Brit. J. Pharmacol. (1962), **19**, 326–336.

OCCURRENCE AND SOME PROPERTIES OF ELEDOISIN IN EXTRACTS OF POSTERIOR SALIVARY GLANDS OF ELEDONE

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(*Received July* 24, 1962)

The posterior salivary glands of *Eledone*, alone among the tissues of this molluscan species, contain *eledoisin*, an endecapeptide with activity on blood vessels and extravascular smooth muscles. The salivary glands of *Octopus vulgaris* and *O. macropus* lack the polypeptide. Eledoisin is present, in amounts ranging from 20 to 160 μg/g fresh tissue, at all stages of growth and in every season; however, conspicuous differences in the eledoisin content were observed in different groups of animals. Eledoisin is fairly stable in intact salivary glands stored on ice. The most suitable solvent for its extraction is 70 to 80% methanol. Better results are also obtained with boiling diluted acetic acid, than with ethanol, whereas extraction with acetone gives unsatisfactory yields. Eledoisin is stable in neutral or slightly acid medium, but is rapidly destroyed by strong acids and even more rapidly by strong alkalis. It is quickly and completely inactivated by chymotrypsin, and somewhat more slowly and incompletely by trypsin. Carboxypeptidase is ineffective. Passage of crude salivary extracts of *Eledone* through an alumina column yields preparations of eledoisin which may be considered pure from a biological point of view.

It has been known for more than 10 years that the posterior salivary glands of *Eledone moschata* and *E. Aldrovandi* (two molluscan species belonging to the octopod Cephalopoda) contain a principle which possesses a powerful vasodilator and hypotensive action and potently stimulates some extravascular smooth muscles (Erspamer, 1949). This principle was first called moschatin and later was renamed *eledoisin*, in order to avoid confusion with the vegetable alkaloid moschatin from *Achillea moschata*. It was soon apparent that it was a polypeptide.

Eledoisin has now been isolated in a pure form, and its amino-acid composition and sequence have been fully elucidated (Erspamer & Anastasi, 1962). Eledoisin is an endecapeptide having the following amino-acid sequence:

Pyr. Pro. Ser. Lys. Asp (OH), Ala. Phe. Ileu. Gly. Leu. Met-NH_2
(Pyr. = pyroglutamyl).

The structure proposed has been confirmed by synthesis (Sandrin & Boissonnas, 1962).

This paper describes the occurrence and distribution of the polypeptide in *Eledone* under different conditions and gives some information on the different extraction procedures, on the stability of the active polypeptide and, finally, on a simple method for obtaining eledoisin in a biologically pure form.

METHODS

Reagents and solvents used in this investigation were usually obtained from Merck and were of the analytical grade. 5-Hydroxytryptamine creatinine sulphate and octopamine (*p*-hydroxyphenylethanolamine)hydrochloride were synthesized at the Farmitalia Research Laboratories, Milan; histamine dihydrochloride and tyramine hydrochloride were purchased from Hoffman-La Roche, Basle; crystalline trypsin and chymotrypsin from Princeton Lab. Products, Princeton, N.J.; and carboxypeptidase from Fluka A.G., Switzerland. We prepared pure natural eledoisin using a procedure to be described in detail elsewhere; synthetic eledoisin was kindly supplied by Dr R. A. Boisonnas, Sandoz, Basle.

Quantitative estimation of eledoisin was carried out by bioassay, using the isolated atropinized large intestine of the rabbit, the isolated guinea-pig ileum and the dog blood pressure (Erspamer, 1949; Erspamer and Falconieri Erspamer, 1962). Both natural and synthetic eledoisin served as standard. 5-Hydroxytryptamine and histamine were similarly estimated by bioassay using the rat uterus preparation and the guinea-pig ileum, respectively.

Semi-quantitative estimation of tyramine and octopamine was carried out on paper chromatograms, by comparing visually both size and intensity of colour of the spots given by different amounts of tissue extracts with those produced by known amounts of pure substances. Octopamine spots were developed by either the Pauly reagent (diazotized sulphanilic acid and sodium carbonate) or the Gibbs reagent (dichloroquinonechlorimide and sodium carbonate), tyramine and histamine spots by the Pauly reagent, 5-hydroxytryptamine spots by either the Gibbs reagent or the NNCD reagent (2-chloro-4-nitro-1-diazobenzene-α-naphthalene sulphuric acid), purchased from Fluka A.G. Chromatograms were run on Whatman no. 1 paper using as solvents the *n*-butanol:acetic acid:water mixture (5:1:4) and the methylamine (30% watery solution):*n*-butanol mixture (3:8).

