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EFFECT OF INCREASED INTRALUMINAL PRESSURE ON THE RELEASE OF ACETYLCHOLINE FROM THE ISOLATED GUINEA-PIG ILEUM

BY

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Release of acetylcholine from the isolated incubated guinea-pig ileum has been repeatedly shown (Schaumann, 1956, 1957; Johnson, 1963a, b; Chujyo, 1952, 1953). Coaxial electrical stimulation of the isolated guinea-pig ileum was also found to release acetylcholine (Paton, 1957, 1963; Harry, 1962). The origin of the liberated acetylcholine has been attributed either to nervous structures (Schaumann, 1957; Paton, 1957; Johnson, 1963b; Harry, 1962) or to non-nervous elements (Feldberg & Lin, 1950; Chujyo, 1953). Morphine inhibits the release of acetylcholine in incubated guinea-pig ileum both during spontaneous activity and during electrical coaxial stimulation (Schaumann, 1957; Paton, 1957).

The present experiments were undertaken in order to test whether there is a correlation between acetylcholine release from the isolated guinea-pig ileum and increase in intraluminal pressure in the gut or peristaltic activity. We hoped that these experiments might provide more supporting data on the role of acetylcholine in the peristaltic reflex, because the increased intraluminal pressure is physiologically the most appropriate stimulus of peristalsis.

METHODS

The isolated guinea-pig ileum was prepared in the way described in a previous paper (Varagić & Kažić, 1965). A piece of gut 6–8 cm long, weighing approximately 440 mg, was immersed in an isolated organ bath of 40 ml., containing Tyrode solution which was oxygenated with a mixture of oxygen (95%) and carbon dioxide (5%). The solution also contained neostigmine methylsulphate 50 μg/l.

The ileum was arranged in such a way as to allow direct measurement of the peristaltic propulsive activity. The intraluminal pressure in the gut was kept either at zero, 22 or 40 mm water.

Before taking the samples of fluid for estimation of acetylcholine the ileum was left in the bath for 40–60 min. During this period the bath fluid was washed out every 15 min. The samples for estimation of acetylcholine were collected in the following way. The bath was washed out and fresh Tyrode solution was left in it for 10 min. At the beginning of this period of time the intraluminal pressure was set at the appropriate level. After 10 min the interaluminal pressure was again dropped to zero and the preparation left at zero pressure for another 2 min to allow diffusion of acetylcholine. Exactly 12 min from the beginning of the collection period, 15 ml. of bath fluid was withdrawn by pipette. The samples so collected as well as the appropriate dilutions of acetylcholine standard were kept at room temperature until the biological estimation of acetylcholine was completed—usually within 2.5 hr of collection.

Acetylcholine was estimated on the isolated guinea-pig ileum (test-ileum) which was immersed in an isolated 4 ml. organ bath containing Tyrode solution together with neostigmine methylsulphate 10 μg/l. and morphine hydrochloride 10 mg/l. The temperature of the bath was 36° C and it was oxygenated with a mixture of oxygen and carbon dioxide. The preparation was kept in the bath for 60 min before estimations were started.

Identification. Three tests were used for identification of acetylcholine in the bath fluid: (1) block of both standard and test fluid responses by a standard concentration of atropine (1 μg/ml.) on the isolated guinea-pig ileum; (2) potentiation of both standard and test fluid responses by neostigmine (10 ng/ml.) on a separate isolated guinea-pig ileum; (3) abolition of responses to both standard and test fluid by alkalinization and boiling (2 drops of N-NaOH and boiling for 3 min).

The amount of released acetylcholine is expressed (as acetylcholine hydrochloride) in ng/g of fresh tissue/min.

The following substances were used: acetylcholine hydrochloride, neostigmine methylsulphate, atropine sulphate, morphine hydrochloride and triethylcholine chloride (supplied by Dr. J. M. Simister, Ward, Blenkinsop & Company, Wembley).

