Br. J. Pharmac. (1969), 37, 234–244.

The action of eledoisin on the peristaltic reflex of guinea-pig isolated ileum

D. B. BELESLIN*

Department of Pharmacology, Medical Faculty, Beograd, Yugoslavia

1. Low concentrations of eledoisin, acting from the serosal surface of the guinea-pig isolated ileum, had no effect on the peristaltic reflex, whereas in high concentrations it depressed or sometimes abolished this reflex.

2. Eledoisin stimulated the peristaltic activity of the guinea-pig ileum subjected to continuously raised intraluminal pressure. This stimulatory effect could be produced regularly when the interval between additions of eledoisin was at least 20 min. When added at shorter intervals, tachyphylaxis to eledoisin developed, but its ability to promote peristaltic activity was restored by the addition of an anticholinesterase to the bath fluid. During tachyphylaxis to eledoisin, nicotine and dimethylphenylpiperazinium were unable to restore peristalsis.

3. When the peristaltic reflex had been abolished by ganglion-blocking agents, morphine and morphine-like substances, or adrenaline, eledoisin restored the reflex. Eledoisin was much more effective in overcoming the blocking effect of hexamethonium, tetraethylammonium and azamethonium than that of nicotine or dimethylphenylpiperazinium.

4. Eledoisin did not antagonize the inhibitory effects of atropine, hyoscine and hyoscine butylbromide.

5. It is suggested that the stimulant effect of eledoisin may be due to a direct action on the smooth muscle and a release of acetylcholine from postganglionic nerve endings, but an effect on the intestinal ganglion cells cannot be excluded.

In the past 10 years a number of polypeptides of relatively low molecular weight have been the subject of many pharmacological and physiological studies, especially with regard to their effects on vascular and non-vascular smooth muscles. This research received added impetus from progress made in the elucidation of the structures of these polypeptides and their subsequent synthesis. Eledoisin, a highly active endecapeptide, was originally isolated from the posterior salivary glands of the molluscs *Eledone moschata* and *Eledone aldrovandi* (Erspamer & Anastasi, 1962; Anastasi & Erspamer, 1962). Erspamer & Erspamer (1962) studied the effects of eledoisin on a number of isolated smooth muscle preparations and found that it had

* Present address: Laboratory of Neuropsychology, Department of Psychology, Purdue University, Lafayette, Indiana 47907, U.S.A.

a potent stimulating action on gastrointestinal smooth muscle. Moreover, in unanaesthetized dogs, subcutaneous injections of eledoisin 100 μg/kg produced vomiting and elimination of formed stools followed by watery diarrhoea and accompanying tenesmus. More recently, Bauer, Gmeiner & Winkler (1966) observed that, when injected or infused intravenously into anaesthetized rabbits and cats, eledoisin increased intestinal motility.

The present experiments were performed to define the sites of action of eledoisin in the guinea-pig isolated ileum, and to attempt an analysis of the mechanism whereby it influences peristalsis.

Methods

The method of Trendelenburg (1917) was used to study the effect of eledoisin on the peristaltic reflex in the guinea-pig isolated ileum. Volume changes in the intestinal segment were recorded by means of a float recorder (Stephenson, 1948) and contractions of the longitudinal muscle by an isotonic lever.

The intestine was suspended in a 20 ml. bath containing Tyrode solution gassed with oxygen ; its temperature was kept at 36° C. The bath fluid was renewed every 10 min. All drugs were added to the bath fluid and therefore acted from the serosal surface.

The peristaltic reflex was elicited by increasing the intraluminal pressure by 3–4 cm H_2O for about 90 sec ; the increase in pressure was kept constant throughout an experiment. In another series of experiments, intraluminal pressure was maintained at 4 cm H_2O for the whole experiment.

The following substances were used: synthetic eledoisin (Sandoz), hexamethonium bromide, tetraethylammonium chloride, azamethonium chloride, nicotine hydrogen tartrate, dimethylphenylpiperazinium iodine (DMPP), morphine hydrochloride, codeine phosphate, methadone hydrochloride (Heptanon " Pliva "), pethidine hydrochloride (Petantin " Galenika "), atropine sulphate, adrenaline hydrochloride, noradrenaline bitartrate, hyoscine hydrobromide, hyoscine butylbromide. The following anti-cholinesterases were used, N-*p*-chlorophenyl-N-methylcarbamyl-*m*-hydroxyphenyltrimethylammonium bromide (RO-2-1250), 1 : 5-di-(*p*-N-allylmethylaminophenyl)-penta-3-one dimethobromide (BW 284 C51), dimethylcarbamyl-(2-hydroxy-5-phenylbenzyl)-trimethylammonium bromide (RO-2-0683), eserine sulphate and neostigmine methylsulphate ; the first two compounds inhibit true cholinesterase and the third pseudo cholinesterase (Austin & Berry, 1953 ; Hawkins & Gunter, 1946 ; Hawkins & Mendel, 1949). All drug concentrations refer to the salt, except those of eledoisin, which refer to the peptide.