RESULTS

Eledoisin and amine content of batches of posterior salivary glands of Eledone *collected at different times*

All the material used in this study was collected at the Observatory of Marine Biology of the University of Parma at S. Margherita Ligure. The salivary glands were removed from live animals (*Eledone moschata* and *E. Aldrovandi*) immediately after the arrival of the fishing-vessels. The tissue was extracted for 24 to 48 hr with 3 to 4 vol. of pure methanol and then for another 24 to 48 hr with 2 to 3 vol. of 80% methanol. The combined filtrates were kept in the refrigerator.

Lot 1 (April, 1959). 680 pairs of glands, removed from 184 kg *Eledone*, weighed 644 g (yield 3.5 g/kg body weight); the average weight of a pair of glands was 0.94 g, the average weight of an *Eledone* specimen 270 g.

Lot 2 (July, 1959). 1,107 pairs of glands, removed from 318 kg *Eledone*, weighed 1,145 g (yield 3.6 g/kg body weight). Average weight of a pair of glands was 1 g, the average weight of a specimen 287 g.

Lot 3 (September and October, 1959). 10,143 pairs of glands, removed from 1,450 kg *Eledone*, weighed 5,369 g (yield 3.7 g/kg body weight); the average weight of a pair of glands was 0.53 g, the average weight of a specimen 143 g. After extraction with methanol the dry salivary tissue weighed 966 g (18% of the fresh tissue). The methanol extract, in its turn, left a dry residue of 59 mg/g fresh tissue. Approximately 15% of this residue was made up of lipids extractable with petroleum ether.

Lot 4 (September, 1960). 6,829 pairs of glands, removed from approximately 1,600 kg *Eledone*, weighed 5,540 g (yield in glands 3.46 g/kg body weight); the average weight of a pair of glands was 0.81 g, the average weight of a specimen 230 g. After extraction with methanol the dry salivary tissue weighed 1,060 g (19%).

Table 1 shows the content of eledoisin and amines in these four batches of posterior salivary glands.

TABLE 1

THE CONTENT OF ELEDOISIN AND AMINES IN DIFFERENT LOTS OF POSTERIOR SALIVARY GLANDS OF *ELEDONE*

Posterior salivary glands	Content of active compounds (in μg/g fresh tissue)				
	Eledoisin	5-Hydroxy-tryptamine	Octop-amine	Tyr-amine	Hist-amine
Lot 1 (April 1959)	110–120	370	85	30	240
Lot 2 (July 1959)	70–75	280	100	40	300
Lot 3 (Sept. 1959)	90–100	430	110	60	280
Lot 4 (Sept. 1960)	20–25	320	70	35	220

The eledoisin content was fairly even in the salivary glands gathered throughout 1959, whereas it was unexpectedly low in the large batch gathered in September, 1960. The reason for this low content is completely obscure, the more so as the amine content was within normal limits.

Methanol extracts of lots 2 and 3 were combined to form the standard extract *Eledone 1959*, which served as a base for the isolation of pure eledoisin and for the preparation of biologically pure eledoisin, suitable for pharmacological studies.

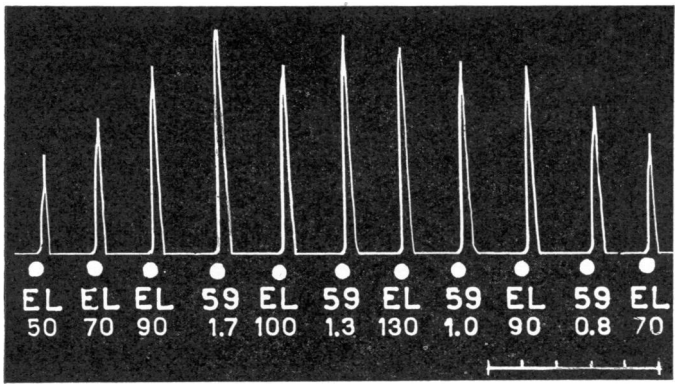

Fig. 1. Guinea-pig ileum suspended in 10 ml. Krebs solution at 32° C. Time, 1 min. Quantitative estimation of eledoisin in the *Eledone 1959* standard extract (59). EL=synthetic eledoisin. Numerals represent ng of pure eledoisin and mg of fresh *Eledone* salivary tissue, respectively. The *Eledone 1959* extract corresponding to 1 mg fresh tissue had the same activity as 100 to 110 ng synthetic eledoisin.