RESULTS

Intraluminal pressure and acetylcholine output

A rise in intraluminal pressure was found to produce an increase in the output of acetylcholine from the isolated guinea-pig ileum. Thus, at zero pressure, the output of acetylcholine was 8.5 ± 0.4 (S.E.) ng/g/min. When the intraluminal pressure was raised to 22 mm water, the output of acetylcholine rose to 17.3 ± 1.8 ng/g/min. Raising the intraluminal pressure to 40 mm water produced an increase of the acetylcholine output to 30.9 ± 4.5 ng/g/min. These values represent means $\pm$ S.E. from ten experiments. A linear dependence is obtained if these values are plotted against the intraluminal pressure (Fig. 1).

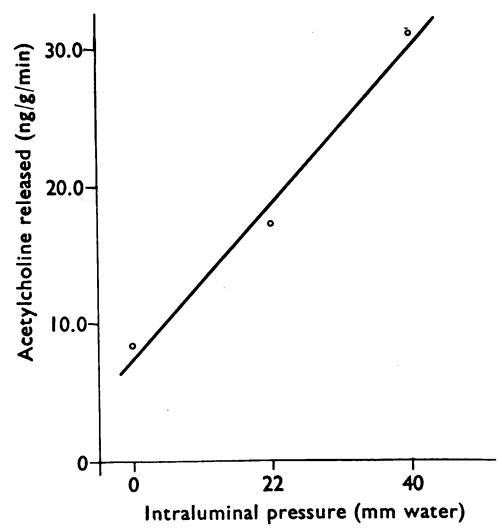

Fig. 1. Effect of increased intraluminal pressure on the acetylcholine release from the isolated guinea-pig ileum.

An increase in the intraluminal pressure to 22 and 40 mm water produced peristaltic activity accompanied by a significant propulsive activity. After raising the intraluminal pressure to 22 mm water, ten to twenty peristaltic waves were recorded in 10 min periods, producing a propulsive activity of 3.0–7.2 ml. Raising the intraluminal pressure to 40 mm water produced eighteen to forty-two peristaltic waves with a propulsive activity amounting to 6–12 ml. A typical experiment is shown in Fig. 2 I. This type

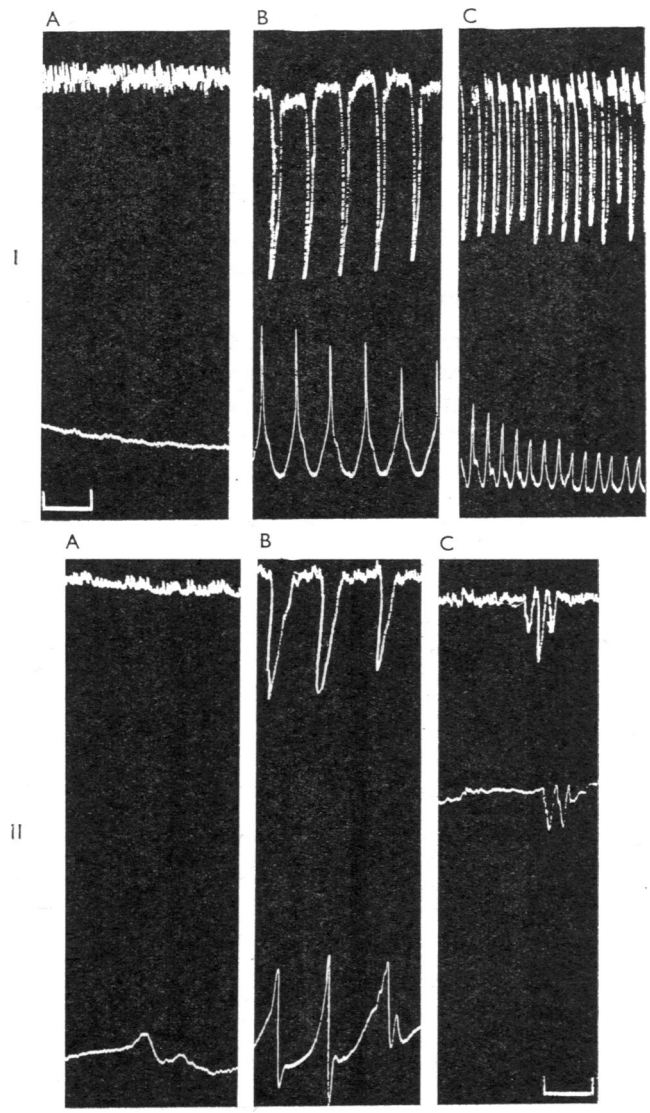

Fig. 2. Effect of increased intraluminal pressure on the peristaltic activity of the isolated guinea-pig ileum. A, control at zero pressure; B, 22 mm water; C, 40 mm water. Upper records in I and II: contractions of the circular muscle. Lower records in I and II: contractions of the longitudinal muscle. Anticholinesterase was present in the bath both in I and II. Time: 1 min.