Results

Effect of eledoisin on the peristaltic reflex

Eledoisin (0·005–0·1 μg/ml.) produced a pronounced and sustained contraction of the longitudinal muscle of the ileum, but had no effect on the peristaltic reflex. In spite of this contraction of the longitudinal muscle, full and regular peristaltic waves appeared when the peristaltic reflex was initiated by raising the intraluminal pressure (Fig. 1). In a higher concentration (1 μg/ml.), eledoisin depressed and sometimes

abolished the peristaltic reflex. Ten minutes after washing out the peptide, the peristaltic reflex had returned to normal.

Effect of eledoisin on peristaltic activity caused by a prolonged rise of intraluminal pressure

When the intraluminal pressure was raised by a few cm H_2O for 3 hr or longer, the ileal segment showed peristaltic activity which at first was regular but later consisted of single or repetitive waves spaced at irregular intervals (Feldberg & Lin, 1949; Beleslin & Varagić, 1963). Once this irregular activity appeared, the ileal segment was very sensitive to the effect of drugs which stimulate peristalsis. In such conditions, eledoisin (0·025–0·1 μg/ml.) caused an immediate increase in peristaltic activity and in the tone of both longitudinal and circular muscle layers (Fig. 2). This effect was obtained with intraluminal pressures of 1–3 cm H_2O.

Tachyphylaxis to eledoisin

The first time eledoisin was applied to an ileal segment, it caused a series of peristaltic waves lasting for 3 to 8 min. Subsequently, identical doses of the peptide became less and less effective; the peristaltic activity was more irregular, less frequent and shorter in duration (Fig. 2C) until finally only a single wave was evoked accompanied by a sustained contraction of the longitudinal muscle (Fig. 2D).

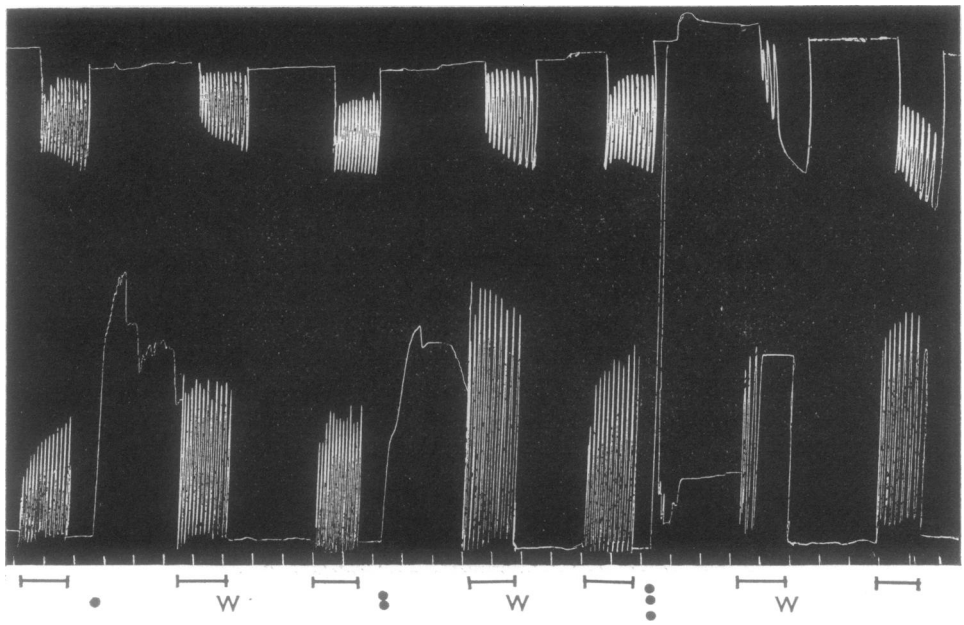

FIG. 1. Guinea-pig isolated ileum. Tracings in this and subsequent figures: intraluminal volume changes shown in upper record, increase in filling downwards; contractions and relaxations of the longitudinal muscle in lower record, contractions upwards. At horizontal bars intraluminal pressure raised to 30 mm H_2O. At one dot, 0·001 μg/ml., at two dots, 0·1 μg/ml., and at three dots, 1 μg/ml. of eledoisin added to the bath fluid. W, Washing out of eledoisin. Time marker in min.

This is consistent with the well known fact that when the peristaltic reflex becomes fatigued, the longitudinal muscle still responds to distension. It was therefore pertinent to inquire how much fatigue contributes to the tachyphylaxis to eledoisin.

