Accumulation of N^1-acetylspermidine in heart and spleen of isoprenaline-treated rats

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N^1-Acetylspermidine is not detectable in rat heart, but its content greatly increases after a single injection of isoprenaline (10 mg/kg), reaching a concentration of about 10 nmol/g of tissue 4 h after the treatment. Part of the accumulated N^1-acetylspermidine was split to putrescine. Isoprenaline also caused an increase of N^1-acetylspermidine in the spleen, where its concentration increased 3.5-fold 6 h after the catecholamine. The accumulation of N^1-acetylspermidine was dependent on the dose of isoprenaline in both the heart and the spleen, and was strongly inhibited by β-antagonists and inhibitors of protein sythesis.

INTRODUCTION

The synthesis of N^1-acetylspermidine in vertebrates is known to be enhanced by toxic agents [1–7], but there is also evidence that spermidine acetylation may be part of the physiological response of a tissue to hormonal stimulation. Spermidine/spermine N^1-acetyltransferase activity is increased in target tissues by growth hormone [2], secretin [8], insulin and glucagon [9], and $1\alpha,25$-dihydroxycholecalciferol [10]. The formation of N^1-acetylspermidine is the limiting step in the polyamine-interconversion pathway [11] and may have a role in the regulation of polyamine concentrations [12, 13]. However, the exact role of spermidine acetylation is not yet fully understood.

Polyamine synthesis and content in the mammalian heart are affected by numerous factors that influence cardiac function and growth [14]. Heart ODC activity may be elevated via sympathetic stimulation by various drugs and stress conditions causing accumulation of putrescine, and several studies have focused on the relationship between increases in polyamine concentration and cardiac hypertrophy [14]. However, there is no information about the factors that affect spermidine acetylation in the heart. In the present study, we report that the β-adrenergic agonist isoprenaline causes the accumulation of N^1-acetylspermidine in the rat heart. A similar effect was also found in the spleen, where polyamine synthesis is activated by adrenergic stimulation [15].

MATERIALS AND METHODS

Female Wistar rats (weighing 200–250 g) were treated with a single intraperitoneal injection of DL-isoprenaline dissolved in 0.9% NaCl at the doses indicated in the text, whereas the control rats received the vehicle only. Similarly all other injections were intraperitoneal, with 0.9% NaCl as the solvent. The rats were killed at various times after treatment and the organs were rapidly removed, rinsed in chilled 0.9% NaCl, blotted and

homogenized (glass/glass apparatus) in 4 vol. of 0.3 M-$HClO_4$. The homogenates were centrifuged at 10000 g for 30 min. N^1-Acetylspermidine and polyamine concentrations in the acid extracts were measured by reversed-phase h.p.l.c. after reaction with dansyl chloride and with 1,8-diamino-octane as internal standard. A detailed description of sample preparation and chromatographic analysis is given in [16].

For studying the metabolism of labelled spermidine in vivo in the heart, the rats were given 10 μCi (117 nmol) of [tetramethylene-^{14}C]spermidine 2 h after isoprenaline or saline. The animals were killed 2 h later and the hearts were processed as described for acetylspermidine analysis. The acid extract (5 ml) was adjusted to pH 12–13 with 3 M-NaOH, and the polyamines were extracted with 2×4 vol. of water-saturated butan-1-ol. The organic phase was evaporated and the residue dissolved in 0.2 ml of water. After dansylation, a quantity corresponding to 0.5 g of tissue was injected into the chromatograph for polyamine separation [16]. The column eluate was collected in 0.25 ml fractions, to which 5 ml of Aquasure was added for radioactivity counting. The recoveries of polyamines by this procedure, determined by adding [^{14}C]putrescine, [^{14}C]spermidine and unlabelled N^1-acetylspermidine to an acid extract obtained from the heart of a control rat (which does not contain endogenous detectable N^1-acetylspermidine), were $68 \pm 4\%$, $61 \pm 4\%$ and $56 \pm 5\%$ respectively (means $\pm$ S.D.; $n = 6$). ODC activity was determined as in [17].

Polyamines, acetyl-polyamines, DL-isoprenaline, actinomycin D, cycloheximide and propanolol were from Sigma. [tetramethylene-^{14}C]Spermidine (85.2 Ci/mol) and Aquasure were from New England Nuclear, and solvents for h.p.l.c. were Aldrich products.