Extracts of posterior salivary glands of *Eledone*, as well as of *Octopus vulgaris*, contain besides 5-hydroxytryptamine considerable amounts of another unknown 5-hydroxyindole, the identification of which is in progress.

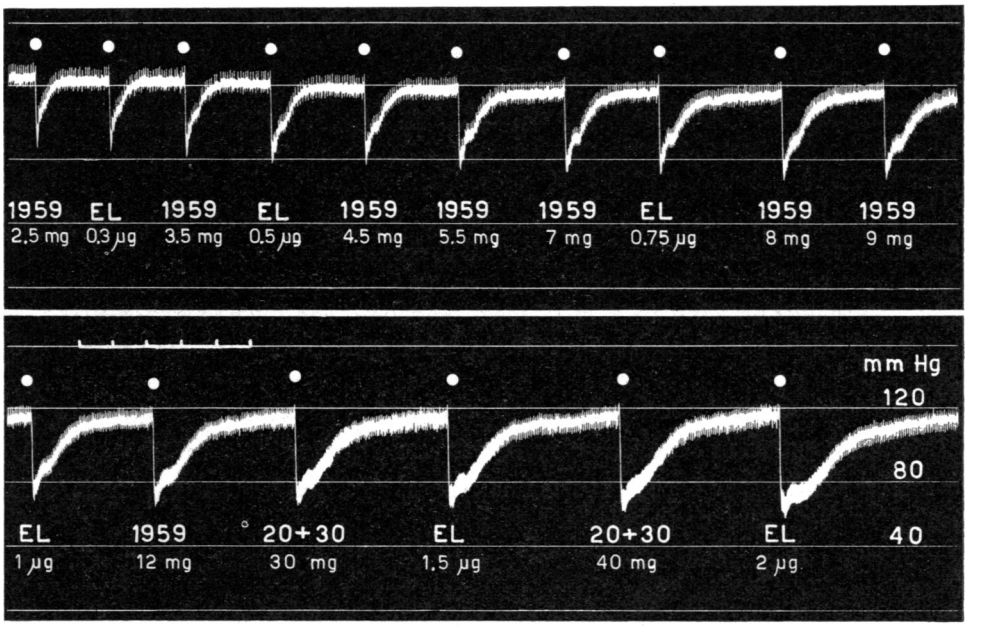

Fig. 2. Blood pressure of a dog anaesthetized with pentobarbitone (30 mg/kg, intravenously) and pre-treated with 0.2 mg/kg atropine sulphate, intramuscularly. Time, 2 min. Quantitative estimation of eledoisin in the *Eledone 1959* standard extract (1959) and in the eluates from an alumina column obtained with 30 and 20% ethanol, at 4° C. (20+30). EL=synthetic eledoisin. Numerals represent µg of pure eledoisin and mg of fresh *Eledone* salivary tissue, respectively. The *Eledone 1959* extract corresponding to 10 mg fresh tissue showed the same activity as 1.1 µg synthetic eledoisin; the 20+30 eluate corresponding to 35 mg tissue was equiactive to 1.5 µg eledoisin.

Eledoisin and amine content of Eledone *specimens of different size*

Table 2 gives the result of eledoisin estimations in specimens of *Eledone* of different size; the content of 5-hydroxytryptamine, octopamine, histamine and tyramine are also shown.

Completely developed adult specimens had an average weight of 290 g; 1,107 pairs of glands weighed 1,145 g (1 g per pair of glands, and 3.6 g per kg body weight). After methanol extraction the dry tissue weighed 21% of the fresh tissue.

