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THE EFFECT OF ANGIOTENSIN ON THE PERISTALTIC REFLEX OF THE ISOLATED GUINEA-PIG ILEUM

BY

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Angiotensin is a polypeptide with hypertensive and smooth muscle stimulating effects. Two forms of angiotensin are known: angiotensin I and angiotensin II. The active form is the octapeptide, angiotensin II. The analogue of angiotensin II containing L-val_5 instead of L-$ileu_5$ has quantitatively different pharmacological effects (Meier, Tripod & Studer, 1958).

Some polypeptides are known to influence peristaltic activity. The peristaltic reflex, when abolished by fatigue, by external or internal application of 5-hydroxytryptamine (5-HT), or by lowering the bath temperature, is restored by substance P (Beleslin & Varagić, 1958). Bradykinin in high concentrations depresses or abolishes the peristaltic reflex of the isolated guinea-pig ileum (Beleslin, Bogdanović & Radmanović, 1964).

In the present experiments an attempt was made to analyse the mechanism of action of synthetic val_5 angiotensin II (Ciba) on the peristaltic activity.

METHODS

For recording peristaltic activity of the guinea-pig ileum the method of Trendelenburg (1917) was used. Simultaneous kymograph records were obtained of the volume changes using a Stephenson's float recorder (Stephenson, 1948) and of the isotonic movements of the longitudinal muscle.

The peristaltic reflex was elicited by increasing the intraluminal pressure to a constant height (30 to 50 mm H_2O) for about 90 sec at constant time intervals.

The intestine was suspended in Tyrode solution aerated with oxygen. The volume of the bath was 20 ml., and its temperature was kept at 36° C. The bath fluid was renewed every 10 min. Drugs used to depress or inhibit the peristaltic activity were kept in the bath for 3 min before the peristaltic reflex was elicited. All drugs acted from the serosal surface of the isolated guinea-pig ileum.

Substances used: synthetic val_5 angiotensin II (Hypertensin, Ciba), hexamethonium bromide, tetraethylammonium chloride, azamethonium chloride, *d*-tubocurarine chloride, morphine sulphate, adrenaline hydrochloride, noradrenaline bitartrate and atropine sulphate. All values except those for angiotensin refer to the salts.

RESULTS

Angiotensin contracted the longitudinal muscle of the guinea-pig ileum preparation but had no effect on the normal peristaltic reflex elicited on raising the intraluminal pressure except in high concentrations (5×10^{-6} g/ml.) which caused a strong and sus-