RESULTS

When the acid extracts from the hearts of rats which had been treated with 10 mg of isoprenaline/kg body wt. were analysed for polyamine content by reversed-phase h.p.l.c., a peak appeared in the chromatograms with the

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Abbreviations used: ODC, ornithine decarboxylase (EC 4.1.1.17); dansyl-, 5-dimethylaminonaphthalene-1-sulphonyl-; DFMO, α-difluoromethylornithine.

same retention time as bidansyl-N^1-acetylspermidine. The peak disappeared upon acid hydrolysis of the extract, and, when the compound was collected and analysed by two-dimensional silica-gel t.l.c. by a procedure highly specific for the separation of dansyl derivatives of acetyl-polyamines [3], about 90% of the fluorescence co-migrated in the position of authentic N^1-acetylspermidine. The material present in the peak also gave one major spot with the mobility of

N^1-acetylspermidine on t.l.c. with chloroform/propan-2-ol (10:1, v/v) as solvent.

Direct evidence about the formation of N^1-acetylspermidine in the heart was obtained by administering a tracer dose of [^{14}C]spermidine (10 μCi) to rats 2 h after they had been treated with isoprenaline (10 mg/kg); 2 h later the rats were killed, and the radioactivity present in the polyamine pool of the hearts was determined as described in the Materials and methods section. A peak
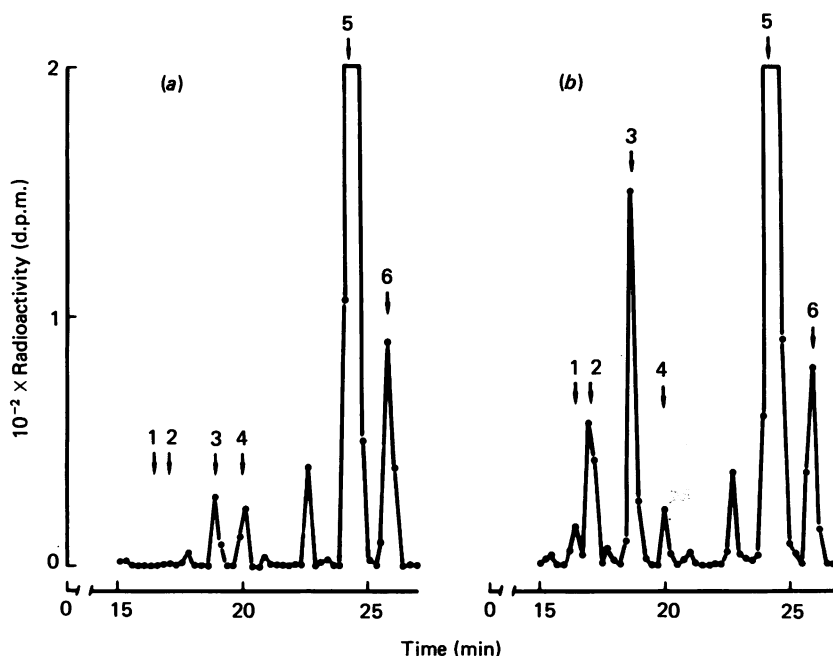

