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OCCURRENCE OF CAERULEIN IN EXTRACTS OF THE SKIN OF *HYLA CAERULEA* AND OTHER AUSTRALIAN HYLIDS

BY

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Methanol extracts of the skin of *Hyla caerulea*, an Australian frog, contain a principle which displays a conspicuous hypotensive effect in the dog and other experimental animals and possesses a formidable stimulant action on some extravascular smooth muscles and on certain secretions, especially those of gastric and pancreatic juice (Erspamer, Bertaccini, De Caro, Endean & Impicciatore, 1967).

This principle, called caerulein, has recently been isolated in a pure form and its amino-acid composition and sequence have been fully elucidated (Anastasi, Erspamer & Endean, 1967, 1968).

It may be seen from the amino-acid sequence that caerulein shows a close resemblance to gastrins, especially to gastrins II.

SO_3H
|
Pyr-Gln-Asp-Tyr-Thr-Gly-Trp-Met-Asp-Phe-NH_2 Caerulein

SO_3H
|
Pyr-Gly-Pro-Trp-$(Glu)_5$-Ala-Tyr-Gly-Trp-Met-Asp-Phe-NH_2 Human gastrin II

(Pyr = pyroglutamyl)

The structure proposed for caerulein has been confirmed by synthesis (Bernardi, Bosisio, De Castiglione & Goffredo, 1967).

This paper describes the occurrence of caerulein in skins from different batches of *Hyla caerulea* and from other Australian species of *Hyla*, the distribution of caerulein in different regions of the skin, the behaviour of the decapeptide during drying of the skin, and the occurrence in the skins of Australian hylids of other active constituents, such as indolealkylamines and imidazolealkylamines.

METHODS

Amphibian material

The following Hyla material was used in this study.

1. Fifteen adult specimens of *Hyla caerulea* captured in May, 1964. The total weight of the dried skins was 13.5 g and the average weight of one skin was 0.9 g.

2. 303 adult specimens of *H. caerulea* captured in December, 1964, and January, 1965. 179 specimens were males (total weight of the dried skins, 249 g ; average weight of one skin, 1.4 g) and 124 were females (total weight of dried skins, 206 g ; average weight of one skin, 1.66 g). After separate study initially, the skins from male and female specimens were combined to constitute pool 1965.

3. 682 adult specimens of *H. caerulea* caught in October and November, 1965. 380 specimens were males (total weight of the dried skins, 521 g ; average weight of one skin, 1.34 g) and 302 specimens were females (total weight of the dried skins, 537 g ; average weight of one skin, 1.77 g). After separate study, the skins from male and female specimens were combined to constitute pool 1966.

4. Six large adult specimens of *H. caerulea* (total weight of the dried skins, 19.9 g) captured in May, 1965. The skins were minced with scissors and the skin fragments were thoroughly mixed. Part of the material was extracted with 80% methanol and part with 70% acetone in order to determine the relative efficiency of the two solvents in the extraction of caerulein.

5. Twelve adult specimens of *H. caerulea* (total weight of the dried skins, 20 g) captured in November, 1965. The skins were extracted with 80% methanol and 70% acetone, as described for 4.

6. Ten specimens of *H. caerulea* captured in November, 1965. The thick skin of the back (10.9 g) was separated from the thin skin of the legs and abdomen (9.85 g) and the two portions were extracted separately.

7. Six male specimens of *H. caerulea*, captured in March, 1965. In each case, the fresh skin was divided longitudinally into two symmetrical (right and left) parts immediately after the animal was killed. Three right moieties and three left moieties were weighed (16.2 g) and extracted immediately with methanol, the other moieties were weighed (15.6 g), and subjected to extraction with methanol after they were dried (4.4 g). Six female specimens captured at the same time were treated in exactly the same way. The weight of the six fresh skin moieties subjected to immediate extraction was 7.83 g, the weight of the other six fresh skin moieties was 8.49 g when fresh and 2.4 g after drying before extraction with methanol.

