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OCCURRENCE IN HIGH CONCENTRATIONS OF N^1-ACETYLSPERMIDINE AND *sym*-HOMOSPERMIDINE IN THE HAMSTER EPIDIDYMIS

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SUMMARY: In mature hamster epididymis several unknown peaks were observed on our high-performance liquid chromatograms in addition to the common polyamines, putrescine, spermidine and spermine. Three of the peaks were identified as N^1-acetylspermidine, N^1-acetylspermine and *sym*-homospermidine by means of thin-layer chromatography, gas chromatography-mass spectrometry and acid hydrolysis. The concentrations of N^1-acetylspermidine and *sym*-homospermidine were highest in the distal caput epididymidis among epididymal regions studied. This is the first report to show that *sym*-homospermidine occurs in mammalian tissues.

Polyamines such as putrescine, spermidine and spermine have been found in almost all mammalian tissues and are thought to be involved in various cellular processes (1, 2). Other polyamines such as *sym*-homospermidine, norspermidine and norspermine have never been detected in mammalian tissues. Cellular levels of acetylated polyamines are quite low or undetectable, although the acetylation of polyamines has been observed in lymphocytes (3), the Chinese hamster ovary (4), the rat liver (5-6) and the rat kidney (7) under varied conditions. Acetylpolyamines appear to be detectable only in situations where polyamine degradation is greatly enhanced (8).

In the experiments described in this paper, we observed in the hamster epididymis several basic compounds other than the three common polyamines, putrescine, spermidine and spermine. We report here direct evidence for the occurrence of acetylpolyamines and *sym*-homospermidine in the sexually mature hamster epididymis.

MATERIALS AND METHODS

Chemicals

N^1- and N^8-Acetylspermidine were kindly supplied by Dr. T. Nakajima from Osaka University and *sym*-homospermidine by Dr. T. Oshima from

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Mitsubishi-Kasei Institute of Life Sciences. N-Acetylputrescine and N^1-acetylspermine were synthesized in our laboratories according to the method of Dubin and Rosenthal (9). 2-Hydroxyputrescine was synthesized by ammoniolysis of 1,4-dibromo-butan-2-ol (10). Norspermidine and norspermine were purchased from Eastman Organic Chemicals and acetyl-1-^{14}C CoA was from New England Nuclear.

Extraction and isolation of polyamines and other basic compounds

Male hamsters were reared under a long daily photoperiod (16 hr light : 8 hr darkness). Epididymides from hamsters of various ages were homogenized in 4 volumes of 0.5 N $HClO_4$ after being washed in a saline solution. After centrifugation, the supernatants were applied on a Dowex 50 W column to remove free amino acids and to concentrate polyamines (11). The eluate with 6 N HCl was evaporated to dryness under reduced pressure. The residue was reconstituted in a minimum amount of distilled water and 200 µl aliquots were loaded on a column (9 x 120 mm) of cation-exchange resin (Hitachi Custom 2612). Polyamines were eluted and separated utilizing 0.35 N citrate buffer (pH 5.25) containing 1.6 M potassium chloride and 2% n-propanol. Some samples were hydrolyzed in the presence of 6 N HCl at 110°C for 16 hr before or after this chromatographic separation.

Identification of polyamines and unknown substances

Polyamines were analyzed by high-performance liquid chromatography using cation-exchange resin (62210F, Kyowa Seimitsu, Co. Ltd., Tokyo). The buffers used were 0.35 N potassium citrate buffer containing 1) 0.5 M KCl (pH 5.55), 2) 2.1 M KCl (pH 5.63) or 3) 2.4 M KCl (pH 5.73). The elution pattern of the amines was followed by o-phthalaldehyde. With this method, amounts as little as 5 pmoles can be detected.

The identity of acetylpolyamines was confirmed by thin-layer chromatography on silica gel G (12). The solvent system used was chloroform-methanol-ammonium hydroxide (2:2:1, v/v). Authentic compounds were run simultaneously and their location was determined by spraying the plate with 0.1% ninhydrin in 80% ethanol-20% acetic acid. The polyamine acetyltransferase activity of the epididymis was determined after Matsui and Pegg (5).

The presence of *sym*-homospermidine was confirmed by gas chromatography spectrometry. The N-trifluoroacetyl derivatives of polyamines were prepared as described by Gehrke *et al.* (13). The mass spectra of the polyamine derivatives were measured using a JEOL JMS-DX 300 gas chromatograph-mass spectrometer.