TABLE 2

THE CONTENT OF ELEDOISIN AND AMINES IN THE POSTERIOR SALIVARY GLANDS OF *ELEDONE* SPECIMENS OF DIFFERENT SIZE

Size of *Eledone* specimens (in parentheses average body weight and weight of a pair of posterior salivary glands)	Content of active compounds (in µg/g fresh tissue)				
	Eledoisin	5-Hydroxytryptamine	Octopamine	Tyramine	Histamine
Large, adult specimens (290 g; 1 g)	70–75	280	100	40	300
Young, medium-sized specimens (37 g; 0·27 g)	150–155	125	12	25	200
Young, small specimens (21 g; 0·14 g)	65	70	6	5	130

Medium-sized specimens had an average weight of 37 g; 70 pairs of glands weighed 19 g (0.27 g per pair of glands, and 7.3 g per kg body weight). Dry tissue weighed, after methanol extraction, 15% of the fresh tissue.

Small specimens had an average weight of 21 g; 70 pairs of salivary glands weighed 10 g (0.14 g per pair of glands, and 6.6 g per kg body weight). Dry tissue weighed, after methanol extraction, only 11% of the fresh tissue.

The eledoisin content of the posterior salivary glands was maximal in young, medium-sized animals, and even in the smallest specimens examined it was as high as in large adult animals. In sharp contrast to this finding the amine content was strikingly higher in adult than in younger animals. The difference, however, was less conspicuous for histamine than for the other amines.

Eledoisin content of other Eledone *tissues and of tissues of other molluscs*

Methanol extracts of the following tissues were examined:

Eledone moschata: hepatopancreas, intestines, gills, heart, kidneys, gonads, eyes, tentacle musculature, optic ganglia.

Octopus vulgaris and *O. macropus:* posterior salivary glands, hepatopancreas, intestines, gills, kidneys, heart, tentacle musculature.

Murex trunculus, M. brandaris, Euthria cornea, Dolium galea, Vulgocerithium vulgatum: hypobranchial body, viscera, foot musculature.

Aplysia limacina: mantle, salivary glands.

Ostrea edulis, Mytilus galloprovincialis: musculature, gills, viscera.

None of these tissues contained detectable amounts of eledoisin. In fact, the stimulant action of these extracts on the rabbit large intestine was always at least 5,000 to 10,000 times less intense than that of extracts of salivary glands of *Eledone*, and some extracts (e.g. those of optic ganglia), instead of stimulating, inhibited the gut preparation. It therefore appears that eledoisin has a very restricted distribution in the molluscan body, so far having been found only in the posterior salivary glands of *Eledone*.

Extraction of eledoisin from fresh salivary glands by different solvents and extraction procedures

A batch of 128 g of posterior salivary glands of *Eledone* obtained from 171 specimens was divided into 4 parts: the first part (35 g) was extracted with 4 vol. of pure methanol, and after 24 hr re-extracted with another 3 vol. of 80% methanol; the second part (30 g) was extracted exactly as above, using absolute ethanol instead of methanol; the third part (30 g) was extracted with pure acetone and then with 80% acetone; finally, the fourth part (33 g) was extracted first with 4 vol. 60% acetone and then with 3 vol. 50% acetone. The experiment was repeated on another large batch of glands.

The best yield of eledoisin was always obtained with methanol. Taking this yield as 100, the percentage recovery of eledoisin with the other solvents was as follows: ethanol 50 to 70, pure acetone 25 to 33, 60% acetone 60 to 65.

In other similar experiments it was found that: (a) extraction with 19 vol. of acetone, instead of 4 vol., further reduced the yield of eledoisin to 15% ; (b) homogenizing the glands in acetone moderately improved the yield of eledoisin, increasing it from 30 to 45% when 4 vol. of acetone was used, and from 15 to 25% when 19 vol. of acetone was employed ; homogenizing the glands in methanol did not improve the yield of eledoisin any further ; (c) boiling with 4 vol. of 0.3% acetic acid for 15 min followed, after 24 hr, by another treatment with boiling diluted acetic acid gave a 80 to 90% yield of eledoisin ; (d) boiling with 0.3% acetic acid salivary glands already exhausted by methanol extracted only traces (<0.5%) of eledoisin. The same negative result was obtained when tissues previously treated with diluted acetic acid were extracted with methanol. This suggests that with both solvents extraction was complete.