Fig. 1. Metabolism of exogenous [^{14}C]spermidine in the rat heart

Control (a) and isoprenaline (10 mg/kg)-treated rats (b) received 10 μCi of [^{14}C]spermidine 2 h after treatment. The radioactivity in the polyamine fractions separated by h.p.l.c. was measured 2 h later. The numbers indicate the positions of polyamines as determined by fluorescence markers: 1, N^8-acetylspermidine; 2, N^1-acetylspermidine; 3, putrescine; 4, cadaverine; 5, spermidine; 6, spermine. Peaks 3 and 4 in (a) represent a very small contamination of the [^{14}C]spermidine preparation.
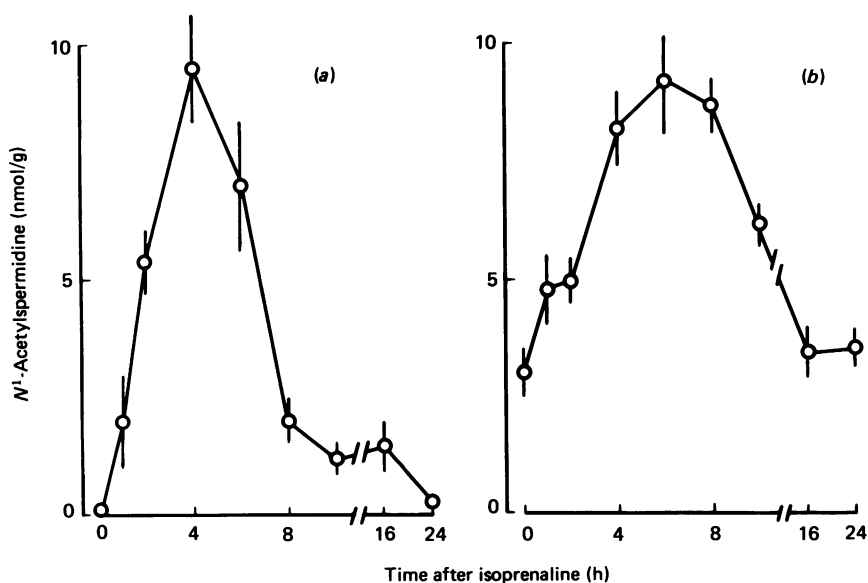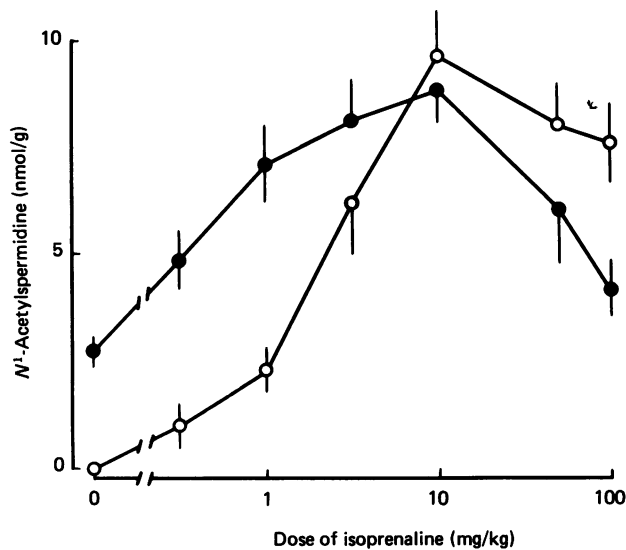

Fig. 2. Effect of isoprenaline on N^1-acetylspermidine concentration in rat heart (a) and spleen (b)

Rats were treated with isoprenaline (10 mg/kg), and the content of N^1-acetylspermidine was determined at the times shown. Results are means ± s.e.m. for four determinations.

Table 1. Effect of DFMO on putrescine accumulation in the heart of isoprenaline-treated rats

DFMO was administered by intraperitoneal injection of 200 mg/kg body wt. 1 h before isoprenaline (10 mg/kg). Polyamine concentrations were measured 2 and 4 h after isoprenaline treatment. Results are means ± s.e.m. for four rats; n.d., not detected.

Treatment	Polyamine (nmol/g)	
	N^1-Acetylspermidine	Putrescine
Control	n.d.	7.1 ± 1.0
Isoprenaline 2 h	5.8 ± 0.6	17.3 ± 2.2
Isoprenaline + DFMO 2 h	5.5 ± 0.4	8.8 ± 0.6
Isoprenaline 4 h	10.3 ± 1.3	33.7 ± 4.1
Isoprenaline + DFMO 4 h	8.2 ± 0.9	12.2 ± 1.7

Fig. 3. Effect of the dose of isoprenaline on N^1-acetylspermidine accumulation in rat heart and spleen

N^1-Acetylspermidine was measures in the heart (○) and in the spleen (●) 4 h after isoprenaline treatment. Results are means ± s.e.m. for three determinations.

of radioactivity was found in the extracts obtained from the rats treated with isoprenaline in the position of N^1-acetylspermidine (Fig. 1). The putrescine peak was also labelled in these animals, showing that the conversion of spermidine into putrescine was effective.

Fig. 2(a) shows the time course of N^1-acetylspermidine content in the heart after a single injection of isoprenaline (10 mg/kg). The concentration of N^1-acetylspermidine, which was not detectable in the control heart, increased to a maximum after 4 h and thereafter declined rapidly. The increase in acetyl-spermidine preceded the peaks of ODC activity and putrescine concentration, which were at 6 and 8 h respectively (results not shown).

In order to distinguish between the putrescine formed *de novo* by ODC and that coming from spermidine degradation, rats were treated with DFMO, a potent irreversible inhibitor of ODC, plus isoprenaline. ODC induction was abolished by DFMO, preventing the synthesis of putrescine from ornithine, and 4 h after isoprenaline the concentration of putrescine only increased by about 70%, compared with the 450% increase in the absence of DFMO (Table 1). This shows that putrescine synthesis was chiefly due to ODC activity, even when N^1-acetylspermidine reached its maximum concentration.