of activity was observed in most of the experiments. In a few experiments, regular peristaltic activity was only observed after raising the intraluminal pressure to 22 mm water, whereas further increase in pressure to 40 mm produced only an increase in tone of the longitudinal muscle, but no contractions of the circular muscle as shown in Fig. 2 II. Nevertheless, in all these experiments, whether peristaltic activity occurred or not, the output of acetylcholine was increased and showed direct dependence on the level of the intraluminal pressure.

Effect of repeated rises in intraluminal pressure

In this series of experiments the intraluminal pressure was repeatedly raised in the course of 180 min from the beginning of incubation of the intestine. The samples were collected in the manner described in METHODS. In a separate series of experiments the intraluminal pressure was either raised to 22 and 40 mm water, or it was kept at zero level and samples were taken 60, 90, 120 and 180 min after the beginning of incubation. The intraluminal pressure was increased only during the collection period and between these periods it was kept at zero level.

The output of acetylcholine increased with repeated raising of the intraluminal pressure (Fig. 3). In a group of three experiments at zero pressure the acetylcholine output was increased from 10.4 ± 1.1 ng/g/min (at 60 min after the beginning of incubation) to 12.7 ± 2.7 ng/g/min (at 180 min after the beginning of incubation). In a second group of four experiments, in which the time course was the same and in which the intraluminal pressure was intermittently raised to 22 mm water, the acetylcholine output was increased

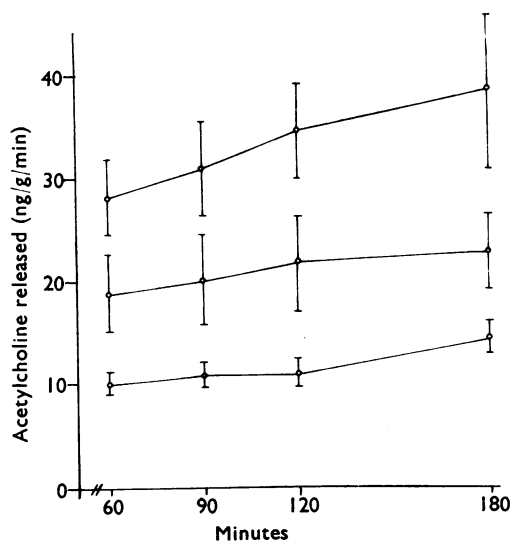

Fig. 3. Effect of repeated raising of the intraluminal pressure in the course of 180 min on the amount of acetylcholine released from the isolated guinea-pig ileum. The lines represent (from the bottom line upwards) the amount of acetylcholine ±S.E. at pressures of zero, 22 and 40 mm water, respectively.

from 19.1 ± 3.1 ng/g/min to 22.7 ± 3.1 ng/g/min. In a third group of five experiments, in which the intraluminal pressure was intermittently raised to 40 mm water, the acetylcholine output was increased from 28.2 ± 3.6 ng/g/min to 38.2 ± 7.1 ng/g/min.

Effect of morphine

Morphine is known to block the peristaltic activity of the isolated guinea-pig ileum (Trendelenburg, 1917) and to inhibit the release of acetylcholine both in incubated guinea-pig ileum and during electrical coaxial stimulation (Schaumann, 1957; Paton, 1957). The present experiments show that morphine reduced the release of acetylcholine produced by raising the intraluminal pressure.

In a series of five experiments (Fig. 4) control samples were taken at intraluminal pressures of zero, 22 and 40 mm water from the beginning of incubation and in the same preparations, using the same procedure, samples were taken while morphine (5 μg/ml.) was present in the bath fluid. Morphine was added to the bath 3 min before raising the intraluminal pressure—that is, before the beginning of the collection period. In all these experiments raising of the intraluminal pressure did not produce any peristaltic activity while morphine was present in the bath.