8. Six specimens of *H. caerulea*, captured in March, 1966. They were treated as described for 7. The weight of the six fresh skin moieties subjected to immediate extraction was 25.7 g, the weight of the other six skin moieties was 23.6 g when fresh and 6.8 g after drying.

9. Eight specimens of *H. caerulea*, captured in July, 1966. They were treated as described for 7. The weight of the eight fresh skin moieties subjected to immediate extraction was 12.08 g, the weight of the other eight skin moieties was 11.15 g when fresh and 3.7 g after drying.

10. Twelve specimens of *H. caerulea*, captured in March and May, 1967. They were treated as described for 7. The weight of the twelve fresh skin moieties subjected to immediate extraction was 38.14 g, the weight of the other twelve skin moieties was 35.76 g when fresh and 11.74 g after drying.

11. Three specimens of *Hyla infrafrenata*, captured in North Queensland in January, 1965. The average weight of one dried skin was 1.25 g.

12. Six specimens of *Hyla rothi*, captured in North Queensland in January, 1965. The average weight of one dried skin was 0.19 g.

13. Two specimens of *Hyla chloris*, captured in December, 1965. The average weight of one dried skin was 0.19 g.

14. Fifty-one specimens of *Hyla dentata*, captured in December, 1965. The average weight of one dried skin was 0.074 g.

15. Two specimens of *Hyla verreauxi*, captured in December, 1964, and in April, 1966, respectively. The average weight of one dried skin was 0.065 g.

16. Two specimens of *Hyla phyllochroa*, captured in December, 1964. The average weight of one dried skin was 0.04 g.

17. Eighteen specimens of *Hyla latopalmata*, captured in December, 1964. The average weight of one dried skin was 0.08 g.

18. Fifty-four specimens of *Hyla bicolor*, captured in December, 1964, and December, 1965. The average weight of one dried skin was 0.03 g.

19. 116 specimens of *Hyla pearsoniana* captured in December, 1964, and January, 1966. The average weight of one dried skin was 0.038 g.

20. Twenty-four specimens of *Hyla peroni*, captured in December, 1964, and December, 1965. The average weight of one dried skin was 0.22 g.

21. Fifty-five specimens of *Hyla rubella*, captured in April, 1964, and December, 1965. The average weight of one dried skin was 0.062 g.

22. Thirty-nine specimens of *Hyla lesueuri* captured in November and December, 1964. The average weight of one dried skin was 0.13 g.

23. Sixty-nine specimens of *Hyla gracilenta*, captured in December, 1965. The average weight of one dried skin was 0.09 g.

24. Six specimens of *Hyla nasuta*, captured in February, 1964, and December, 1965. The average weight of one dried skin was 0.2 g.

25. Eighteen specimens of *Hyla gilleni*, captured in April, 1967, near Alice Springs, Central Australia. The average weight of one dried skin was 1.2 g.

Unless otherwise stated, all specimens were adult and were captured in regions near Brisbane, Queensland, Australia.

Extracts of fresh skins were prepared in Australia by extracting twice with five parts (w/v) of methanol the skins which had been removed from the animals immediately after killing them. The skins destined to be dried were carefully spread out and dried in the shade. Soon after their arrival in Italy, by air mail, they were minced with scissors and then immersed in twenty parts of methanol 80% (more seldom acetone 70%). The liquid was decanted after a week, and the skins were treated for another week with fifteen to twenty parts of the solvent. The methanol extracts, yellow or brownish in colour, were mixed and filtered. Kept in dark bottles and refrigerated (+4° C), they can be stored for months and years without appreciable loss of activity.

Reagents and drugs

Analytical grade reagents and solvents were used throughout the investigation.