Isoprenaline also caused an accumulation of N^1-acetylspermidine in the spleen (Fig. 2b). Both N^1- and N^8-acetylspermidine have been found in the spleen of normal rats [4, 16], but only the concentration of the N^1 isomer was substantially enhanced by the treatment.

The extent of the accumulation of N^1-acetylspermidine caused by isoprenaline in both the heart and spleen was dose-dependent, as shown in Fig. 3, and the acetyl-polyamine enhancement was evident even with the lowest dose tested (0.3 mg/kg). Doses higher than 10 mg/kg did not cause a further rise in the acetyl-polyamine concentration.

Table 2. Effect of propranolol, cycloheximide and actinomycin D on N^1-acetylspermidine content in heart and spleen of isoprenaline-treated rats

Propranolol (20 mg/kg), cycloheximide (20 mg/kg) or actinomycin D (2 mg/kg) were given respectively 30 min, 10 min and 60 min before isoprenaline. N^1-Acetylspermidine was measured 4 h after isoprenaline. Results are means ± s.e.m. for four rats; n.d., not detected.

Treatment	N^1-Acetylspermidine (nmol/g)	
	Heart	Spleen
Control	n.d.	2.8 ± 0.3
Isoprenaline	9.3 ± 1.1	8.9 ± 0.8
Isoprenaline + propranolol	1.0 ± 0.4	2.2 ± 0.5
Isoprenaline + actinomycin D	2.2 ± 0.6	8.8 ± 1.3
Isoprenaline + cycloheximide	n.d.	1.3 ± 0.2

The β-antagonist propranolol and cycloheximide strongly inhibited the increase of N^1-acetylspermidine in both the heart and spleen, whereas actinomycin D was very effective at inhibiting N^1-acetylspermidine accumulation in the heart, but not in the spleen (Table 2).

DISCUSSION

Isoprenaline causes a β-receptor-mediated induction of ODC activity, with subsequent polyamine accumulation in the heart [18, 19]. We have found that N^1-acetylspermidine also greatly increases in response to the catecholamine. The increase in N^1-acetylspermidine is very rapid and precedes the increases in ODC activity and putrescine. Generally, acetylation of spermidine causes an increase in putrescine concentration via the acetylase/oxidase pathway, and it is interesting that isoprenaline produces an active conversion of spermidine into putrescine in the parotid gland [20]. The catecholamine also causes an activation of the conversion of spermidine into putrescine in the heart; however, when the synthesis of putrescine de novo from ornithine was prevented by DFMO, the increase in putrescine was no more than 20% of that in the absence of the ODC inhibitor, at both 2 and 4 h after isoprenaline. Therefore, spermidine degradation is only responsible for a small part of the putrescine formed during this time.

The activity of polyamine oxidase is very low in the heart compared with other tissues [21], and this fact might limit polyamine interconversion. On the other hand, it appears from the data of Fig. 1 and Table 1 that a considerable amount of the N^1-acetylspermidine formed within 4 h after isoprenaline treatment is converted into putrescine, suggesting that also the limited availability of N^1-acetylspermidine (maximum content of about 10 nmol/g) may be responsible for the low extent of polyamine interconversion. In this respect, the use of recently developed inhibitors of polyamine oxidase [22, 23] should be very useful and should allow a precise determination of the actual amount of N^1-acetylspermidine that is formed and degraded.

A series of events are necessary for N^1-acetylspermidine accumulation (Table 2), i.e. isoprenaline binding to β-receptors, DNA transcription (at least in the heart) and protein synthesis. These data suggest that the catecholamine acts by inducing a protein responsible for the formation of N^1-acetylspermidine, probably the cytosolic spermidine N^1-acetyltransferase described in other tissues [1, 2, 5–10].

N^1-Acetylspermidine concentration is also increased by isoprenaline in the spleen, but here the accumulation of N^1-acetylspermidine is slower than it is in the heart. Furthermore, actinomycin D does not inhibit the acetyl-polyamine enhancement in the spleen, suggesting that different mechanisms may be involved in the regulation of N^1-acetylspermidine formation in the heart and spleen.

Isoprenaline and other catecholamines exert large effects on some functions of the spleen, especially the capacity of storing and releasing blood cells, through their action on the capsula and the vascular system of the spleen [24]. Catecholamines can also elicit a large increase in ODC activity and putrescine concentration in this organ [15]. Therefore it seems that N^1-acetylspermidine formation may be part of the general response of target tissues to β-receptor stimulation, as well as ODC induction [25]. The significance of this response and the possible correlation with cell growth at present remain unknown, but a linkage with hypertrophy development is possible.

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