It is obvious that methanol is the best solvent for extraction of eledoisin. Methanol extraction is considerably more reliable than extraction with boiling diluted acid owing to its extreme simplicity ; moreover the extracts can be stored for long periods, and the solvent easily removed.

Acetone or methanol extracts of posterior salivary glands kept in the refrigerator at 3 to 4° C were highly stable for years. Eledoisin activity of an acetone extract prepared in 1951 (*p*H 6.1) appeared to be virtually unchanged after 10 years, and the same could be observed for a methanol extract (*p*H 6.5) after storage for 3 years. But even aqueous solutions of the dry residue left by evaporation of the acetone extract suffered only very slow loss of activity, if kept under sterile conditions either in the refrigerator or at room temperature. In fact, residual activity after 10 years of storage was 70 to 80 and 40 to 60%, respectively.

Inactivation of eledoisin by salivary tissue

A collection of 350 g of posterior salivary glands of *Eledone*, obtained from 470 animals, was divided into 8 parts. One part was treated immediately with 4 vol. methanol, and served as control ; five parts were immersed, in bottles, into ground ice and then extracted with methanol after ½, 1, 2, 4 and 12 days ; two parts were kept at room temperature for 12 and 24 hr, respectively, before methanol extraction.

Glands kept in ice for ½, 1 and 2 days possessed the same eledoisin activity as fresh control tissue ; glands stored in ice for 4 to 12 days showed 85 to 90 and 60%, respectively, of the activity of fresh glands. The loss of activity was more rapid at room temperature, being 20 to 25% after 12 hr and 50% after 24 hr. Homogenizing posterior salivary glands *per se* produced a conspicuous loss of activity ; viz., as much as 90% when the homogenizer flask was not cooled with ice, and 30 to 40% when it was cooled with ice. Moreover, activity of homogenates stored in ice disappeared more rapidly than that of control whole glands.

In order to investigate the inactivation of eledoisin by posterior salivary gland tissue, 11.2 g of glands was homogenized in ice with 4 vol. of 1% sodium chloride solution, and the resulting homogenate was brought to 112 ml. with the same saline solution. Of the homogenate (*p*H 6.4) 10 ml. was treated immediately with 4 vol. methanol, the other 100 ml. was incubated in a water bath at 37° C ; 10 ml. aliquots were taken off at different times and treated with methanol. For comparison 15 g of

Eledone hepatopancreas was homogenized in 135 ml. of 1% sodium chloride solution ; 90 ml. of the homogenate was then added to 10 ml. of physiological saline in which the residue left by evaporation of the methanol extract of 10 g salivary tissue had been previously dissolved. Reduction of activity produced by the two homogenates is shown in Fig. 3.

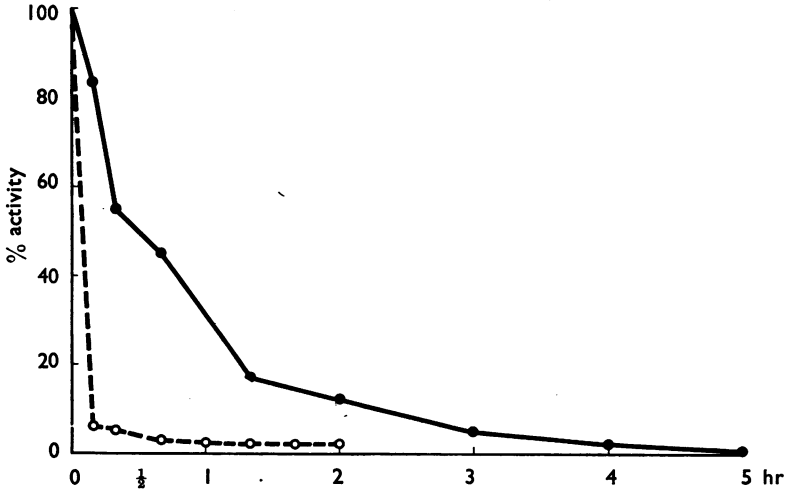

Fig. 3. Inactivation of eledoisin in homogenates of posterior salivary glands (● —— ●) and of hepatopancreas (○ - - - ○) of *Eledone*. Incubation was carried out at *p*H 6.4 and at 37° C for 10 min to 5 hr.

