Short communications

The effect of desensitization to adenosine triphosphate on the peristaltic reflex in guinea-pig ileum

A. H. WESTON

Department of Pharmacology, Materia Medica & Therapeutics, Stopford Building, University of Manchester, Manchester M13 9PT

Contractions of the longitudinal muscle of guinea-pig ileum were inhibited by adenosine triphosphate. This inhibition was prevented by specific desensitization of the tissue to adenosine triphosphate whilst the peristaltic reflex was unaffected. The results suggest that the transmitter substance released from the intramural inhibitory neurones activated during peristalsis is not adenosine triphosphate.

The peristaltic reflex in the guinea-pig ileum consists of an initial contraction of the longitudinal muscle followed by a contraction of the circular muscle, raising the intraluminal pressure. During circular muscle contraction, the contraction of longitudinal muscle is inhibited by the action of intramural, inhibitory nerves (Kosterlitz, 1967 ; Kottegoda, 1970). The inhibitory transmitter is not adrenergic (Kosterlitz, 1967) but otherwise its identity is unknown. Electrical stimulation of intramural nerves in some intestinal smooth muscle preparations produces inhibitory responses which, on pharmacological analysis, seem to be non-cholinergic and non-adrenergic in character (Bennett, Burnstock & Holman, 1966 ; Campbell, 1970 ; Weston, 1971). It is not unreasonable to assume, therefore, that the inhibitory neurones activated by electrical stimulation are the same as those mediating inhibition during peristalsis. Burnstock, Campbell, Satchell & Smythe (1970) have suggested that adenosine triphosphate (ATP) is the transmitter released from the intramural inhibitory nerves, and Holman & Hughes (1965) have shown that a tissue can be specifically desensitized to the inhibitory effects of exogenous ATP. The purpose of the present investigation was to discover whether the guinea-pig ileum could be desensitized to the inhibitory effects of ATP and, if so, to assess the effects of desensitization on the peristaltic reflex in this tissue.

Methods.—Segments of guinea-pig ileum were set up at 37° C for production of peristaltic reflexes (Trendelenburg, 1917). Longitudinal and circular muscle tension changes were recorded with an isometric transducer and pressure transducer respectively in conjunction with a potentiometric recorder. The peristaltic reflex was initiated by raising intraluminal pressure by 0·5–1 cm H_2O. This procedure was carried out under three different conditions: (1) in Krebs solution to produce a control reflex, (2) in Krebs solution containing hexamethonium (1 μM) to prevent circular muscle contraction and to produce a prolonged spasm of the longitudinal muscle (Kottegoda, 1970), (3) in Krebs solution containing acetylcholine (5 μM) to desensitize the longitudinal muscle to acetylcholine and allow examination of the inhibition of longitudinal muscle tension during circular muscle contraction (Schaumann, Jochum & Schmidt, 1953).

The inhibitory effects of exogenous ATP were examined in the presence of hexamethonium 1 μM. Noradrenaline was used as a control. Intraluminal pressure was raised and 10 s later the tissue was exposed to ATP or noradrenaline and the resulting decrease in longitudinal muscle tension was recorded. The pressure increase was maintained for 40 s and repeated every 10 minutes. During this period, the Krebs solution was changed three times.

Tissues were desensitized to ATP by exposing them to Krebs solution containing ATP (100 μM). When hexamethonium was used as an antagonist and when ATP and acetylcholine were used as desensitizing agents, these drugs were added to the Krebs solution and a 30 min equilibration period was allowed before the experiment was continued.

Results.—The increase in intraluminal pressure required to initiate a peristaltic reflex ranged from 0·5–1·5 cm H_2O. This increase was constant for a given preparation ; if the pressure was raised above the threshold no increase in the maximum response of either longitudinal or circular muscle layers was observed.

In the presence of hexamethonium (1 μM), an increase in the intraluminal pressure produced a rise in longitudinal muscle tension which was maintained for as long as the rise in pressure. There was no active rise in intraluminal pressure due to ganglion blockade in the excitatory pathway (Kottegoda, 1970). Both ATP (0·1–2·5 μM) and noradrenaline (0·005–0·125 μM) produced a concentration-dependent reduction of the rise in longitudinal muscle tension. In sufficient concentration, ATP produced a complete inhibition of the spasm whereas noradrenaline was less effective. In the presence of hexamethonium (1 μM) + ATP (100 μM), inhibitory responses to ATP (0·1–2·5 μM) were abolished, whereas those to noradrenaline were unaffected (8 experiments, Fig. 1B). In the presence of normal Krebs solution, the effects of desensitization were abolished in about 45 minutes.

In 8 experiments in normal Krebs solution, a rise in the intraluminal pressure produced a peristaltic reflex consisting of an increase in longitudinal muscle tension followed by an increase in circular muscle tension. The spasm of the longitudinal muscle was inhibited during circular muscle contraction. When the preparation