Pure caerulein was obtained by a procedure described in detail elsewhere (Anastasi *et al.*, 1967, 1968). Synthetic caerulein was prepared at the Farmitalia Laboratories for Basic Research, Milan. Parallel assay revealed that it possessed the same biological activity as natural caerulein.

Other drugs were obtained from the following sources: crystalline trypsin and chymotrypsin from Princeton Laboratory Products, Princeton, N.J., U.S.A.; crystallized and lyophilized type VII subtilisin from the Sigma Chemical Co., St. Louis, Missouri, U.S.A.; NNCD reagent (2-chloro-4-nitrobenzenediazoniumnaphthalene-2-sulphonate) from Hopkins & Williams Ltd., Chadwell Heath, Essex, England.

Bioassay

Quantitative estimation of caerulein was carried out by bioassay, using dog blood pressure and, subordinately, the contraction of the *in situ* gall bladder of the guinea-pig.

5-Hydroxytryptamine and histamine were also estimated by bioassay, using the rat uterus preparation, and the guinea-pig ileum in the presence of 2-bromo lysergic acid diethylamide.

Paper chromatography

The estimations of 5-hydroxytryptamine (5-HT) and histamine made using bioassay procedures were checked by paper chromatography. Semi-quantitative estimations of the two amines were carried out by making a visual comparison of the size and colour intensity of spots yielded by different amounts of tissue extracts with those produced by known amount of 5-HT and histamine. Histamine spots were developed with the Pauly reagent (diazotized sulphanilic acid and sodium carbonate) and 5-HT spots with either the NNCD reagent (0.2–0.3% in 0.1 N HCl) or the Gibbs reagent (dichloroquinonechlorimide and sodium carbonate).

For the estimation of 5-HT, paper chromatograms were run with the *n*-butanol : acetic acid : water mixture (40 : 10 : 50), for the estimation of histamine with the *n*-butanol : 30% methylamine mixture (80 : 30) or with the methylethylketone : pyridine : water : 30% methylamine (65 : 15 : 10 : 0.5) mixture.

Further details of the methods can be found in previous publications (Erspamer, Vitali, Roseghini & Cei, 1967 ; Anastasi & Erspamer, 1962 ; Anastasi *et al.*, 1968).

RESULTS

Caerulein and amine content of different batches of dried and fresh skin of Hyla caerulea

The content of caerulein, 5-HT and histamine in the various batches of *Hyla caerulea* skin examined is shown in Table 1.

TABLE 1

CONTENT OF CAERULEIN AND AMINES IN DIFFERENT BATCHES OF *HYLA CAERULEA* SKIN

* Figures indicate the weight ratio of fresh skin: dried skin. In square parentheses is the content of active compounds in dried skin calculated as fresh skin. Amines are always expressed as free bases.

Skin			Content of active compounds (in μg/g tissue)		
Batch	Condition		Caerulein	5-HT	Histamine
1 (May 1964)	Dried		700		
2 (Pool 1965)	Dried		790	220	190
3 (Pool 1966)	Dried		1300	450	310
4 (May 1965)	Dried		950	240	420
5 (November 1965)	Dried		1200	235	240
7 (March 1965)	Dried	3·6*	800 [225]	210 [60]	300 [85]
	Fresh		300	40	55
7 (March 1965)	Dried	3·5*	700 [200]	350 [100]	315 [90]
	Fresh		300	60	50
8 (March 1966)	Dried	3·1*	1900 [610]	520 [170]	530 [170]
	Fresh		1000	90	115
9 (July 1966)	Dried	3*	1500 [500]	650 [220]	480 [160]
	Fresh		900	200	140
10 (March, May 1967)	Dried	3·05*	850 (285]	600 [195]	390 [130]
	Fresh		400	160	120

It can be seen from Table 1 that moderate losses (25–40%) of caerulein occurred during drying of the skins. These losses do not, however, impair the general validity of the analytical data obtained. Also, it is possible to relate the caerulein concentration in dry skin to its concentration in fresh skin with reasonable accuracy.