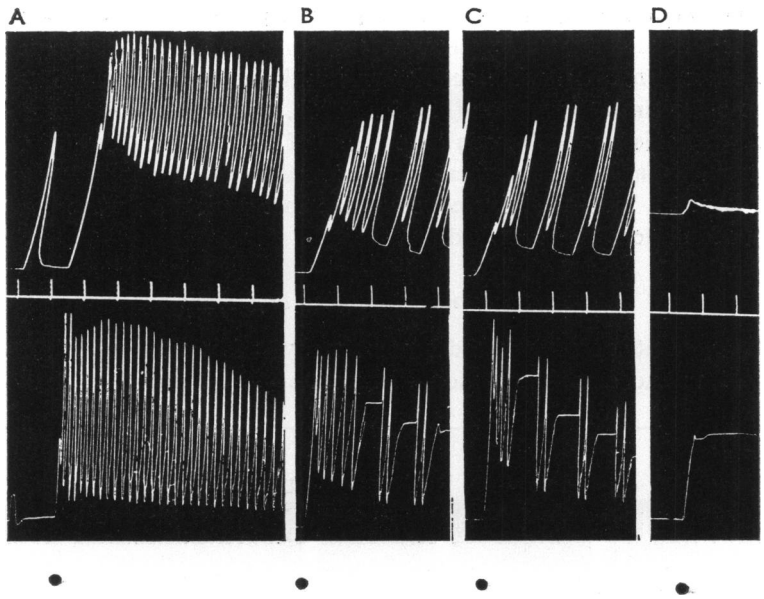

FIG. 2. Guinea-pig isolated ileum subjected to constant intraluminal pressure of 30 mm H_2O for 140 min. At dots, eledoisin 0·05 μg/ml. added to the bath. A, First; B, second; C, fifth; D, seventh addition of eledoisin. All but first addition were made 5 min after the last peristaltic wave evoked by a previous dose had subsided. Time marker in min.

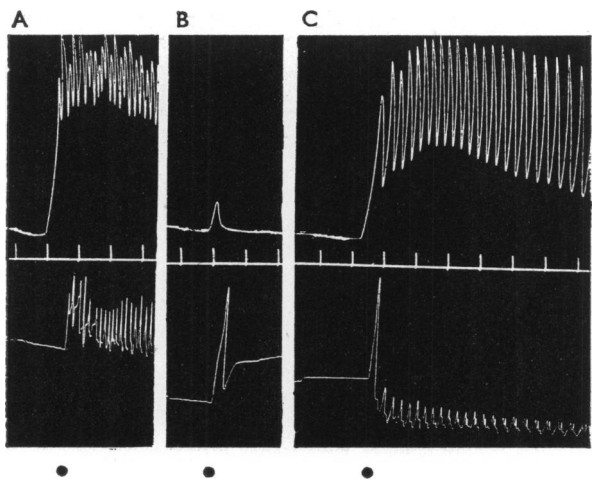

FIG. 3. Guinea-pig isolated ileum subjected to constant intraluminal pressure of 30 mm H_2O. At dots, additions of eledoisin (0·075 μg/ml.); A, First; B, sixth addition, 5 min after last peristaltic wave evoked by fifth addition had subsided. Between B and C neostigmine (0·0001 μg/ml.) added to the bath; C, 5 min later. Time marker in min. Similar effects were obtained with RO-2-1250 (0·001–0·02 μg/ml.), BW 284 C51 (0·001–0·02 μg/ml.), eserine (0·001–0·003 μg/ml.).

In most experiments, maximal attenuation of the peristaltic response to eledoisin was achieved after the fifth or sixth dose, although there was some variation. To distinguish fatigue from tachyphylaxis, preparations whose responsiveness to eledoisin had become maximally attenuated were exposed to a suitable concentration of acetylcholine, arecoline or an anticholinesterase drug, agents which are known to stimulate peristalsis (Beleslin & Varagić, 1963). If such treatment failed to initiate peristaltic activity, the preparation was considered to have become fatigued ; if, on the other hand, peristaltic activity was initiated, it was assumed that tachyphylaxis to eledoisin had occurred (Fig. 3). Thus, it was found that in most experiments tachyphylaxis was the reason for the loss of sensitivity to eledoisin. In order to avoid tachyphylaxis, a subsequent dose of eledoisin was not added to the bath before at least 20 min had elapsed since the last peristaltic wave evoked by a previously added dose had subsided.