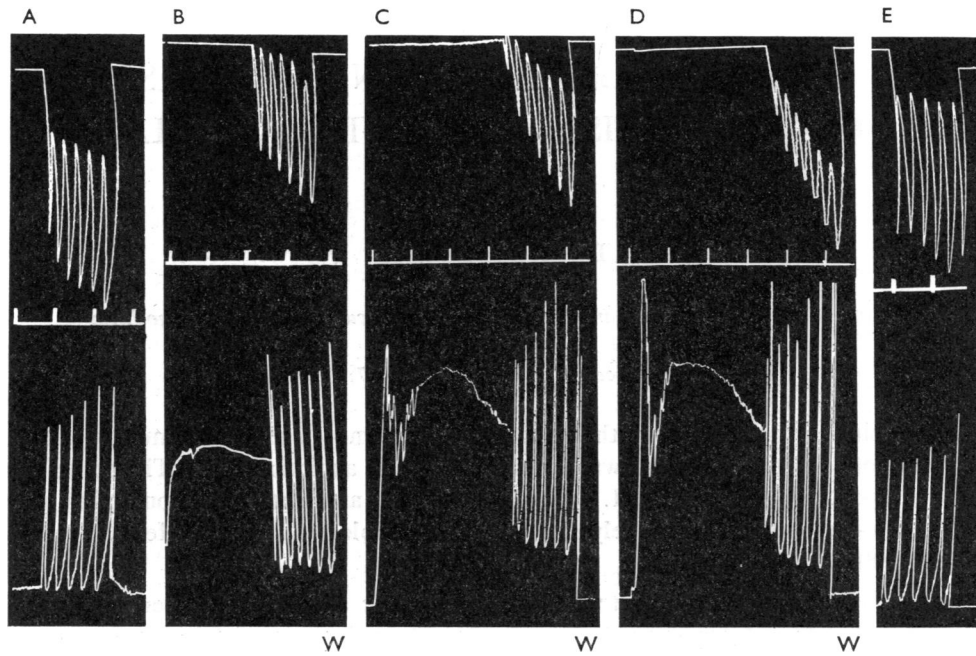

Fig. 1. Isolated guinea-pig ileum preparation. Intraluminal volume changes shown in upper record, contractions and relaxations of longitudinal muscle in lower record. Peristaltic reflex elicited by raising intraluminal pressure to 40 mm water in the absence (A and E) and in the presence of angiotensin in a concentration of 5×10^{-8} g/ml. (B), 5×10^{-7} g/ml. (C), and 5×10^{-6} g/ml. (D). W, washing out of angiotensin. Time marker in min.

tained contraction of the longitudinal muscle. They depressed the waves of the circular but not of the longitudinal muscle. This is illustrated in Fig. 1. Record A shows the normal peristaltic reflex on raising the intraluminal pressure. The subsequent records show the contractions of the longitudinal muscle on addition to the bath of angiotensin in a concentration of 5×10^{-8} g/ml. (at B), 5×10^{-7} g/ml. (at C), and 5×10^{-6} g/ml. (at D) as well as the peristaltic reflex elicited each time 3 min later in the presence of these concentrations of angiotensin in the bath. Record E shows the restoration of the depressed peristaltic reflex 20 min after washing out the angiotensin (5×10^{-6} g/ml.).

Ganglion blocking agents

Angiotensin (5×10^{-8} to 5×10^{-6} g/ml.) restored the peristaltic reflex blocked by hexamethonium, tetra-ethylammonium, *d*-tubocurarine and azamethonium. A typical restoration of the azamethonium block is illustrated in Fig. 2. In the presence of azamethonium (3×10^{-5} g/ml.) raising the intraluminal pressure caused only a single peristaltic wave, then the peristaltic reflex was blocked. The addition of angiotensin (5×10^{-7} g/ml.) to the bath at once restored peristaltic activity. This effect, however, was always short-lasting (1–3 min). In the experiment of Fig. 2 peristaltic activity came to an end after

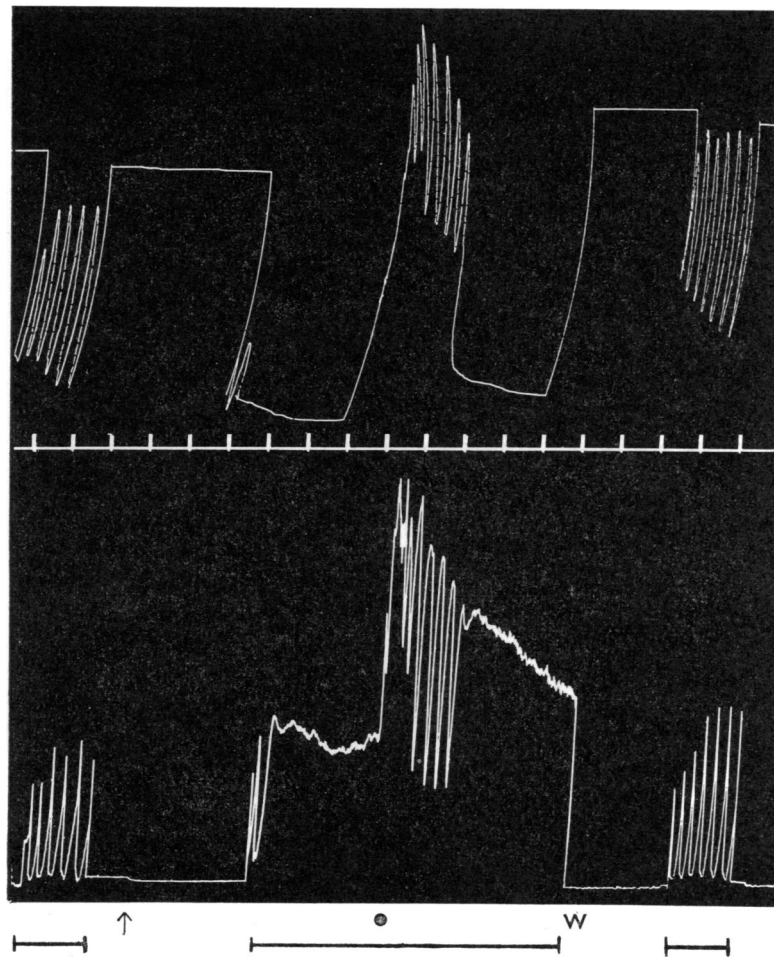

Fig. 2. Isolated guinea-pig ileum preparation. Intraluminal volume changes shown in upper record, contractions and relaxations of longitudinal muscle in lower record. At the horizontal bars intraluminal pressure raised to 35 mm water. ↑, azamethonium 3×10^{-5} g/ml.; ●, angiotensin 5×10^{-7} g/ml. added to the bath; W, both azamethonium and angiotensin washed out. Time marker in min.

