OCCURRENCE OF SYM-HOMOSPERMIDINE IN THE JAPANESE NEWT, *CYNOPS PYRRHOGASTER PYRRHOGASTER*

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1. Introduction

During the course of study on polyamines in lower vertebrates, we encountered the presence of a prominent ninhydrin-positive peak which eluted posterior to spermidine on our amino acid analyzer. The concentration of this peak was particularly high in tissues of the Japanese red-bellied newt, *Cynops pyrrhogaster pyrrhogaster*. Using both amino acid analysis and two-dimensional paper chromatography, we tested the unknown peak in various tissues of the newt. Our results demonstrate that the peak is composed of sym-homospermidine (1,9-diamino-5-azanonane). The presence of this polyamine has not been reported in the animal world, though it was detected in leaves of sandalwood tree [1] and in some algae [2,3]. This paper describes the identification and measurement of sym-homospermidine in the newt.

2. Materials and methods

1,3-Diaminopropane, putrescine, cadaverine, spermidine and spermine were purchased from Nakarai Chemicals; 1,6-diaminohexane, and agmatine sulfate from Wako Pure Chemicals Industries; sym-nor-spermidine (1,7-diamino-4-azaheptane) and sym-nor-spermine (1,11-diamino-4,8-diazaundecane) from Eastman Organic Chemicals, Rochester, NY and histamine dihydrochloride from Merck. sym-Homospermidine (1,9-diamino-5-azanonane) and 1,9-diamino-6-azanonane were kindly supplied by Dr T. Oshima from Mitsubishi-Kasei Institute of Life Sciences, Tokyo and Mr D. F. Worth from Warner-Lambert/Parke-Davis, Detroit, respectively. All other chemicals were the purest available grades from standard commercial sources.

Adult *C. pyrrhogaster pyrrhogaster* collected in Niigata were kindly supplied by Mr S. Tanaka from our Institute of Endocrinology. Tissues from the animals were homogenized in 4 vol. 0.5 N perchloric acid (PCA) and the supernatants (300 μl) were subjected to column chromatography. Polyamine analysis was carried out by a slightly modified method of [4] as in [5]. In addition to the analytical column chromatography, two-dimensional paper chromatography was carried out to identify sym-homospermidine. The PCA extracts were first applied to a short Dowex-50 column to remove ninhydrin-positive contaminants including free amino acids [6]. Polyamines were eluted by 6 N HCl, and the eluate was evaporated to dryness in a rotary evaporatory at 60°C. The mixture of polyamines dissolved in 0.05 N HCl was analyzed by two-dimensional paper chromatography according to [7], which was used by them to identify sym-homospermidine.

3. Results

In addition to the three naturally occurring polyamines, putrescine, spermidine and spermine, a prominent ninhydrin-positive peak appeared after spermidine on column chromatograms when PCA extracts of newt testis and ovary were employed (fig.1). We have never observed such a peak in any of the mammalian tissues. This peak corresponded to