RESULTS

The analysis by high-performance liquid chromatography of crude extracts of the hamster epididymis demonstrated the presence of two unknown components (X1 and X2) in addition to putrescine, spermidine and spermine (Fig. 1, A). Upon acid hydrolysis, X1 disappeared and the spermidine peak increased in height, while the X2 peak remained unchanged (Fig. 1, B). None of the unknown peaks corresponded to 2-hydroxyputrescine, N-acetylputrescine, cadaverine, agmatine, norspermidine or norspermine on the chromatograms. After separation by cation-exchange resin (Hitachi Custom 2612), unknown peak fractions were collected and separately analyzed by high-performance liquid

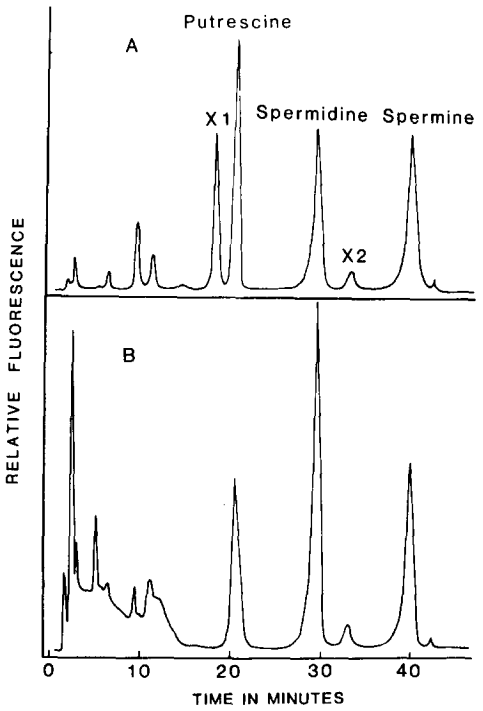

Fig. 1. Separation of polyamines from hamster epididymis by high-performance liquid chromatography. Samples were pretreated with Dowex 50 W to remove most of the amino acids in $HClO_4$ extracts. Polyamines were assayed before (A) and after (B) hydrolysis in 6 N HCl.

chromatography and thin-layer chromatography. The analysis of the fractions containing X1 peak after acid hydrolysis revealed the production of spermidine, showing that the peak contained N^1- and/or N^8-acetylspermidine. Thin-layer chromatography of the X1 peak fractions (Nos. 10 and 11) showed that the peak was identical to N^1-acetylspermidine and that no measurable N^8-acetylspermidine was present (Fig. 2). The regional distribution of this compound was highest in distal caput epididymidis from 60- and 200-day old hamsters (Fig. 3).

The incorporation of acetyl-1-^{14}C CoA into spermidine and spermine was detected in the cytosol fraction of both caput and cauda epididymidis; the rate of acetylation of spermidine was several times greater than that of spermine (Table 1). The activity of polyamine acetyltransferase was much lower in hamster liver than in the epididymis.

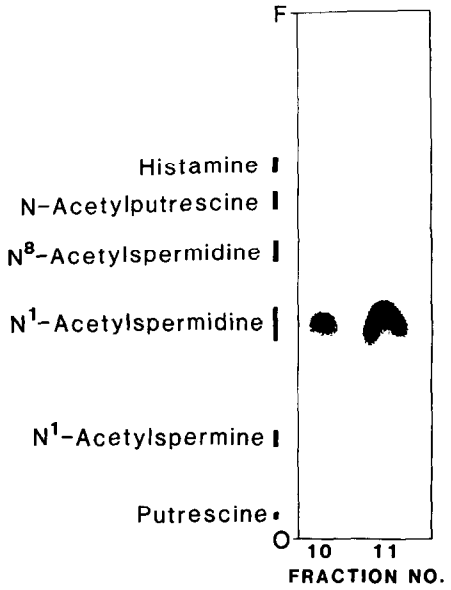

Fig. 2. Thin-layer chromatography on silica gel G of X1 fractions. O, origin; F, solvent front.

The unknown peak X2 behaved chromatographically like authentic *sym*-homospermidine. The eluate containing this component was desalted and analyzed by gas chromatography-mass spectrometry. The identity of

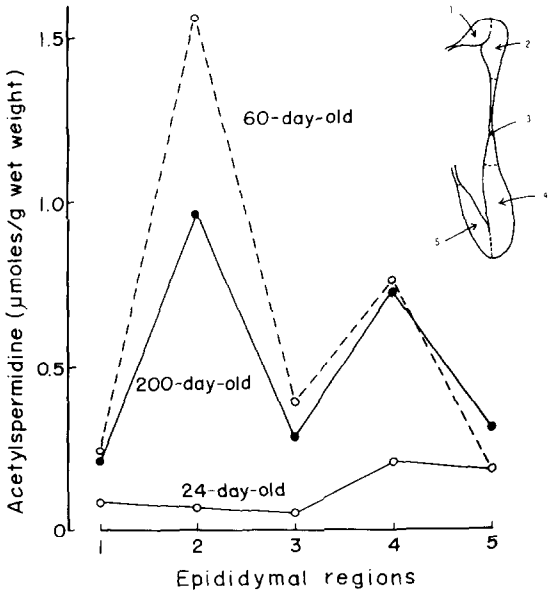

Fig. 3. Regional distribution of N^1-acetylspermidine in hamster epididymidis. 1, proximal caput; 2, distal caput, 3, corpus; 4, proximal cauda and 5, distal cauda.