Interaction between nicotine and eledoisin

When the " sensitized " ileum had become tachyphylactic to eledoisin, it was also unresponsive to the stimulant action of nicotine (20–50 μg/ml.) (Fig. 4). After

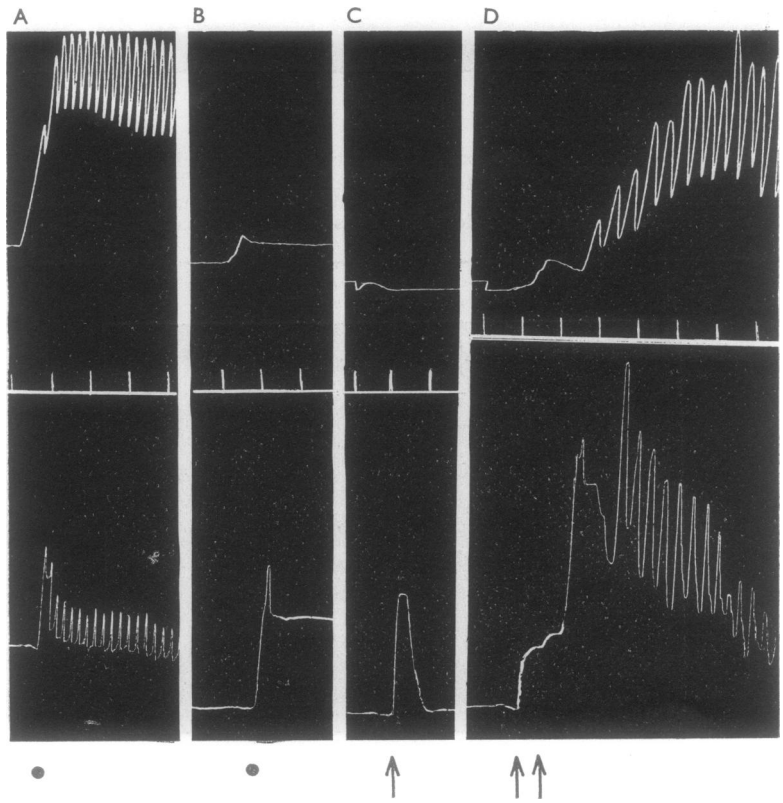

FIG. 4. Guinea-pig isolated ileum subjected to constant intraluminal pressure of 35 mm H_2O. At dots, eledoisin (0·1 μg/ml.) first addition (A) ; eighth addition (B), 5 min after the last peristaltic wave evoked by the seventh addition had subsided (not shown). At single arrow, nicotine (50 μg/ml.) ; at double arrow, RO-2-1250 (0·2 μg/ml.). B, C and D each 5 min apart. Time marker in min.

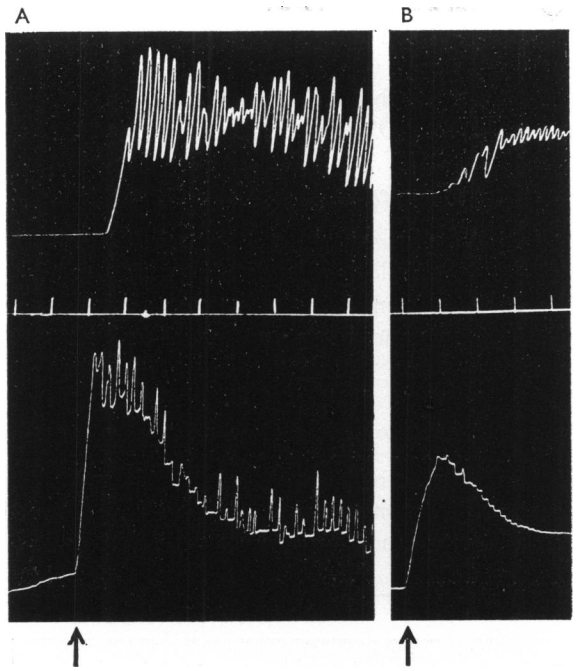

FIG. 5. Guinea-pig isolated ileum subjected to constant intraluminal pressure of 30 mm H_2O. A, 1 hr cooling at 28° C; between A and B, 1 hr further cooling at 15° C. At arrows, eledoisin 0·1 μg/ml. Time marker in min.