As Fig. 4 shows, the output of acetylcholine was decreased by 25% in comparison with controls at zero pressure, by 35% at a pressure of 22 mm water, and by 54% at 40 mm water. All the morphine samples were taken 60 min after the corresponding control samples.

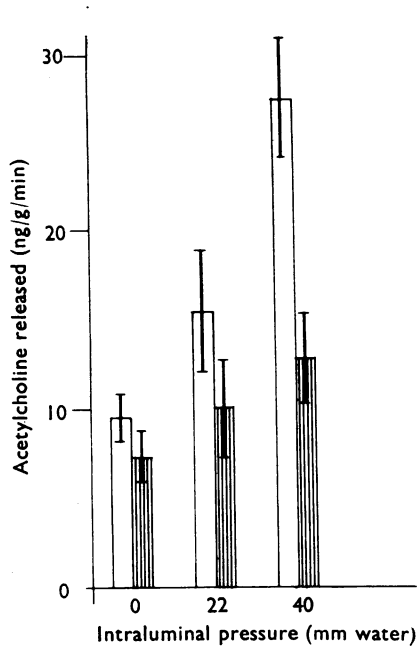

Fig. 4. Effect of morphine on the acetylcholine release produced by increasing intraluminal pressure to 22 and 40 mm water. White columns: acetylcholine output in the control period ±S.E. Striped columns: acetylcholine output while morphine was present in the bath ±S.E. The control samples were obtained at 40, 60 and 80 min from the start of incubation, and the samples in the presence of morphine at 100, 120 and 140 min.

Effect of triethylcholine

It was shown in a previous paper that triethylcholine produces inhibition of peristaltic activity after a certain period of time (Varagić & Kažić, 1967). It was now found that it also produced a decrease in the acetylcholine output from the intestine on raising the intraluminal pressure. The effect of this substance was studied in three series of experiments: at zero intraluminal pressure (three experiments), at a pressure of 22 mm water (four experiments) and at a pressure of 40 mm water (five experiments). The control sample was taken 60 min after the beginning of incubation and immediately afterwards triethylcholine (0.5 mg/ml.) was added to the bath. This substance was washed out and added again every 15 min, so that triethylcholine was always present in the bath until the end of the experiment. Samples were taken 30, 60 and 120 min after addition of triethylcholine to the bath. The intraluminal pressure was increased only during the collection periods. The results are presented in Fig. 5.

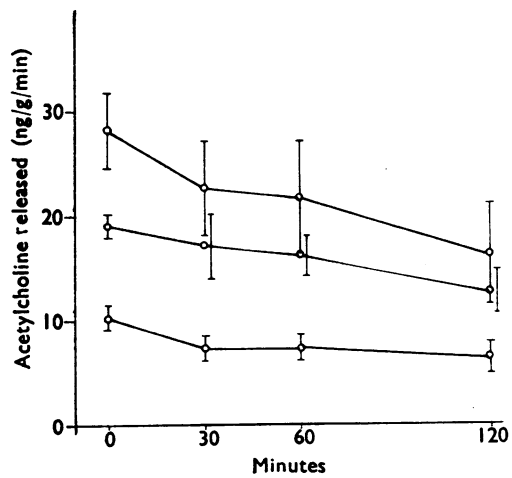

Fig. 5. Effect of triethylcholine on the acetylcholine output produced by increased intraluminal pressure of the isolated guinea-pig ileum. The control output of acetylcholine was recorded at zero time. The lines represent (from below upwards) the amount of acetylcholine ±S.E. released at intraluminal pressures of zero, 22 and 40 mm water, respectively. Triethylcholine was present in the bath throughout the experiments.

There was a decrease in the acetylcholine output which depended on the period of time for which triethylcholine had been in contact with the intestine. Thus, a decrease from 10.4 ± 1.3 ng/g/min to 7.3 ± 1.3 ng/g/min (30 min after addition of triethylcholine), and to 6.4 ± 1.5 ng/g/min (120 min after addition of triethylcholine) were observed in experiments at zero intraluminal pressure. The overall decrease was even more pronounced at 22 mm water intraluminal pressure: from 19.1 ± 1.1 ng/g/min in the control sample to 12.7 ± 2.1 ng/g/min (120 min after addition of triethylcholine). At a pressure of 40 mm water the acetylcholine output was decreased from 28.2 ± 5.1 ng/g/min in the control sample to 16.4 ± 4.1 ng/g/min 120 min after addition of triethylcholine.