On the other hand, a more or less considerable increase in the 5-HT and histamine contents of the skins occurred during drying. This increase, which indicates that amine biosynthesis may continue during drying of the skins, will be studied and discussed in detail elsewhere.

Caerulein and amine content of different regions of the skin of Hyla caerulea *and of skins from male and female specimens*

Table 2 shows the content of active compounds in dorsal and ventral regions of the skin from a batch of *Hyla caerulea* and the content of these compounds in skins from batches of male and female specimens.

TABLE 2

CONTENT OF CAERULEIN AND AMINES IN DIFFERENT REGIONS OF *HYLA CAERULEA* SKIN AND IN THE SKIN OF SPECIMENS OF DIFFERENT SEX

Batch (dried skins)	Content of active compounds (in μg/g dried skin)		
	Caerulein	5-HT	Histamine
6 (November 1965)			
Dorsal skin	2450	300	650
Ventral skin	300	450	80
2 (Pool 1965)			
Male	770–810	220	140
Female	770–810	220	240
3 (Pool 1966)			
Male	1350	400	300
Female	1250	500	330
7 (March 1965)			
Male	800	210	300
Female	700	350	315

It is apparent that there were no differences, related to sex, in the caerulein and amine content of the skin. There were, however, marked differences in the distribution of these compounds between the thick skin from dorsal regions of specimens and the thinner skin from ventral regions (Fig. 1). Whereas skin from ventral regions contained barely 10–13% of the caerulein and histamine present in skin from dorsal regions, it contained 50% more 5-HT than is present in skin from dorsal regions. The significance of the differences in the respective concentrations of the two amines in dorsal and ventral regions of the skin is obscure but is possibly related to their function.

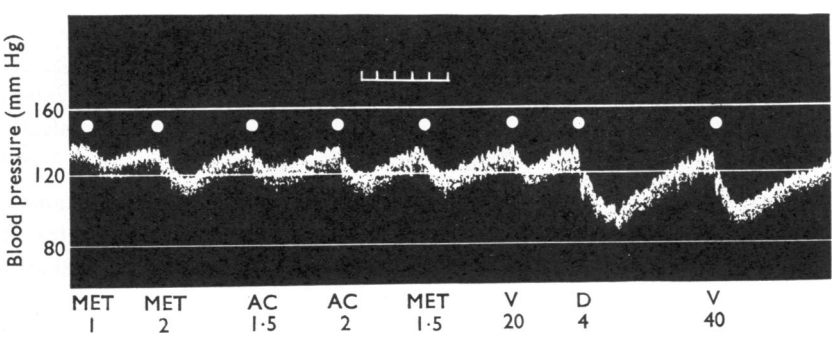

Fig. 1. Blood pressure of a dog anaesthetized with sodium pentobarbitone (30 mg/kg, intravenously) and previously treated with atropine sulphate (0.2 mg/kg intramuscularly). Time, 1 min. MET, Methanol extract, and AC, acetone extract of aliquots from the same pool of minced skins of *Hyla caerulea*. V, Extract of ventral skin, and D, extract of dorsal skin of the same specimens of *Hyla caerulea*. All doses in mg of dried tissue, intravenously. It may be seen that dorsal skin was more than 10 times as active as ventral skin and that, in this experiment, the acetone extract possessed approximately 85–90% of the activity of the methanol extract.

Content of caerulein-like peptides and content of amines in the skin of other Australian hylids

To date, the dried skins of fifteen Australian species of *Hyla*, in addition to the skins of *Hyla caerulea*, have been available for paper chromatographic and biological screening. Results are shown in Table 3.

TABLE 3

CONTENT OF CAERULEIN-LIKE POLYPEPTIDES AND OF AMINES IN THE SKINS OF DIFFERENT SPECIES OF AUSTRALIAN HYLIDS

Contents are expressed as μg of active compound per g of dried skin. Caerulein-like polypeptides are expressed as caerulein. n.d., Not detectable; —, not tested.