TABLE I

Polyamine acetyltransferase activity in hamster epididymis

Enzyme source	Age (days)	No. of assay	Acetyltransferase activity (d.p.m/10 min incubation)	
			Spermidine	Spermine
Caput	31	5	217.6 ± 10.7	48.3 ± 5.9
	74	5	97.5 ± 12.6	30.7 ± 10.9
Cauda	31	5	182.0 ± 10.8	110.8 ± 13.4
	74	5	107.2 ± 19.2	44.7 ± 18.5

The reaction mixture contained 3 mM spermidine or spermine, 20 µl of cytosol fraction, 100 mM Tris-HCl, pH 7.8 and 50 nCi [^{14}C] acetyl-CoA (8.8 µM) in a total volume of 0.1 ml. Data are shown as means ± S. E. M.

this substance was shown to be *sym*-homospermidine (Fig. 4).

Several other minor peaks were found in some samples. Peak fractions corresponding to the unknown compounds were further analyzed by high-performance liquid chromatography and thin-layer chromatography before and after acid hydrolysis. The peak eluted between putrescine and spermidine was identified as N^1-acetylspermine. Histamine was also identified as one of the constituents of the epididymis.

DISCUSSION

The present study has shown that N^1-acetylspermidine and *sym*-homospermidine occur in quite high concentrations in hamster epididymis. In normal mammalian tissues the concentrations of acetylpolyamines are very low or under the limits of detection (3-8). The hamster epididymis appears to be a good model in which polyamine metabolism via acetylated forms can be evaluated. Possibly the rate of spermidine acetylation is much greater than that of N^1-acetylspermidine degration in hamster epididymis. N^1-Acetylspermidine is known to be converted to putrescine by polyamine oxidase (8).

The occurrence of *sym*-homospermidine has never been reported in mammalian tissues, though it has been found in amphibian and reptile tissues (14, 15) as well as in other organisms (16-19). We were not

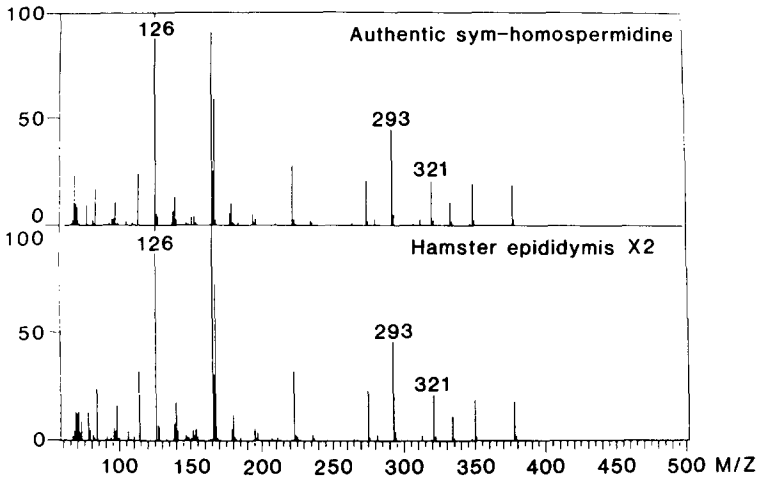

Fig. 4. Mass spectra of authentic *sym*-homospermidine and the unknown compound X2 in the epididymis.

able to exclude the possibility that this polyamine was of exogenous origin and concentrated in the epididymis. However, *sym*-homospermidine was found to be present only in trace amounts in the hamster testis. No detectable amount of this polyamine was found in the pellet chow for the hamster; only putrescine, cadaverine, spermidine and spermine were detected. These results raise the possibility that *sym*-homospermidine is actually formed in the hamster epididymis.

It is interesting to postulate that homospermidine and/or acetylpolyamines play some specific role(s) in the maturation or mobility of spermatozoa in the epididymis. Recently acetylated polyamines were shown to induce ornithine decarboxylase in rat HTC cells (20). The physiological significance of these compounds in the hamster epididymis still remains to be clarified.

ACKNOWLEDGMENTS

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