about 2 min although the increased intraluminal pressure was maintained. Restoration of the peristaltic activity was always initiated by an increase in tone of the circular and longitudinal muscle.

Morphine

Angiotensin (5×10^{-8} to 5×10^{-7} g/ml.) restored the peristaltic reflex blocked by morphine (2 to 5×10^{-7} g/ml.). This effect is illustrated in Fig. 3. Immediately after the addition of angiotensin (5×10^{-8} g/ml.) to the bath the peristaltic reflex blocked by the presence of morphine (3×10^{-7} g/ml.) was restored, regular strong peristaltic waves

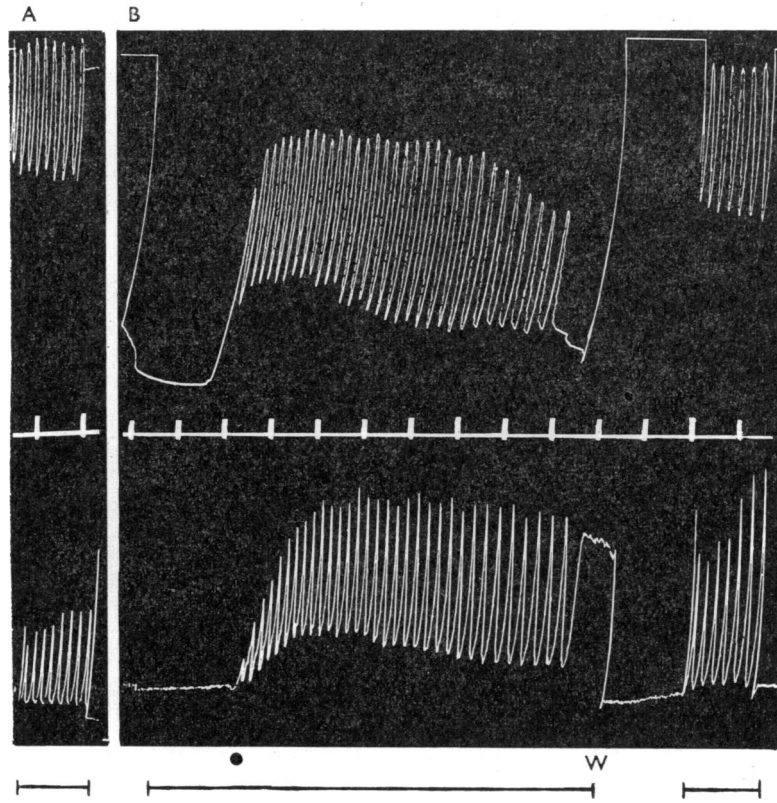

Fig. 3. Isolated guinea-pig ileum preparation. Intraluminal volume changes shown in upper record, contractions and relaxations of longitudinal muscle in lower record. At the horizontal bars intraluminal pressure raised to 40 mm water. Between A and B, morphine (3×10^{-7} g/ml.) and at ● in B angiotensin (5×10^{-8} g/ml.) added to the bath; W, both drugs washed out. Time marker in min.

occurred and continued for about 7 min. During this time the tone of the circular and longitudinal muscle was increased. In other similar experiments restoration by angiotensin of the peristaltic activity blocked by morphine lasted between 7 and 10 min.

Adrenaline

Inhibition of the peristaltic reflex produced by adrenaline was removed by angiotensin (5×10^{-7} to 2×10^{-6} g/ml.). This effect is illustrated in Fig. 4. It shows the inhibition of the reflex in the presence of adrenaline (1×10^{-5} g/ml.) and then the restoration of the peristaltic activity on the addition of angiotensin (2×10^{-6} g/ml.) to the bath. The first effect was a strong contraction of both muscle layers followed by small peristaltic waves which developed into strong regular and full peristaltic waves while the circular muscle relaxed, whereas the longitudinal muscle remained partly contracted. The peristaltic waves continued in this experiment for about 4 min and in other experiments for up to 8 min.