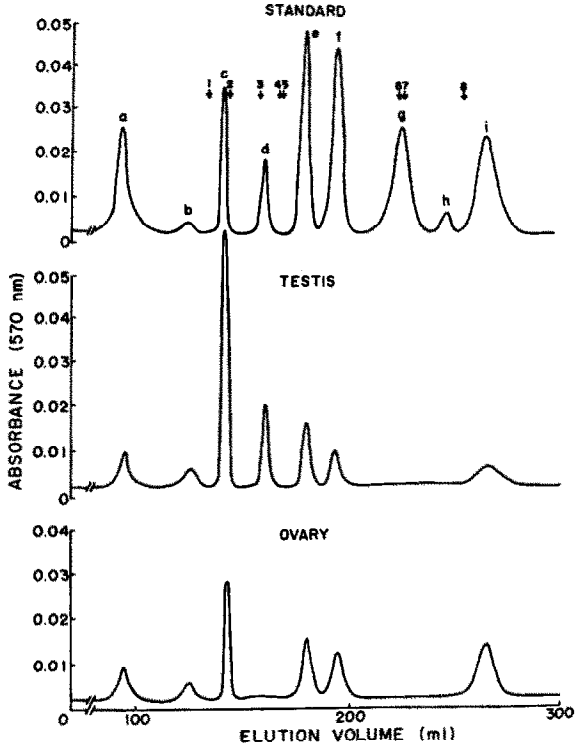

Fig.1. Elution profiles of a standard solution and PCA extracts from newt gonads. The chromatographic separation was carried out at 39.5°C using a Hitachi KLA-3B amino acid analyzer on a column (0.9 × 10 cm) of Hitachi Custom 2612. Amino acids were first eluted by the initial buffer which contained 0.35 N sodium citrate (pH 5.25) and 2% *n*-propanol. Polyamines were then eluted by the second buffer which contained 0.35 N sodium citrate (pH 5.25), 2% *n*-propanol and 2 N sodium chloride. The standard solution (Standard) contained 50 nmol each of authentic compounds. The elution peaks correspond to: (a) arginine; (b) buffer change artifact; (c) putrescine; (d) cadaverine; (e) spermidine; (f) sym-homospermidine; (g) 1,9-diamino-6-azanonane; (h) agmatine; (i) spermine. The numbers above the arrows indicate the peaks of: (1) 1,3-diaminopropane; (2) norepinephrine; (3) kanaymcin; (4) sym-norspermidine; (5) histamine; (6) 1,6-diaminohexane; (7) sym-norspermine; (8) cystamine.

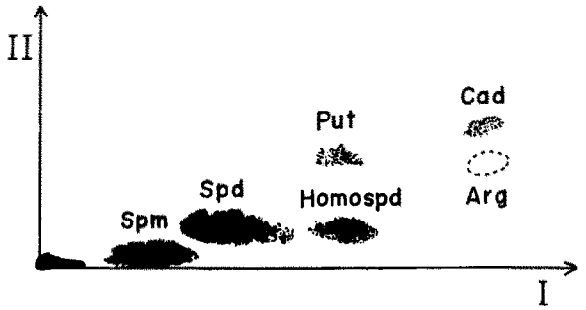

Fig.2. Polyamines in the newt testis. Solvent systems used were: phenol–0.2 M KCl–HCl buffer (pH 1) (50:7, v/v) (I) and 1-butanol–acetic acid–water (4:1:1, v/v) (II) for first and second development, respectively. Spots on paper chromatograms were visualized with a 0.4% solution of ninhydrin in acetone containing 2% collodine. Homospd, sym-homospermidine; Put, putrescine; Cad, cadaverine; Spd, spermidine; Spm, spermine, Arg, arginine.

that of authentic sym-homospermidine which was found to elute just after spermidine. None of the other authentic polyamines tested cochromatogramed with sym-homospermidine. Although we did not test, acetylpolyamines were shown to elute before putrescine [8]. The unidentified peak was not reduced after hydrolysis by 6 N HCl at 108°C, excluding the possible presence of glutathionyl-spermidine [9]. Furthermore, periodate oxidation of the extract from the newt testis yielded no products, showing that coexistence of hydroxy-polyamines was unlikely [10].

To identify the ninhydrin-positive substance, which behaved chromatographically like sym-homospermidine, condensed extracts were subjected to two-dimensional paper chromatography [7]. A typical paper chromatogram of polyamines in the newt testis is shown in fig.2. In addition to the widely occurring polyamines (putrescine, cadaverine, spermidine and spermine), one spot was found, which was identical with that of authentic sym-homospermidine. No other spots except the one near the origin were observed on chromatograms. These results showed that polyamines in the newt could be determined quantitatively by our amino acid analysis alone, although some of the polyamines did not separate adequately.

Table 1 summaries the polyamine assay of some of the newt organs. sym-Homospermidine was detected in other organs such as lung, heart, oviduct, seminiferous tubule and abdominal gland. Cadaverine content was much greater in the testis than in any other organs. A great seasonal variation was observed in the testicular content of cadaverine. There was a

Table 1
Distribution of polyamines in some organs of Japanese newts

Organ	Sex	Polyamine (μmol/g wet wt)				
		Put	Cad	Spd	Homospd	Spm
Testis	M	1.57	3.46	0.19	0.12	0.07
Ovary	F	0.69	n.d.	0.23	0.17	0.19
Liver	M	0.47	0.10	0.67	0.06	0.18
	F	0.87	0.03	0.78	0.07	0.22
Spleen	M	1.25	0.47	0.91	0.27	0.26
	F	1.38	0.18	1.03	0.23	0.33
Kidney	M	0.88	0.27	0.57	0.12	0.17
	F	0.43	n.d.	0.56	0.09	0.23
Gut	M	1.08	0.25	0.70	0.06	0.19
	F	0.84	0.08	0.29	0.07	0.10
Brain	M	0.25	n.d.	0.27	0.09	0.26
	F	0.23	n.d.	0.34	0.07	0.21

M, male, F, female, n.d., not detectable (<0.01 μmol/g). Adult newts were collected in Nov. 1977

clear sex difference in cadaverine content of liver, spleen, kidney and gut. No other rare polyamines such as sym-nor-spermidine and sym-nor-spermine were detected in any of the organs examined including the gut with its contents.