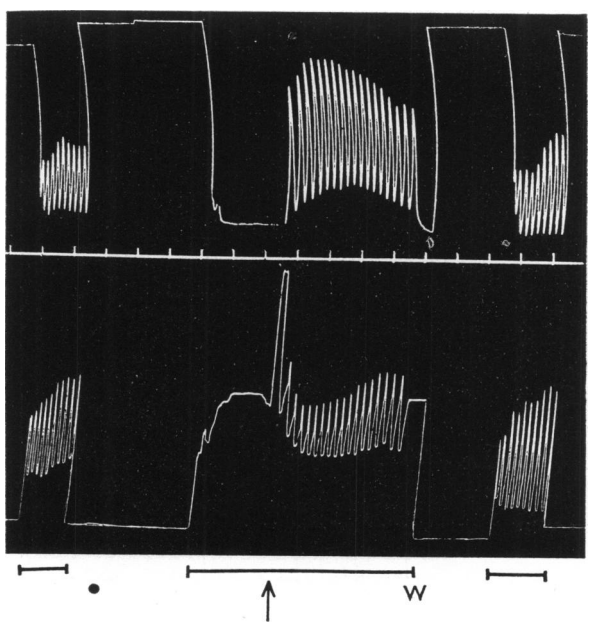

FIG. 6. Guinea-pig isolated ileum. At horizontal bars intraluminal pressure raised to 30 mm H_2O. At dot, tetraethylammonium (50 μg/ml.); at arrow, eledoisin (0·2 μg/ml). W, Washing the drugs out. Time marker in min.

maximum attenuation of the response to eledoisin had been obtained (B), nicotine produced only a short-lived contraction of the longitudinal muscle layer and had no effect on the circular muscle layer (C). Yet shortly afterwards, addition of the anti-cholinesterase, RO-2-1250 to the bath, (D), evoked vigorous and regular peristaltic waves. In similar experiments, DMPP was as ineffective as nicotine.

Effect of lowering of the bath temperature

When the bath temperature was between 22° and 28° C, eledoisin produced a few regular peristaltic waves followed by irregular, very often small and uncoordinated waves (Fig. 5A). When the intestine was cooled further to 15°–19° C, eledoisin caused sustained continuous contractions of both circular and longitudinal muscle layers (Fig. 5B).

Effect of ganglion-blocking substances on the action of eledoisin

Eledoisin antagonized the inhibitory effects of hexamethonium, tetraethylammonium and azamethonium on the peristaltic reflex. When, for example, the peristaltic reflex was first abolished by tetraethylammonium, eledoisin caused an

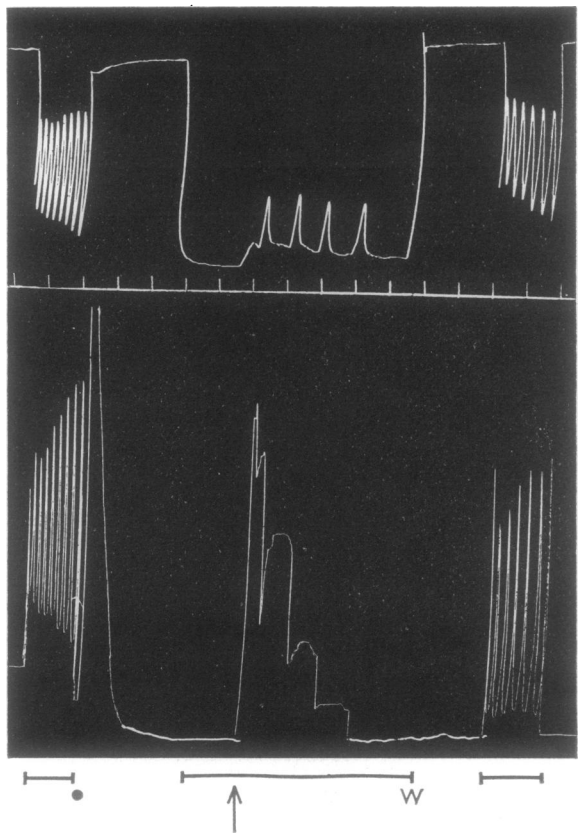

FIG. 7. Guinea-pig isolated ileum. At horizontal bars, intraluminal pressure raised to 30 mm H_2O. At dot, DMPP (20 μg/ml.); at arrow, eledoisin (0·5 μg/ml.). W, Both DMPP and eledoisin washed out. Time marker in min.