Species	Caerulein-like polypeptide	Indolealkylamines			Histamine
		5-HT	*N*-Methyl-5-HT	Bufotenine	
Hyla infrafrenata	850	500	n.d.	n.d.	170
Hyla rothi	160–240	40	n.d.	n.d.	n.d.
Hyla gilleni	35–40	60	n.d.	n.d.	25
Hyla chloris	90	—	—	—	n.d.
Hyla dentata	10–20	250	n.d.	750	n.d.
Hyla verreauxi	50–80	—	—	—	—
Hyla phyllochroa	n.d.	—	—	—	—
Hyla latopalmata	5–7	30	n.d.	n.d.	n.d.
Hyla bicolor	?	n.d.	n.d.	n.d.	n.d.
Hyla pearsoniana	n.d.	n.d.	n.d.	750–2500	n.d.
Hyla rubella	n.d.	20–25	n.d.	60–80	n.d.
Hyla peroni	n.d.	35–250	0–12	10–15	n.d.
Hyla lesueuri	20–30	150–200	n.d.	5	n.d.
Hyla gracilenta	15–20	100	n.d.	25	n.d.
Hyla nasuta	n.d.	0–10	n.d.	n.d.	n.d.

It is apparent from the tabulated data that caerulein-like polypeptides frequently occur in the skin of other Australian species of *Hyla*.

Hyla infrafrenata, which, on morphological grounds, is closely related to *Hyla caerulea*, closely resembled this species both with respect to the content of caerulein-like polypeptides, and also with respect to the content of amines in its skin. The skins of all the other *Hyla* species investigated possessed lower concentrations of caerulein-like polypeptides (Fig. 2). Indeed, these polypeptides could not be detected in some species. Moreover, although histamine did not seem to be present in the skins of these other *Hyla* species (with the exception of *Hyla gilleni*) 5-HT and its *N*-methylated derivatives were well represented.

Some properties of caerulein

Extraction from dried skin by methanol and acetone

Five dried skins of *Hyla caerulea* were finely minced with scissors and the minced fragments were thoroughly mixed. Part of the material was extracted twice with 80% methanol and part was extracted with 70% acetone. A similar experiment was carried out using six dried skins. Results were as follows:

	Contents of active compounds (in μg/g dried skin)		
	Caerulein	*5-HT*	*Histamine*
First experiment			
Methanol extract	950	240	420
Acetone extract	790	300	260
Second experiment			
Methanol extract	1300	230	240
Acetone extract	1200	300	180

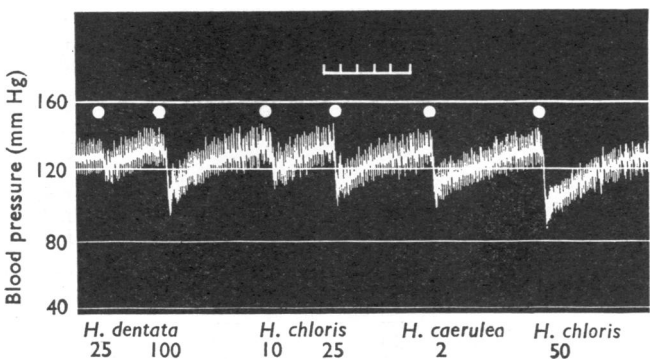

Fig. 2. Blood pressure of a dog anaesthetized with pentobarbitone after treatment with atropine. Time, 1 min. The hypotensive activity of methanol extracts of dried skins of *Hyla dentata* and *Hyla chloris* is compared with that of extracts of dried skins of *Hyla caerulea* pool 1966. All doses in mg of dried tissue, intravenously. *H. dentata* skin possessed approximately 2% and *H. chloris* skin 8% of the activity displayed by *H. caerulea* skin.