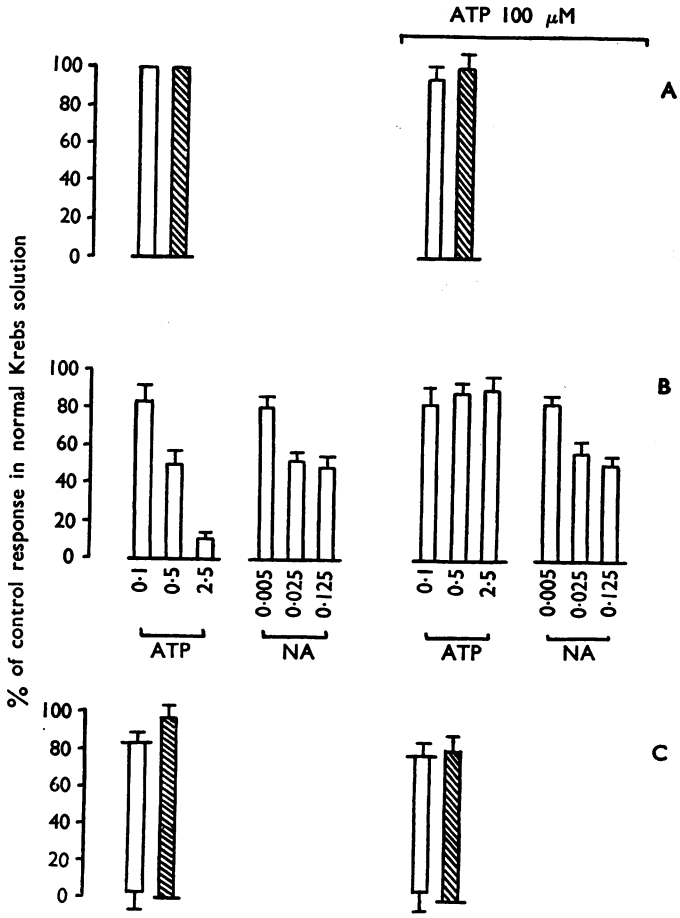

FIG. 1. Effects of desensitization to ATP on the peristaltic reflex in guinea-pig ileum. Tension changes in longitudinal (open columns) and circular (cross-hatched columns) muscle are each expressed in terms of their control values in normal Krebs solution. A. Reflex in normal Krebs solution. B. Effects of ATP and noradrenaline (NA) on longitudinal muscle tension development produced by raising intraluminal pressure. All experiments conducted in Krebs solution containing hexamethonium (1 μM) to abolish circular muscle contraction. Doses shown in μM below columns. C. Reflex in Krebs solution containing acetylcholine (5 μM) to increase resting tension in the longitudinal muscle and to desensitize this layer to acetylcholine. Each vertical bar represents the mean of 8 experiments ± S.E.

had been desensitized to ATP, the characteristics of the peristaltic reflex were unchanged (Fig. 1A).

Eight experiments were conducted in Krebs solution containing acetylcholine (5 μM). In these conditions, a high level of resting tension was maintained in the longitudinal muscle which failed to respond to exogenous acetylcholine (10–500 nM). When the intraluminal pressure was raised to threshold, the circular muscle alone contracted and immediately, tension in the longitudinal layer was reduced. This reduction was maintained for as long as intraluminal pressure was raised. Desensitization to ATP in these preparations produced no change in the pattern of muscular activity elicited by raising the intraluminal pressure (Fig. 1C).

Discussion.—The present experiments have demonstrated the inhibitory effects of ATP in the guinea-pig ileum and have shown that exposure to high concentrations of ATP renders the longitudinal muscle insensitive to further exposure to ATP. Sensitivity to noradrenaline was unchanged. In no experiment was desensitization to ATP effective in modifying the inhibitory components of the peristaltic reflex. It is possible that it is more difficult to desensitize a tissue to the effects of an endogenously-released transmitter, as in the peristaltic reflex, than to exogenous application of that transmitter. However, exposure to high concentrations of acetylcholine abolished both the longitudinal muscle spasm during raised intraluminal pressure and the effects of exogenous acetylcholine. The observations suggest, therefore, that ATP is not the transmitter substance released from the intramural nerves forming the inhibitory pathway of the peristaltic reflex.

I should like to thank Dr. A. J. Watt for his helpful suggestions.

REFERENCES

BENNETT, M. R., BURNSTOCK, G. & HOLMAN, M. E. (1966). Transmission from intramural inhibitory nerves to the smooth muscle of the guinea-pig taenia coli. *J. Physiol., Lond.*, **182**, 541–558.

BURNSTOCK, G., CAMPBELL, G., SATCHELL, D. & SMYTHE, A. (1970). Evidence that adenosine triphosphate or a related nucleotide is the transmitter substance released by non-adrenergic inhibitory nerves in the gut. *Br. J. Pharmac.*, **40**, 668–688.

CAMPBELL, G. (1970). Autonomic nervous supply to effector tissues. In: *Smooth Muscle*, ed. Bülbring, E., Brading, A. F., Jones, A. W. & Tomita, T. London: Arnold.

HOLMAN, M. E. & HUGHES, J. R. (1965). Inhibition of intestinal smooth muscle. *Aust. J. exp. Biol. med. Sci.*, **43**, 277–290.

KOSTERLITZ, H. W. (1967). Intrinsic intestinal reflexes. *Am. J. dig. Dis.*, **12**, 245–254.

KOTTEGODA, S. R. (1970). In: *Smooth Muscle*, ed. Bülbring, E., Brading, A. F., Jones, A. W. & TOMITA, T. London: Arnold.

SCHAUMANN, O., JOCHUM, K. & SCHMIDT, H. (1953). Analgetika und Darmmotorik III Zum Mechanismus der Peristaltik. *Arch. exp. Path. Pharmak.*, **219**, 302–309.

TRENDELENBERG, P. (1917). Physiologische und pharmakologische Versuche über die Dunndarmperistaltik. *Arch. exp. Path. Pharmak.*, **81**, 55–129.

WESTON, A. H. (1971). Inhibition of the longitudinal muscle of rabbit duodenum. *Br. J. Pharmac.*, **43**, 428–429P.

(*Received November* 14, 1972)