Increasing Doses of Substance P

The addition of small doses of substance P into the bath caused an increase in amplitude of the peristaltic waves in three out of seven experiments. In several other experiments the doses of substance P were gradually increased, and a typical response is shown in Figure 1. The addition of 20 units of substance P (at P in A) to the bath caused an immediate reduction in the number of peristaltic waves which was

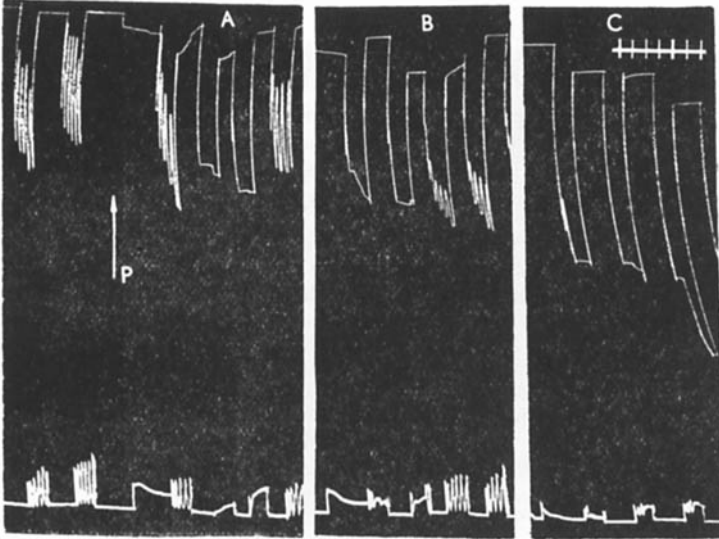


FIG. 1. The effect of increasing doses of substance P on peristalsis. Upper record, peristalsis. Lower record, contractions of the longitudinal muscle. At P in A, 20 units of substance P added to the bath. Between A and B, 30 units and between B and C, 40 units of substance P added to the bath. Time in 1 minute intervals.

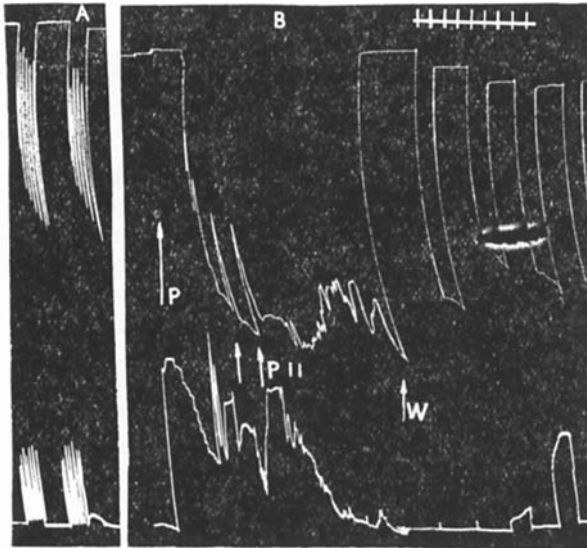


FIG. 2. The effect of intraluminal injection of substance P. Records as in Figure 1. A, the control response. At P in B, 30 units of substance P added to the bath. At the arrow, 0.2 ml. of Tyrode solution introduced intraluminally. At P II, 30 units of substance P introduced intraluminally. W, washing out the bath and gut. Time in 1 minute intervals.

EFFECT OF SUBSTANCE P ON GUINEA PIG ILEUM

followed 15 to 25 minutes later by complete abolition of the reflex, which was reversible, as shown in A. Between A and B, 30 units of substance P were added to the bath, causing a complete block of the reflex with an incomplete recovery, as shown in B. Between B and C, 40 units of substance P was added into the bath, and this dose produced a complete abolition of the reflex lasting more than 60 minutes, as shown in C.

The Effect of Intraluminal Injection of Substance P

To study the site of blocking action of substance P, it was injected intraluminally into gut in which the reflex was blocked by the previous addition of substance P to the bath. Peristalsis was not restored. In some experiments the intraluminal injection produced only irregular and unco-ordinated peristaltic waves. This type of response is shown in Figure 2. At P in B, 30 units of substance P was added into the bath, producing an abolition of the reflex. At P II. in B the same dose of substance P was injected intraluminally. After 2 minutes only the irregular and unco-ordinated peristaltic waves were recorded. The complete block of the reflex was still present 1 hour after washing out substance P.

When larger doses of substance P were used to block peristalsis the intraluminal injection produced no effect.