It appears that salivary tissue was not very rich in eledoisin-inactivating enzymes. Hepatopancreas, on the contrary, produced 90 to 95% inactivation of the polypeptide within only 10 min.

Resistance of eledoisin to proteolytic enzymes and to acid and alkali treatment

The methanol extract corresponding to 70 g of posterior salivary glands was evaporated under reduced pressure and the residue taken up with 70 ml. distilled water.

Chymotrypsin. Aliquots of 0.5 ml. of the aqueous liquid were brought to *p*H 7.5 with sodium carbonate and then, after addition of crystalline chymotrypsin (2, 5, 10 and 100 μg, respectively) incubated for different times in a water bath, at 37° C. Chymotrypsin action was arrested by immersion of the incubation flasks into boiling water, for 3 to 5 min.

Fig. 4 shows that an incubation of 5 min was sufficient for 100 μg chymotrypsin to produce 100% inactivation of the *Eledone* extract, and 10 μg an inactivation of 50%.

Trypsin. A similar experiment was carried out using crystalline trypsin (0.1, 0.2, 0.5, 1 and 10 mg, respectively). An amount of 0.1 to 0.2 mg trypsin produced after 30 min incubation a 25 to 45% inactivation of the *Eledone* extract, 0.5, 1 and 10 mg trypsin a 70 to 90% inactivation.

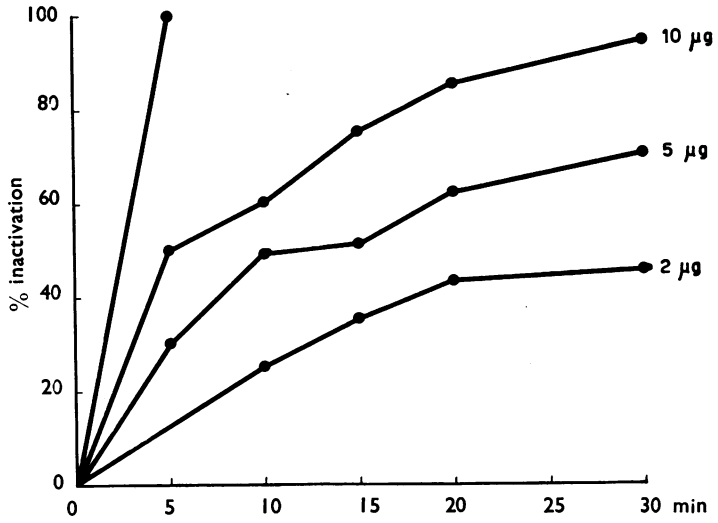

Fig. 4. Inactivation of eledoisin by crystalline chymotrypsin. The extract corresponding to 0.5 g salivary tissue (=50 μg eledoisin) was incubated with 2 to 100 μg chymotrypsin at *p*H 7.5 and at 37° C for 5 to 30 min.

Carboxypeptidase. In a third experiment a preparation of carboxypeptidase (10 and 100 μg) was used. The substrate was brought to *p*H 8.5 and then incubated at 22° C for 1 to 16 hr. No inactivation was observed. The same complete resistance towards the enzyme was shown by pure eledoisin (50 to 200 μg), both natural and synthetic.

Acid and alkali treatment. Aliquots of 3 ml. of the aqueous extract were brought to 6 ml. with: sodium hydroxide, sodium bicarbonate, acetic acid and hydrochloric acid, respectively, to give the final concentrations indicated in Table 3. Residual activity of the extracts, after treatment in a boiling water bath for different times, is shown.

TABLE 3

RESISTANCE OF ELEDOISIN TO ACID AND ALKALI TREATMENT

	Residual activity (in %) after treatment at 100° C for				
	5 min	10 min	30 min	1 hr	2 hr
Sodium hydroxide N	3	2	1	—	—
Sodium hydroxide 0·1 N	10	4	1	—	—
Sodium bicarbonate 0·1 N	—	90	75	55	20
Hydrochloric acid N	50	25	10	3	1
Hydrochloric acid 0·1 N	85	60	35	20	5
Acetic acid 0·1 N	—	—	85	75	60

Eledoisin, although rapidly inactivated both by strong acids and strong alkalis, is clearly more resistant to acid than to alkali treatment.