DISCUSSION

The present experiments show that an increase of intraluminal pressure is an appropriate stimulus for acetylcholine release from the isolated guinea-ileum. The amount of liberated acetylcholine is proportional to the level of intraluminal pressure.

An increased intraluminal pressure is known to be physiologically the most appropriate stimulus for producing peristaltic activity. In these experiments, in which neostigmine was present in the bath, raising the intraluminal pressure usually produced a regular peristaltic propulsive activity. In a few experiments at high intraluminal pressure, however, again in the presence of neostigmine, no peristaltic activity occurred but only an increase in tone of the longitudinal muscle. Irrespective of whether peristalsis was present or not, acetylcholine output was increased and it was dependent on the height of the intraluminal pressure. It seems therefore that the increased intraluminal pressure and not the peristaltic activity itself is essential for producing increased acetylcholine release from the isolated ileum. Chujyo (1952) has already shown an increased liberation of acetylcholine from passively distended intestine.

If the collection periods are kept constant and acetylcholine output is followed up to 3 hr after the beginning of incubation of the intestine, a tendency of increased output of acetylcholine with time is observed. This finding can probably be explained by a more complete action of the anticholinesterase if the time of its contact with the preparation is prolonged. Paton (1963) has pointed out that a certain time is necessary for the action of anticholinesterase to become complete. On the other hand, Johnson (1963b) has shown that acetylcholine output (in pg/mg/min) fell progressively during the experiment if collection periods were not of equal length.

Morphine has been known to inhibit the liberation of acetylcholine either from a distended (Schaumann, 1956) or undistended guinea-pig ileum (Schaumann, 1957). This substance is also known to have a depressant action on the postganglionic cholinergic nerve fibres of the guinea-pig intestine, reducing both the resting output of acetylcholine and the output from the nerve endings when they are coaxially stimulated (Paton, 1957). The results of the present experiments indicate that morphine also inhibits the release of acetylcholine produced by raising the intraluminal pressure in the intestine. It therefore seems possible that the increased intraluminal pressure produces activation of the cholinergic nerve fibres and leads to an increased output of acetylcholine. Several authors have postulated the nervous origin of acetylcholine in the gut (Johnson, 1963b ; Harry, 1962 ; Paton, 1957 ; Schaumann, 1957) ; this is contrary to the views of Feldberg and Lin (1950) and Chujyo (1953).

Triethylcholine has been shown to depress the synthesis of acetylcholine by organized nervous tissue (Bull & Hemsworth, 1963) and to produce a decrease in the acetylcholine output from the isolated diaphragm of the rat (Bowman & Hemsworth, 1965) and from the perfused superior cervical ganglion of the cat (Matthews, 1966). This substance was also found to inhibit peristaltic activity of the isolated guinea-pig ileum (Varagić & Kažić, 1967). The present experiments show that triethylcholine decreased acetylcholine output from the guinea-pig intestine both at zero and at an increased intraluminal pressure. It is therefore possible that the peristaltic block produced by triethylcholine was actually the result of a decrease in the acetylcholine synthesis in the nerve structures which belong to or take part in the peristaltic reflex arc.

SUMMARY

1. A rise in intraluminal pressure was found to produce increased acetylcholine release from the isolated guinea-pig ileum. The amount of acetylcholine liberated was proportional to the height of intraluminal pressure.

2. When the intraluminal pressure was repeatedly raised for a standard period in the course of 3 hr, the acetylcholine output became progressively greater.

3. The increase of acetylcholine output produced by raising the intraluminal pressure was reduced in the presence of morphine. Triethylcholine abolished the increase with time and progressively diminished acetylcholine output.

4. It is concluded that increased intraluminal pressure produces activation of cholinergic nerve structures in the intestine, thus leading to increased acetylcholine release from nerve fibres. These experiments indicate that the increased intraluminal pressure, as the most appropriate stimulus of peristalsis, acts by activating cholinergic processes in the peristaltic reflex arc.

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