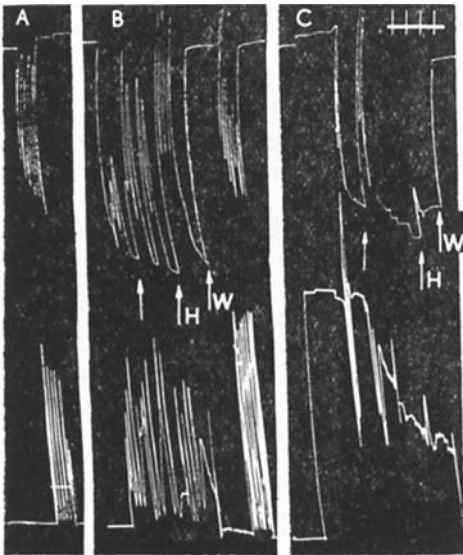


FIG. 3. The effect of intraluminal injection of 5-HT. Records as in Figure 1. A, the control response. Between A and B, 30 units and between B and C, 50 units of substance P added to the bath. At the arrow, 0.2 ml. of Tyrode solution introduced intraluminally. At H, 2 µg. of 5-HT introduced intraluminally. W, washing out the bath and gut. Time in 1 minute intervals.

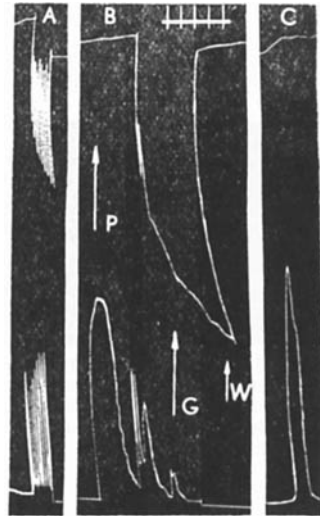


FIG. 4. The effect of nicotine. A, control response. Records as in Figure 1. At P, 100 units of substance P added to the bath. At G, 0.2 mg. of nicotine added to the bath. W, washing out the bath. C, 0.2 mg. of nicotine added to the bath without raising the intraluminal pressure. Time in 1 minute intervals.

The Effect of Intraluminal Injection of 5-HT

Intraluminal injection of substance P had been found to restore peristalsis previously blocked by high doses of 5-HT in the bath¹, but we have now found that intraluminal injection of 5-HT restored peristalsis only if small doses of substance P were used. With large doses of substance P in the bath, the intraluminal 5-HT failed to produce peristalsis. A typical experiment is shown in Figure 3. Between A and B, 30 units of substance P was added to the bath. This dose caused only a partial block of peristalsis, and the intraluminal injection of 2 μ g. 5-HT (at H in B) caused peristalsis. Between B and C, 50 units of substance P was added to the bath and caused a complete block on which 2 μ g. of 5-HT had no effect, as shown in C. Similar findings were observed with doses of 5-HT up to 0.2 mg.

The Effect of Nicotine

After blocking the peristaltic reflex with a high dose of substance P in the bath, nicotine added to the bath caused no effect. This is shown in Figure 4. The response to 0.2 mg. of nicotine in the presence of 100 units of substance P is shown in B. The same dose of nicotine was added to the bath 25 minutes later, after substance P had been washed out, and it caused a contraction.

Where doses of substance P less than 100 units were used to abolish peristalsis, the response to nicotine was reduced but not abolished.

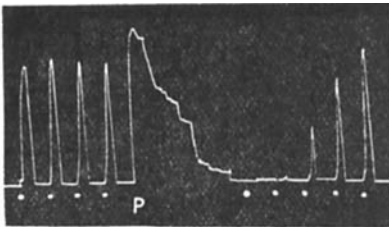
Abolition of the Response to Substance P by Substance P Itself

FIG. 5. Abolition of the response to substance P by a high dose of substance P itself. At dots, 4 units of substance P added to the bath (10 ml.). At P, 50 units of substance P added to the bath.

was abolished; it became normal 25 minutes after washing out the high dose.

The isolated guinea pig ileum was arranged to record contractions of the longitudinal muscle only. It was found that a high dose of substance P abolished the response of the gut to the small doses of the same substance. A typical experiment is shown in Figure 5. The small dose of substance P (4 units) was added to the bath at 6-minute intervals. At P, 50 units substance P was added to the bath and washed out after 7 minutes. The response to the small dose of substance P

DISCUSSION

Small doses of substance P when acting on the outside of the intestine *in situ* can increase the tone and spontaneous rhythmic activity². The present work on the isolated guinea pig ileum has in part confirmed this finding, and it has also been found that high doses of substance P may abolish the peristaltic reflex when acting on the outside of the isolated

EFFECT OF SUBSTANCE P ON GUINEA PIG ILEUM

guinea pig ileum. This was not preceded by a stimulating action, which is in contrast to the response to intraluminal substance P, where it always caused an increase of peristaltic activity before abolition of the reflex¹.

It was also found on the Magnus preparation that a high dose of substance P can block the response of the gut to a small dose of the same substance. This effect is probably due to the saturation of the substance P receptors in the plain muscle cells. So far it seems that the blocking effect of substance P takes place somewhere between the synapse in the peristaltic reflex arc and the effector cell.

Acknowledgements. 5-Hydroxytryptamine creatinine sulphate was kindly supplied by Farmitalia, Milan, and substance P by Dr. B. Pernow, Stockholm.

REFERENCES

1. Beleslin and Varagić, *Brit. J. Pharmacol.*, 1958, 13, 321.
2. Gernandt, *Acta physiol. scand.*, 1942, 3, 270.