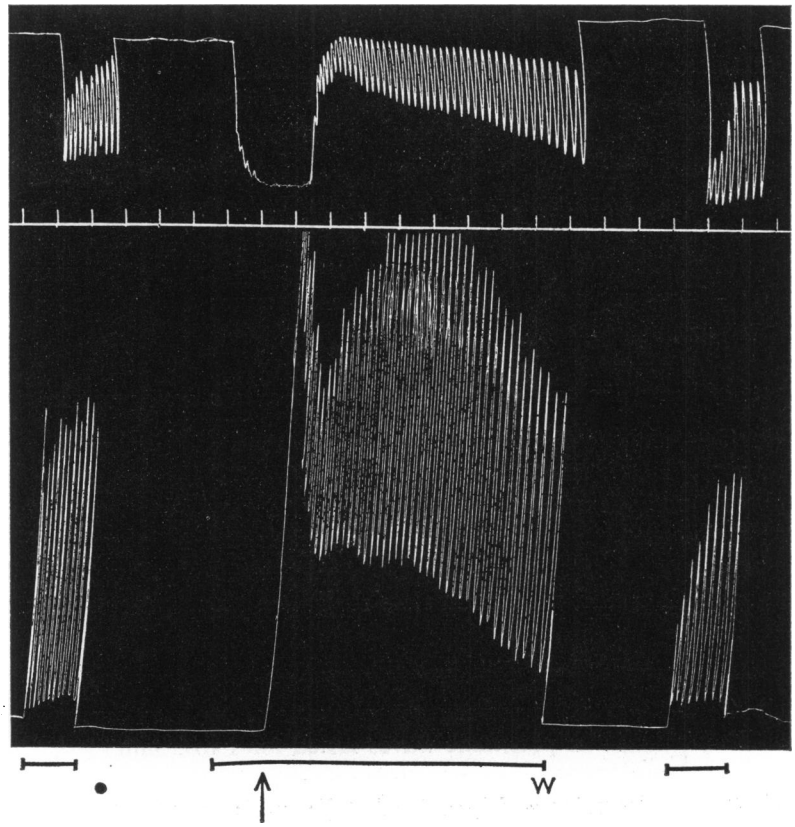

FIG. 8. Guinea-pig isolated ileum. At horizontal bars intraluminal pressure raised to 30 mm H_2O. At dot, morphine (0·2 μg/ml.); at arrow, eledoisin (0·4 μg/ml.). W, Both morphine and eledoisin washed out. Time marker in min.

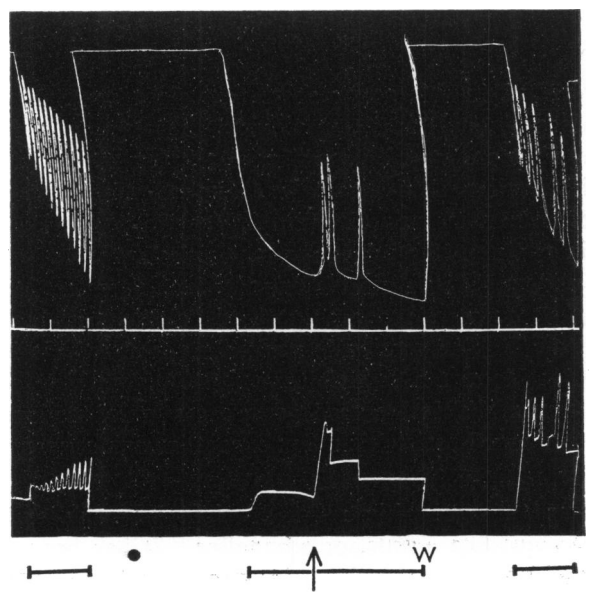

FIG. 9. Guinea-pig isolated ileum. At horizontal bars, intraluminal pressure raised to 30 mm H_2O. At dot, hyoscine butylbromide (20 μg/ml.); at arrow, eledoisin (0·4 μg/ml.). W, Washing out the drugs from the bath. Time marker in min.

almost immediate reappearance of large, regular peristaltic waves which continued for a few minutes. During this time, the tone of the longitudinal muscle was increased much more than that of the circular muscle (Fig. 6).

In similar experiments with hexamethonium (30–500 μg/ml.), azamethonium (100–500 μg/ml.) and tetraethylammonium (50–500 μg/ml.), eledoisin (0·4–1 μg/ml.) caused the peristaltic waves to reappear. On the other hand, when nicotine (10–100 μg/ml.) and DMPP (10–100 μg/ml.) were used to block the peristaltic reflex, eledoisin (0·4–1 μg/ml.) evoked only a few small waves which were not typical peristaltic waves (Fig. 7).

Effects of morphine and morphine-like drugs on the action of eledoisin

Eledoisin (0·2–0·5 μg/ml.) was found to restore the peristaltic reflex previously blocked by morphine (0·005–0·2 μg/ml.), codeine (50–100 μg/ml.), pethidine (0·2–0·5 μg/ml.) or methadone (0·01–0·02 μg/ml.). This is illustrated in Fig. 8 for morphine ; eledoisin immediately caused large, regular peristaltic waves. When the peristaltic reflex was inhibited by pethidine, the peristaltic waves evoked by eledoisin were often small, irregular, with incomplete relaxation between contractions.

Effect of adrenaline on the action of eledoisin

Eledoisin (0·01–1 μg/ml.) restored the peristaltic reflex previously blocked by adrenaline (5 μg/ml.). The peristaltic waves lasted for 1 to 3 min.