Treatment with diazonium salts. Adding diazotized *p*-nitroaniline or diazotized sulphanilic acid to *Eledone* aqueous extracts produced no inactivation of eledoisin, either in acid or alkaline medium. On the other hand, 5-hydroxytryptamine, octopamine, tyramine and histamine were completely inactivated.

Partial purification of crude extracts on an alumina column

Passage through an alumina column was the first step in the purification and isolation procedure of eledoisin. It was carried out in order to obtain large amounts of biologically pure eledoisin, i.e. of eledoisin with no or negligible contamination by other active compounds, especially amines.

In a typical experiment 750 ml. of methanol extract, corresponding to 150 g salivary tissue, was evaporated, at 45 to 50° C under reduced pressure, to 50 to 60 ml. and the remaining aqueous liquid extracted repeatedly with petroleum ether in order to remove fats. The distillation was then continued until the residue was of syrupy consistence. The residue was taken up, in a warm water bath, by stirring in 100 ml. of 96% ethanol. The abundant precipitate found after storing overnight in the refrigerator was discarded and the limpid liquid passed through an alumina column (alkaline aluminium oxide Merck, for chromatography), 3.3 cm in diameter. The alumina weighed 140 g and reached a height of 21 cm in the column. Elution was performed in a refrigerated room, at 3 to 4° C, with decreasing concentrations of ethanol, and the eluate was collected in fractions of 200 ml. each.

Each fraction was assayed biologically for its eledoisin content (expressed in percentage of the eledoisin content of the standard *Eledone* extract) and was chromatographed on paper after having been suitably concentrated (1 ml. liquid = 10 to 15 g fresh salivary tissue), for the detection of amines. Results are shown in Table 4.

TABLE 4

CHROMATOGRAPHY ON AN ALUMINA COLUMN OF A CRUDE SALIVARY EXTRACT OF *ELEDONE*. RECOVERY OF ELEDOISIN AND AMINES IN THE ELUATE FRACTIONS OBTAINED WITH DESCENDING CONCENTRATIONS OF ETHANOL

	Dry residue (in mg per g fresh tissue)	Eledoisin content (in %)	Occurrence of amines			
			5-Hydroxy-tryptamine	Octop-amine	Tyr-amine	Hist-amine
Standard extract	59	100				
Eluates						
95% ethanol	—	<0·1	Traces	0	0	Traces
90% ethanol	—	<0·1	0	0	0	Traces
80% ethanol	8	1·2	++++	+	++	++
70% ethanol	11·6	2	+++	+	+	+++
70% ethanol						
60% ethanol	2·2	0·2	+	Traces	0	0
60% ethanol						
50% ethanol	0·7	7	Traces	0	0	0
50% ethanol						
40% ethanol	2·1	51	Traces	0	0	0
40% ethanol						
30% ethanol	1·8	12	0	0	0	0
30% ethanol						
Dist. water	—	1	0	0	0	0

From the Table it may be seen that: (a) 50% of eledoisin was recovered in the 40% ethanol eluates, which left a dry residue of barely 2.1 mg/g fresh tissue, as opposed to the 59 mg/g left by the standard extract; this represents a 14-fold purification; (b) 40% ethanol eluates, although containing conspicuous amounts

of amino-acids and other inactive constituents (approximately 97 to 98% of the dry residue) may be considered to be a source of biologically pure eledoisin ; in fact, the traces of 5-hydroxytryptamine present in these eluates (0.2 to 0.3 μg/g) do not interfere, except in very rare cases, with the pharmacological effects of eledoisin ; (c) the bulk of amines was eluted by 80 and 70% ethanol.

In 36 experiments of chromatography on alumina columns recovery of eledoisin varied between 55 and 85%. When chromatography was carried out at room temperature instead of in the refrigerator, maximum elution of eledoisin occurred with 60 or 50% ethanol and maximum elution of amines with 90 and 80% ethanol.

In one experiment the dry residue left by the standard crude methanol extract was taken up in 80% ethanol, instead of 95% ethanol. In this instance there was practically no absorption of eledoisin by the alumina. In fact 80% of the polypeptide appeared already in the second fraction of the 80% ethanol eluate.