Both sym-homospermidine and cadaverine were also detected in some tissues of other amphibians, such as *Xenopus laevis*, *Rana brevipoda porosa*, *Hyla arborea japonica* and *Hynobius*, the details of which will be presented elsewhere.

4. Discussion

The results indicate that the unknown compound on our chromatograms of newt tissues is sym-homospermidine (1,9-diamino-5-azanonane). To our knowledge, occurrence of this polyamine has not been reported in lower vertebrates. This polyamine was first isolated from leaves of sandalwood tree [1] and later detected in various algae [2,3]. Recently new polyamines such as sym-nor-spermidine and sym-nor-spermine have been reported in thermophilic bacteria [11,12], in the white shrimp [13] and in arthropods [14]. However, neither of them, nor sym-homospermidine has been reported in any tissues of vertebrates. Since this polyamine was found not only in the gut but in all other tissues examined and detected even in *Xenopus laevis* reared in our Institute feeding pellet chow for trout, it is highly possible that it is synthesized in amphibian tissues but not of exogenous origin. One cannot exclude, however, the possibility that this polyamine is derived from diet and/or is formed by the intestinal flora. These possibilities are currently under investigation.

Since cadaverine was detected in appreciable amounts in the newt, one would expect biosynthesis of 1,9-diamino-6-azanonane by the action of amino-propyltransferase as shown in *Escherichia coli* [15,16]. However, the latter polyamine was not detected in our study. It is tempting to speculate that cadaverine plays some specific role in reproduction of amphibians. Further studies are needed to clarify physiological significance of cadaverine and sym-homospermidine as well as other polyamines in amphibians.

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References

[1] Kuttan, R., Radhakrishnan, A. N., Spande, T. and Witkop, B. (1971) Biochemistry 10, 361–365.
[2] Kneifel, H. (1977) Chemiker-Zeitung 101, 165–168.
[3] Rolle, I., Hobucher, H.-E., Kneifel, H., Paschold, B., Riepe, W. and Soeder, C. J. (1977) Anal. Biochem. 77, 103–109.
[4] Kremzner, L. T. (1973) in: Polyamines in Normal and Neoplastic Growth (Russell, D. H. ed) pp. 27–40, Raben Press, New York.
[5] Matsuzaki, S., Kakegawa, T., Suzuki, M. and Hamana, K. (1978) Endocrinol. Japon. 25, 129–139.
[6] Inoue, H. and Mizutani, A. (1973) Anal. Biochem. 56, 408–416.
[7] Subramanian, N. and Rao, M. V. L. (1955) J. Sci. Ind. Res. 14C, 56–58.
[8] Tabor, H. and Tabor, C. W. (1973) Anal. Biochem. 55, 457–467.
[9] Tabor, C. W. and Tabor, H. (1970) Biochem. Biophys. Res. Commun. 41, 232–238.

[10] Rosano, C. L., Hurwitz, C. and Bunce, S. C. (1978) J. Bacteriol. 135, 805–808.
[11] Oshima, T. (1975) Biochem. Biophys. Res. Commun. 63, 1093–1098.
[12] De Rosa, M., De Rosa, S. and Gambacorta, A. (1976) Biochem. Biophys. Res. Commun. 69, 253–261.
[13] Stillway, L. W. and Walle, T. (1977) Biochem. Biophys. Res. Commun. 77, 1103–1107.
[14] Zappia, V., Porta, R., Carteni-Farina, M., De Rosa, M. and Gambacorta, A. (1978) FEBS Lett. 94, 161–165.
[15] Dion, A. S. and Cohen, S. S. (1972) Proc. Natl. Acad. Sci. USA 69, 213–217.
[16] Bowman, W. H., Tabor, C. W. and Tabor, H. (1973) J. Biol. Chem. 248, 2480–2486.