It can be seen that methanol is more suitable than acetone for the extraction of caerulein and histamine. Indeed, acetone is obviously not suitable for the extraction of histamine. On the other hand, a more complete extraction of 5-HT is given by acetone than by methanol.

Resistance to proteolytic enzymes

Chymotrypsin. The dried residue of a methanol extract of 0.1–0.2 g of dried *Hyla caerulea* skin was taken up with 2 ml. of phosphate buffer at pH 7.6 and, after addition of 100 μg of crystalline chymotrypsin, was incubated in a water bath at 38° C for 30–60 min. Chymotrypsin action was arrested by immersion of the incubation flask in boiling water for 3–5 min. Residual activity on dog blood pressure was less than 1% of the original activity.

Trypsin. A similar experiment was carried out using crystalline trypsin (1–2 mg). As much as 75–90% of the original activity remained.

Subtilisin. Incubation of 100 μg of caerulein with 5–10 μg of subtilisin for 2 hr, at 37° C and pH 8–8.5, produced a total inactivation of the polypeptide.

Results similar to those recorded above were obtained when pure caerulein was exposed to chymotrypsin or trypsin.

Treatment with diazonium salts

The addition of 0.5 ml. of an 0.2% solution of NNCD reagent in 0.1 N HCl to the residue of an extract of *Hyla caerulea* skin (0.1–0.2 g dried tissue) which had been taken up in distilled water reinforced the yellow orange colour of the liquid. At the same time, the activity of the extract on dog blood pressure was abolished. Obviously the inactivation of the material was the result of a reaction of diazonium salt with the tryptophanyl residue of the caerulein molecule. Similar results were obtained with pure caerulein (Fig. 3).

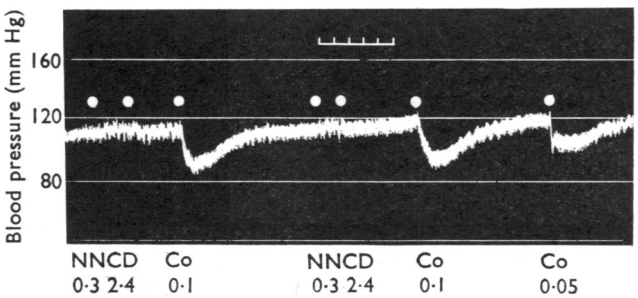

Fig. 3. Blood pressure of a dog anaesthetized with pentobarbitone after treatment with atropine. Time, 1 min. Co, Control caerulein ; NNCD, caerulein treated with the NNCD reagent. All doses in μg/kg, intravenously. The NNCD reagent produced a complete inactivation of caerulein.

Acid treatment

Treatment of a crude extract of *Hyla caerulea* skin or pure caerulein with glacial acetic acid in boiling water bath caused a prompt loss of activity in both cases. The loss was greater for pure caerulein (95–97% inactivation after 5–10 min), however, than for the crude extract (80–90% inactivation after 10–15 min). Similarly, a 95–98% inactivation of caerulein was obtained when the polypeptide was exposed to 0.1 N HCl at 100° C for 30 min.

It is likely that the observed decay in activity was caused by splitting off of the sulphate radical of the tyrosinyl residue in the caerulein molecule. Indeed, desulphated caerulein was 50–100 times less active than caerulein on the dog blood pressure (Anastasi *et al.*, 1968).

Treatment with hydrogen peroxide

Incubation at room temperature for 1–3 hr with 0.5–1% hydrogen peroxide of either crude extracts of *H. caerulea* skin or pure caerulein resulted, in both cases, in a 95–99% loss of hypotensive activity (Fig. 4). The inactivation observed probably results from the presence in the caerulein molecule of methionine which, after mild oxidation, is converted to the corresponding sulphoxide (Mutt, 1964).