Besides methanol extracts, acetone extracts of posterior salivary glands were subjected to column chromatography, using not only alkaline but also neutral and acid alumina. The eledoisin content of the standard acetone extract was 22% of that of the standard methanol extract. Chromatography was carried out at 3 to 4° C. Elution of eledoisin from the alkaline alumina column occurred with 30% ethanol and recovery was 90% ; from both neutral and acid alumina elution occurred only with distilled water, and recoveries were 85 and 75%, respectively. It therefore appears that the polypeptide is absorbed more tenaciously by neutral and acid alumina than by alkaline alumina. The fact that following chromatography of acetone extracts elution of eledoisin was obtained only with lower concentrations of ethanol may be explained by the different composition of acetone extracts which, for example, certainly contained less amino-acids than the methanol extracts.

DISCUSSION

The only tissues of *Eledone* where eledoisin was found were the posterior salivary glands. The salivary glands of *Octopus vulgaris* and *O. macropus* were completely lacking in the substance.

Like other related biogenic polypeptides, eledoisin could be best extracted from the tissues by boiling with diluted acid or by methanol. With ethanol and, still more, with acetone extraction was incomplete. Salivary tissue was apparently poor in proteolytic enzymes capable of attacking eledoisin ; hence the stability of the polypeptide in this tissue. When salivary glands were kept at approximately 0° C no appreciable loss of eledoisin activity occurred for some days.

The significance of eledoisin in the salivary glands is obscure. In preliminary experiments no detectable action of the polypeptide was observed on the *Eledone* gut or gill musculature. It is tempting to speculate that eledoisin has no significance for *Eledone*, except that it represents a chain of amino-acids either used in the synthesis of proteins or originating from breakdown of proteins. That eledoisin has something to do with protein synthesis or breakdown also seems to be suggested by the occurrence of large amounts of the polypeptide in the salivary glands of very young specimens of *Eledone*, at a stage of growth where the amine content in the tissue was very low. The likelihood of any general biological significance of

eledoisin is lessened by the circumstance that the polypeptide is present only in the salivary glands of *Eledone*, while lacking in those of other octopod cephalopods. However, the possibility cannot be excluded that the function of eledoisin in *Eledone* is taken over in other cephalopods by related polypeptides which escape our attention simply because, unlike eledoisin, they are inactive on the vascular and extravascular smooth muscle preparations examined.

Moreover, the finding that an eledoisin-like polypeptide occurs also in some lower vertebrates (Erspamer, Cei & Bertaccini, forthcoming publication) makes one hesitant to rule out the possibility that eledoisin may have some function in the *Eledone* salivary glands and/or organism.

Extracts of salivary glands contain, in a free form, large amounts of numerous amino-acids, among which are included all those which constitute the eledoisin molecule; they also contain other inactive polypeptides the study of which will probably offer a key to the understanding of the biosynthesis and perhaps the significance of eledoisin. It will also be of interest to see whether breakdown of proteins from the salivary glands by enzymes gives rise to eledoisin.

It will be seen that the biogenic polypeptide which is most similar to eledoisin in both its pharmacological properties and its behaviour towards proteolytic enzymes is substance P. Absorption on alumina column and subsequent elution with decreasing concentrations of ethanol can help in distinguishing the two polypeptides. In fact, whereas crude substance P is completely absorbed on alkaline alumina when dissolved in 80% ethanol or methanol and then eluted only by 20% ethanol and distilled water (Dahlstedt, v. Euler, Lishajko & Östlund, 1959), eledoisin is adsorbed on alumina only when dissolved in 95% ethanol and is then eluted by 60 to 30% ethanol.

Treatment with proteolytic enzymes permits an easy distinction between eledoisin and bradykinin: the former is attacked by trypsin and is resistant to carboxypeptidase, the latter shows the opposite behaviour.

We are indebted to and wish to thank Professor B. Schreiber, Director of the Institute of Zoology, University of Parma, for his hospitality at the Marine Biological Observatory of S. Margherita Ligure. We also wish to thank Dr M. B. Nobili for her untiring assistance in the collection of the salivary glands. This work was supported by a grant from the Rockefeller Foundation, New York.

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