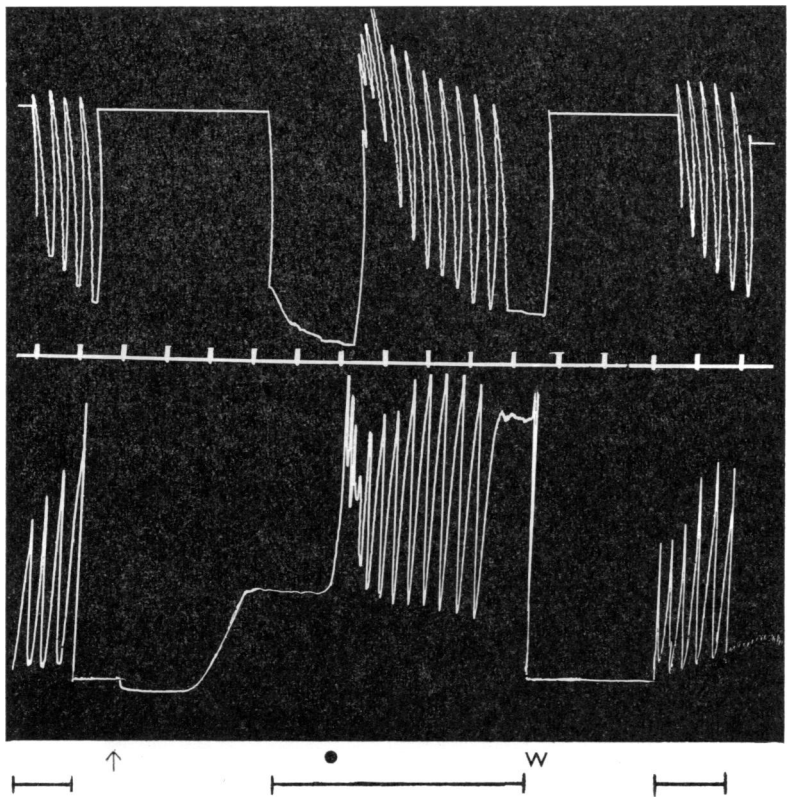

Fig. 4. Isolated guinea-pig ileum preparation. Intraluminal volume changes shown in upper record, contractions and relaxations of longitudinal muscle in lower record. At the horizontal bars intraluminal pressure raised to 40 mm water. At ↑, adrenaline (1×10^{-5} g/ml.) and at ● angiotensin (2×10^{-6} g/ml.) added to the bath ; W, both drugs washed out. Time marker in min.

Atropine

The inhibition of the peristaltic reflex by atropine was also removed by angiotensin (5×10^{-8} to 5×10^{-7} g/ml.). The effect is illustrated in Fig. 5. Restoration of the peristaltic reflex by angiotensin lasted 3 min and in other experiments up to 6 min.

DISCUSSION

On the guinea-pig intestine preparation the only effect on the peristaltic reflex exerted by angiotensin when acting from the serosal surface was a depression of the waves of the circular muscle during the emptying phase. This depression which occurred only with concentrations of angiotensin which caused powerful contraction of the longitudinal muscle is probably an unspecific effect similar to the unspecific inhibition observed by Kosterlitz & Robinson (1957) with other substances that cause powerful contraction of the longitudinal muscle in this preparation. Yet, although either ineffective or causing