Paper chromatography

Caerulein present in crude extracts of *H. caerulea* skin (0.05–0.1 g dry skin) could be visualized easily on paper chromatograms run with the *n*-butanol : acetic acid : water mixture if the NNCD reagent or the *p*-dimethylaminobenzaldehyde reagent were used as

locating reagents. With these reagents caerulein gave orange and violet spots respectively because of the presence in the caerulein molecule of the tryptophanyl residue. In the above solvent system caerulein had an R_F value of 0.19–0.22. The threshold amount of caerulein which yielded on paper appreciable colour reactions with the locating reagents used was 10–20 μg. Frequently, the caerulein spot was accompanied by minor spots which yielded the same colour reactions. Possibly these attendant spots are caused by the presence in the crude extracts of tryptophan-containing peptides related to caerulein. One of these peptides might be represented by desulphated caerulein ($R_F = 0.48$–0.54).

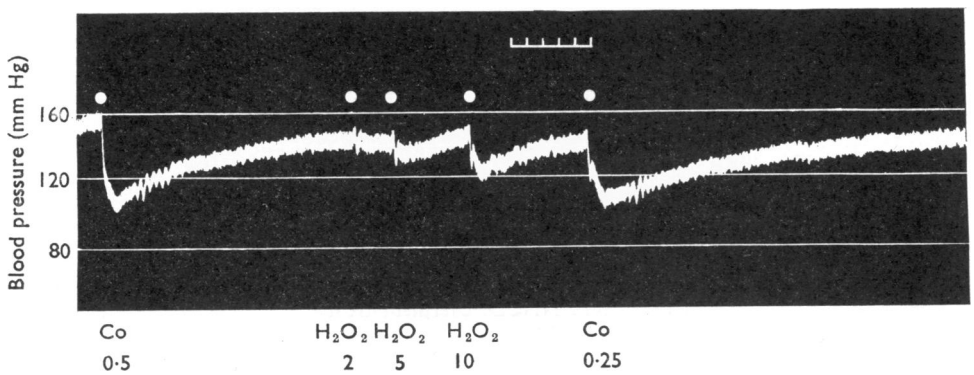

Fig. 4. Blood pressure of a dog anaesthetized with pentobarbitone after treatment with atropine. Time, 1 min. Co, Control caerulein ; H_2O_2, caerulein treated with hydrogen peroxide. All doses in μg/kg, intravenously. Hydrogen peroxide produced a nearly complete (>98%) inactivation of caerulein.

Thin-layer chromatography

In ascending thin-layer chromatography, caerulein had an R_F of 0.2 with the solvent system *n*-butanol : acetic acid : water (4 : 1 : 1) and of 0.7 with 80% aqueous ethanol (Anastasi *et al.*, 1968).

Paper electrophoresis

Because of the presence in its molecule of the tyrosinyl *O*-sulphate residue, caerulein migrated in high voltage electrophoresis towards the anode at neutral and acidic pH, its position being 0.43 relative to glutamic acid at pH 5.8 and 0.53 relative to cysteic acid at pH 1.9 (Anastasi *et al.*, 1968).

Partial purification of caerulein by passage through an alumina column

A simple procedure often resulting in good recoveries of biologically pure polypeptides involves the chromatography of crude tissue extracts on alumina followed by elution of the polypeptides with descending concentrations of ethanol. The method has proved to be very effective in the isolation of eledoisin, physalaemin and phyllokinin. In the case of caerulein, however, inconsistent and on the whole disappointing results were obtained. Recoveries of caerulein were satisfactory only in a few experiments ; they did not usually

exceed 15–25% of the caerulein present. Moreover, caerulein emerged from the column when low concentrations of ethanol were passed and was accompanied by considerable amounts of contaminants, especially amino-acids.

DISCUSSION

The skin of *Hyla caerulea* contains large amounts of caerulein, a polypeptide possessing very potent and versatile pharmacological activity. The content of caerulein in the skin may be as high as 1,000 μg/g of fresh skin. Skin from dorsal regions of the body of *H. caerulea* is much richer in caerulein than skins from ventral regions.