Effects of atropine, hyoscine and hyoscine butylbromide on the action of eledoisin

When the peristaltic reflex was blocked by atropine (0·3–1 μg/ml.), hyoscine (0·4–1 μg/ml.) or hyoscine butylbromide (20–30 μg/ml.), eledoisin (0·01–1 μg/ml.) did not restore the peristaltic waves (Fig. 9).

Discussion

The results presented in this paper show that eledoisin applied to the serosal surface of the guinea-pig isolated ileum restored vigorous peristaltic activity when fatigue had set in after prolonged distension of the lumen. It is known from our previous results that similar effects are produced by carbachol, pilocarpine, arecoline, anticholinesterases and angiotensin (Beleslin & Varagić, 1963 ; Beleslin, 1966). On the other hand, in the non-fatigued ileum, eledoisin has no effect on the normal peristaltic reflex elicited by raising the intraluminal pressure for short periods ; indeed, when high concentrations are used, eledoisin depresses and sometimes abolishes the reflex. This depression is probably a non-specific inhibition similar to that observed by Kosterlitz & Robinson (1957) after high concentrations of carbachol. Angiotensin, which also produces contraction of the longitudinal muscle, has no effect on the normal peristaltic reflex except in high concentrations (Beleslin, 1968).

Eledoisin restores the peristaltic reflex blocked by ganglion-blocking agents, morphine and morphine-like agents, or adrenaline. It has been found previously that other polypeptides—for example, substance P and angiotensin—restore the peristaltic reflex blocked by fatigue, 5-hydroxytryptamine, lowering of the temperature of the bath, ganglion-blocking substances, morphine, catecholamines or atropine

(Beleslin & Varagić, 1958 ; Beleslin, 1968). On the other hand, bradykinin fails to restore the peristaltic reflex blocked by tetraethylammonium, methadone or atropine (Beleslin, Radmanović & Rakić, 1966).

Feldberg & Lin (1949) have shown that substances which increase smooth muscle tone may elicit the peristaltic reflex when the intraluminal pressure is not raised sufficiently to initiate the reflex. In the present experiments, eledoisin restored the peristaltic reflex when it was blocked by hexamethonium, tetraethylammonium or azamethonium. It is possible that these ganglion-blocking agents reduce the number of stimuli reaching the neuro-effector junction to below threshold and that eledoisin lowers the muscle threshold to the transmitter ; on the other hand, eledoisin does not restore the peristaltic reflex abolished by depolarizing ganglion-blocking agents. An excitatory action on the intestinal ganglion cells cannot be excluded, although Lewis & Reit (1965, 1966) showed that eledoisin does not stimulate the superior cervical ganglion. In this context, it is of interest that bradykinin, which also stimulates the superior cervical ganglion (Lewis & Reit, 1965, 1966), does not restore the peristaltic reflex blocked by tetraethylammonium, methadone or atropine, although it causes a contraction of the longitudinal muscle (Beleslin, Radmanović & Rakić, 1966).

It is known that morphine and morphine-like drugs reduce acetylcholine release in the guinea-pig ileum (Paton, 1957 ; Schaumann, 1957) ; therefore eledoisin may restore the peristaltic waves by lowering the muscle threshold to acetylcholine or by increasing the output of acetylcholine. Beleslin, Bogdanović & Rakić (1964, 1966) found that acetylcholine in high concentrations overcomes the block caused by morphine or methadone, so eledoisin may restore peristalsis in the same way as acetylcholine. The fact that eledoisin does not restore the peristaltic reflex blocked by atropine and atropine-like drugs suggests that eledoisin may act by increasing the output of acetylcholine.

When tachyphylaxis to eledoisin has developed, inhibition of true cholinesterase or pseudo-cholinesterase restores the stimulant effect of eledoisin. This observation is explained best by assuming that the action of acetylcholine and eledoisin are additive.

When, after repeated additions, eledoisin no longer restores the peristaltic reflex of the fatigued ileum, it still has a contracting effect on the longitudinal muscle. This observation raises the possibility that tachyphylaxis to eledoisin occurs mainly at a hypothetical neuronal site of the reflex arc. Beleslin & Vargić (1958) have shown that the receptors for substance P in the peristaltic reflex arc differ from those for 5-hydroxytryptamine. When tachyphylaxis to eledoisin has developed, anticholinesterases, acetylcholine and arecoline still produce peristaltic waves ; this observation suggests that the eledoisin and acetylcholine receptors are not identical.

Synthetic eledoisin was kindly supplied by Dr. E. Stürmer (Sandoz-Basle), and through Dr. B. B. Beleslin, by J. A. Harris, Sandoz Pharmaceuticals, Hanover, New Jersey, U.S.A. This work was supported by the grant of ZMNU, SRS, Yugoslavia.