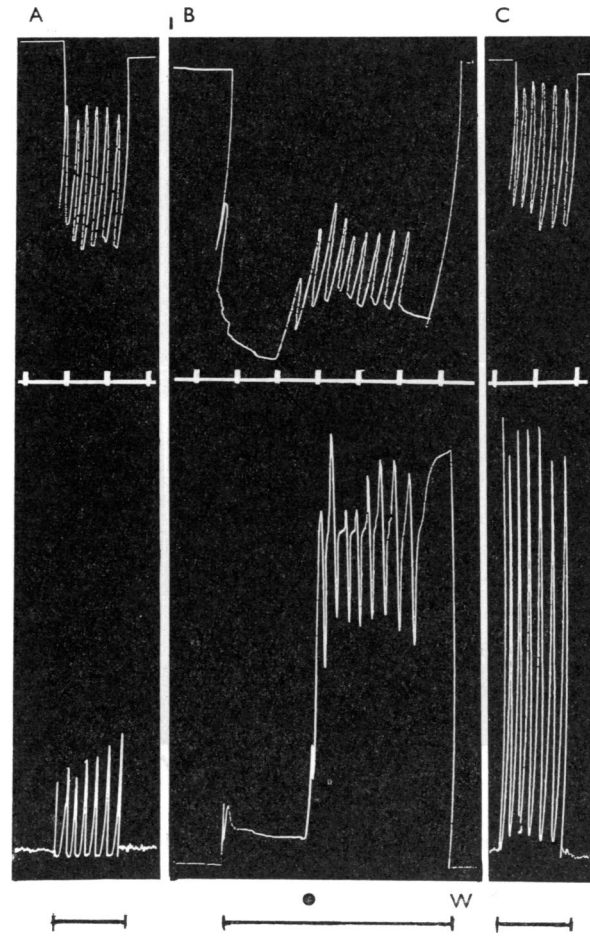

Fig. 5. Isolated guinea-pig ileum preparation. Intraluminal volume changes shown in upper record, contractions and relaxations of longitudinal muscle in lower record. At the horizontal bars intraluminal pressure raised to 40 mm water. Between A and B atropine (5×10^{-7} g/ml.) and at ● angiotensin (5×10^{-8} g/ml.) added to the bath ; W, both drugs washed out. Time marker in min.

depression of the emptying phase of the peristaltic reflex, angiotensin had the property of restoring the reflex blocked by ganglion blocking agents, morphine, adrenaline and atropine. The site of action of angiotensin when restoring the peristaltic reflex blocked by apparently different mechanisms may vary according to the drug used for blocking the reflex.

Restoration of the peristaltic reflex blocked by the ganglion blocking agents hexamethonium, tetraethylammonium and azamethonium, may result from an action of angiotensin on ganglion cells in the intestinal preparation because angiotensin is known to stimulate the ganglion cells of the superior cervical ganglion in cats and rabbits (Lewis & Reit, 1965, 1966). On the other hand, Busse, Wolf & Lendle (1953) have pointed out

that purely smooth muscle stimulating drugs can antagonize the effect of ganglion blocking substances on peristalsis. And in the present experiment, restoration of the block by the ganglion blocking agent was initiated by an increased tone of the circular muscle. The distension caused by the increased tone may stimulate stretch receptors and thereby restore the peristaltic reflex.

Morphine inhibits release of acetylcholine in the isolated guinea-pig ileum preparation, and this effect has been correlated with the blocking effect of morphine on peristalsis (Paton, 1957; Schaumann, 1957). Acetylcholine, in strong concentration, was shown to overcome this block (Beleslin, Bogdanović & Rakić, 1964) and angiotensin may act in the same way as acetylcholine in restoring peristalsis.

Adrenaline inhibits intestinal smooth muscle and this effect may be the principal cause of its ability to block peristalsis, but adrenaline has also an effect on ganglion-cells (Bülbring, 1944) and on the release of acetylcholine (Schaumann, 1958). The adrenaline block is overcome by acetylcholine and anticholinesterases (Beleslin & Varagić, 1964), and angiotensin may again act like acetylcholine in counteracting the block produced by adrenaline. The ability of angiotensin to restore peristalsis blocked by atropine may simply be caused by the distending forces of the increased intraluminal pressure resulting from the contraction of the longitudinal muscle.

According to Robertson & Rubin (1962) the contractile response of angiotensin in the guinea-pig and rabbit ileum results from an action on nerve elements in the intestinal wall, and according to Khairallah & Page (1963), three sites are involved in the contraction of the longitudinal muscle produced by angiotensin. An action on postganglionic nerve endings and on ganglion-cells, both actions resulting in a release of acetylcholine, and an action on smooth muscle. An action of angiotensin at these three sites would also account for its ability to counteract the different forms of block of peristalsis produced by the various drugs.

SUMMARY

1. Angiotensin acting from the serosal surface of the isolated guinea-pig ileum caused contraction of the longitudinal muscle, but had no effect on the peristaltic reflex, except in high concentrations which depressed the waves of the circular muscle.

2. The peristaltic reflex when blocked by ganglion blocking agents, by morphine, adrenaline or atropine, was restored by angiotensin. For this effect an action of angiotensin at three sites is postulated: on postganglionic nerve endings, on ganglion cells and on smooth muscle.

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