Approximately one-third of the caerulein present in fresh skin is inactivated during drying of the skin, probably because of the activity of proteolytic enzymes.

Although experiments designed to determine the most suitable solvent for the extraction of caerulein have not been carried out, it has been found that methanol is more effective than acetone for this purpose. Methanol has also proved more satisfactory than acetone for the extraction of other polypeptides such as eledoisin and physalaemin.

It is possible that caerulein is widely distributed among the Australian species of *Hyla*. The term caerulein should, however, be reserved for the decapeptide possessed by *Hyla caerulea* pending identification of the caerulein-like polypeptides possessed by other species of *Hyla*.

It has also been demonstrated (Erspamer and co-workers, unpublished) that caerulein-like polypeptides are present in the skins of some South American species of *Phyllomedusa* and *Leptodactylus*, a fact which has interesting implications from the viewpoints of amphibian systematics and zoogeography.

The significance of caerulein in the skin of *H. caerulea* and other Australian hylids is obscure, as is also the significance of 5-HT and the biogenic amines which accompany the polypeptides. Liberation of caerulein into the blood stream and hence participation in the regulation of secretory processes associated with the digestive tract may be reasonably excluded. It is possible that caerulein is involved in the regulation of skin permeability to water and to electrolytes, and studies designed to elucidate this possible role of the polypeptide might prove rewarding. Because of the powerful pharmacological activity exhibited by caerulein and the accompanying biogenic amines, their presence in the skin of hylids might serve as a deterrent to potential predators. In this respect is might be noted that *Hyla caerulea* is commonly found in dwellings and suburban gardens in southern Queensland yet it is rarely molested by domestic animals. Snakes, however, have been observed to devour specimens of *Hyla caerulea*.

The results described in this paper represent only a small part of the data we have gathered during studies of biogenic amines and active polypeptides in the amphibian skin. In fact, more than 300 amphibian species collected throughout the world have been subjected to screening. We hope that some credible indication of the function of amines and peptides in the amphibian skin will be found after all the data have been considered and discussed.

SUMMARY

1. The skin of *Hyla caerulea*, an Australian amphibian, contains a decapeptide, caerulein, which possesses a conspicuous hypotensive effect in the dog and other experimental animals, stimulates certain extravascular smooth muscles and displays marked activity on certain secretions associated with the digestive tract. The caerulein content of the skin of adult animals is of the order of 300–1,000 μg/g of fresh skin and of the order of 700–1,900 μg/g of dried skin. Skin from dorsal regions of the body is considerably richer in caerulein than skin from ventral regions. Both methanol and acetone may be used profitably in the extraction of the polypeptide but methanol is the more effective extracting medium. Approximately 25–40% of the activity attributable to caerulein possessed by fresh skin is lost during drying of the skin.

2. Variable amounts of caerulein or caerulein-like polypeptides are present in the skin of other Australian species of *Hyla*. Of the species examined, however, only *Hyla infrafrenata* contains in its skin concentrations of the polypeptide approaching that found in *Hyla caerulea*.

3. In the skins of *Hyla caerulea*, *Hyla infrafrenata* and *Hyla gilleni* caerulein is accompanied by variable amounts of 5-HT and histamine. Although histamine does not seem to be present in the skins of the other species of *Hyla* investigated, 5-HT and also *N*-methylated derivatives of 5-HT are well represented.

4. Caerulein is rapidly inactivated by chymotrypsin and subtilisin, as well as by treatment with acids, diazonium salts and hydrogen peroxide. However, the polypeptide is resistant to trypsin.

5. Caerulein may be visualized on paper chromatograms and pherograms by the characteristic colour reactions of tryptophan. Threshold amounts are 10–20 μg.

6. The possible significance of caerulein in the skin of Australian hylids is discussed.

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