REFERENCES

ANASTASI, A. & ERSPAMER, V. (1962). Occurrence and some properties of eledoisin in extracts of posterior salivary glands of Eledone. *Br. J. Pharmac. Chemother.*, **19**, 326–336.

AUSTIN, L. & BERRY, W. K. (1953). Two selective inhibitors of cholinesterase. *Biochem. J.*, **54**, 695–700.

BAUER, G., GMEINER, R. & WINKLER, H. (1966). Über die Wirkung synthetischer Polypeptide auf den Darm in situ. *Archs int. Pharmacodyn. Thér.*, **159**, 373–385.

BELESLIN, D. (1966). The effect of angiotensin on the peristaltic activity of the isolated guinea-pig ileum under constant intraluminal pressure. *Iugoslav. Physiol. Pharmac. Acta*, **2**, 166–168.

BELESLIN, D. (1968). The effect of angiotensin on the peristaltic reflex of the isolated guinea-pig ileum. *Br. J. Pharmac. Chemother.*, **32**, 583–590.

BELESLIN, D. B., BOGDANOVIĆ, S. B. & RAKIĆ, M. M. (1964). The effect of morphine and anti-cholinesterases on the peristaltic reflex of the isolated guinea-pig ileum. *Archs int. Pharmacodyn. Thér.*, **149**, 457–466.

BELESLIN, D. B., BOGDANOVIĆ, S. B. & RAKIĆ, M. M. (1966). The mechanism and site of action of methadone on the peristaltic reflex of the isolated guinea-pig ileum. *Archs int. Pharmacodyn. Thér.*, **164**, 345–355.

BELESLIN, D. B., RADMANOVIĆ, B. Z. & RAKIĆ, M. M. (1966). Stimulating and paralysing effect of bradykinin on the peristaltic activity of the isolated guinea-pig ileum. *Iugoslav. Physiol. Pharmac. Acta*, **2**, 139–148.

BELESLIN, D. & VARAGIĆ, V. (1958). The effect of substance P on the peristaltic reflex of the isolated guinea-pig ileum. *Br. J. Pharmac. Chemother.*, **13**, 321–325.

BELESLIN, D. & VARAGIĆ. V. (1963). The spontaneous activity and the effect of drugs on the isolated guinea-pig ileum kept under a constant intraluminal pressure. *Acta med. Iug.*, **17**, 336–345.

ERSPAMER, V. & ANASTASI, A. (1962). Structure and pharmacological actions of eledoisin, the active endecapeptide of the posterior salivary glands of Eledone. *Experientia*, **18**, 58–59.

ERSPAMER, V. & ERSPAMER, G. F. (1962). Pharmacological actions of eledoisin on extravascular smooth muscle. *Br. J. Pharmac. Chemother.*, **19**, 337–354.

FELDBERG, W. & LIN, R. C. Y. (1949). The action of local anaesthetics and *d*-tubocurarine on the isolated intestine of the rabbit and guinea-pig. *Br. J. Pharmac. Chemother.*, **4**, 33–44.

HAWKINS, R. D. & GUNTER, J. M. (1946). The selective inhibition of pseudocholinesterase *in vivo*. *Biochem. J.*, **40**, 192–197.

HAWKINS, R. D. & MENDEL, B. (1949). Selective inhibition of true cholinesterase *in vivo*. *Biochem. J.*, **44**, 260–264.

KOSTERLITZ, H. W. & ROBINSON, J. A. (1957). Inhibition of the peristaltic reflex of the guinea-pig ileum. *J. Physiol., Lond.*, **136**, 249–262.

LEWIS, G. P. & REIT, E. (1965). The action of angiotensin and bradykinin on the superior cervical ganglion of the cat. *J. Physiol., Lond.*, **179**, 538–553.

LEWIS, G. P. & REIT, E. (1966). Further studies on the actions of peptides on the superior cervical ganglion and suprarenal medulla. *Br. J. Pharmac. Chemother.*, **26**, 444–460.

PATON, W. D. M. (1957). The action of morphine and related substances on contraction and on acetylcholine output of coaxially stimulated guinea-pig ileum. *Br. J. Pharmac. Chemother.*, **12**, 119-127.

SCHAUMANN, W. (1957). Inhibition by morphine of the release of acetylcholine from the intestine of the guinea-pig ileum. *Br. J. Pharmac. Chemother.*, **12**, 115–118.

STEPHENSON, R. P. (1948). An outflow recorder useful for detecting small amounts of vasopressin. *J. Physiol., Lond.*, **105**, 162–164.

TRENDELENBURG, P. (1917). Physiologische und pharmakologische Versuche über die Dünndarm-peristaltik. *Arch. exp. Path. Pharmak.*, **81**, 55–129.

(*Received April* 14, 1969)