EFFECT OF SPERMINE ON FREE NUCLEOTIDE TURNOVER IN CHICK EMBRYO

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Received 16 January 1971

1. Introduction

Spermine and spermidine are aliphatic polyamines present in animal and plant tissues [1–3]. The exact biological function of these compounds is not yet known but much additional knowledge on their involvement in various biological systems is available. It has been shown that polyamines are essential growth factors for several microorganisms [4, 5], e.g. *Haemophilus parainfluenzae* and *Neisseria perflava*, and they stimulate the development of animal [6] and plant [7] culture cells. Some in vitro experiments showed that polyamines have a stabilizing effect [8] on cellular and subcellular components and nucleic acids, because of their strong polycationic nature. On the other hand, more recent studies indicate that polyamines exert a metabolic action. It has been demonstrated, under several experimental conditions, that polyamines play a role in nucleic acid accumulation [9]. Caldarera and Moruzzi [10, 11] have demonstrated a relationship between polyamines and nucleic acid metabolism of subcellular fractions in chick embryo. They have observed an increase of incorporation of labelled precursors into nucleic acids when the polyamine level was experimentally increased. Under the same experimental conditions, a considerable increase in the total free nucleotide pool was also observed [12]. All previous statements led us to study the relationships between polyamines and acid soluble free nucleotides which are involved both in cellular metabolism and nucleic acid biosynthesis.

2. Materials and methods

Spermine tetrahydrochloride was supplied by Fluka A.G., Buchs, Switzerland; sodium ^{3}H-formate (specific activity 210 mCi/mmole) and 6-^{14}C-orotic acid (specific activity 60.8 mCi/mmole) were obtained from the Radiochemical Centre, Amersham, England. Embryos, at the 8th day of incubation, were obtained from White Leghorn × New Hampshire fertilized eggs purchased from a commercial source and incubated at 39° at a relative humidity of 65% with forced air circulation.

^{14}C-Orotic acid (2.5 μCi) and sodium ^{3}H-formate (20 μCi), in a final volume of 0.2 ml, were injected into the air space; 90 min later, different doses of spermine were injected in the same way, and embryos were killed 45 min later. In another experiment, the most effective dose of spermine was injected 90 min after the supply of labelled precursors, and was allowed to act for different periods of time. At the end of these periods, embryos were freed from the extra-embryonic membranes and yolk sac and killed by chilling in liquid air. The free nucleotides were extracted and separated as described previously [13]. The nucleotide fractions were separated, combined, dried completely in a vacuum oven at 70° and dried samples were redissolved at room temperature in 0.5 ml of water. Finally, 10 ml of a liquid scintillator [14] was added and the radioactivity was measured in a Philips scintillation spectrometer.

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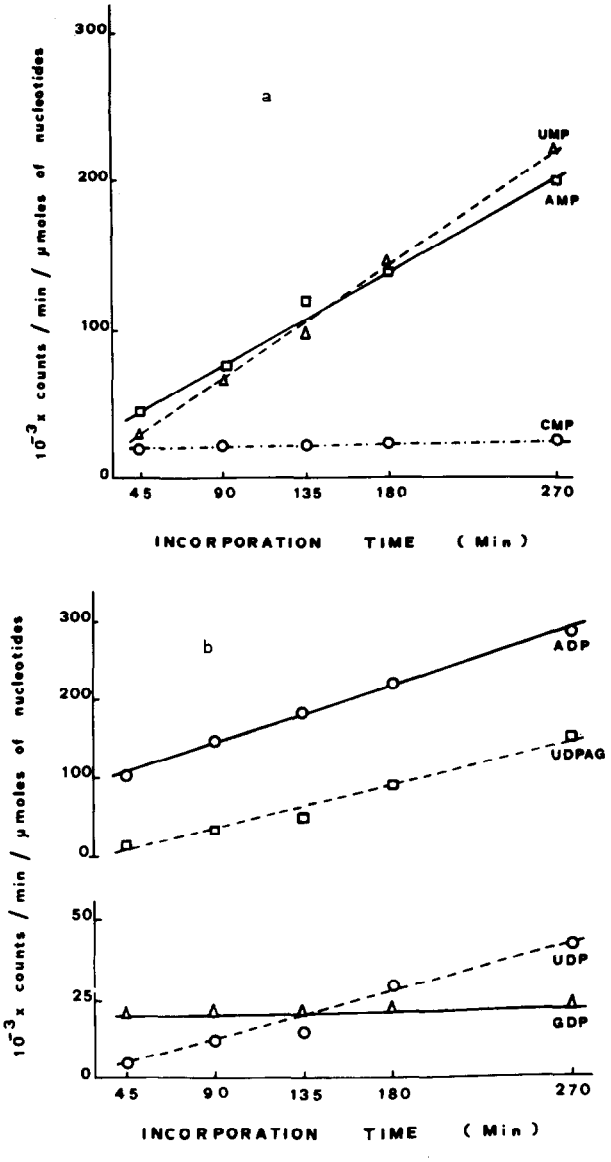

Fig. 1. Rate of incorporation of ^{14}C-orotic acid and ^{3}H-formate in the free nucleotides of the chick embryo. (a) Nucleoside monophosphates; (b) nucleoside diphosphates; (c) nucleoside triphosphates.

3. Results and discussion

We have studied the turnover of acid-soluble free nucleotides in chick embryos throughout the incorporation of labelled precursors, ^{14}C-orotic acid and ^{3}H-formic acid. Fig. 1 shows the results expressed as specific activity of the free nucleotides of chick embryo, measured at different times after injection of labelled precursors. Uridylic and adenylic nucleotides show a higher specific activity than cytidylic and guanylic nucleotides. This can be related to the different metabolic conditions of the chick embryo at this stage of development.

Fig. 2 shows the result of different doses of spermine (50, 250, 500 nmoles) on the incorporation of the labelled precursors into purine and pyrimidine nucleotides. Small doses of polyamine produce an appreciable increase in the incorporation rate, this increase reaches a maximum with 250 nmoles polyamine and the effect is again smaller after injection of higher doses of spermine. This latter effect may be because the polyamine injected in high concentrations is rapidly oxidized to aminoaldehydes which are known to be toxic [15]. Finally we have considered the effect of spermine on the incorporation of labelled precursors into the free nucleotide pool for different periods of time after injection. Table 1 shows that the effect of spermine was greatest 45 min after injection. The increase of specific activity was greatest for nucleoside triphosphates, particularly for CTP (+248%) and UTP (+326%). The general and progressive decline of specific activity observed if the embryos were killed after a longer time than 45 min after injection of spermine may be related to the hypothesis that amine oxidase activity is

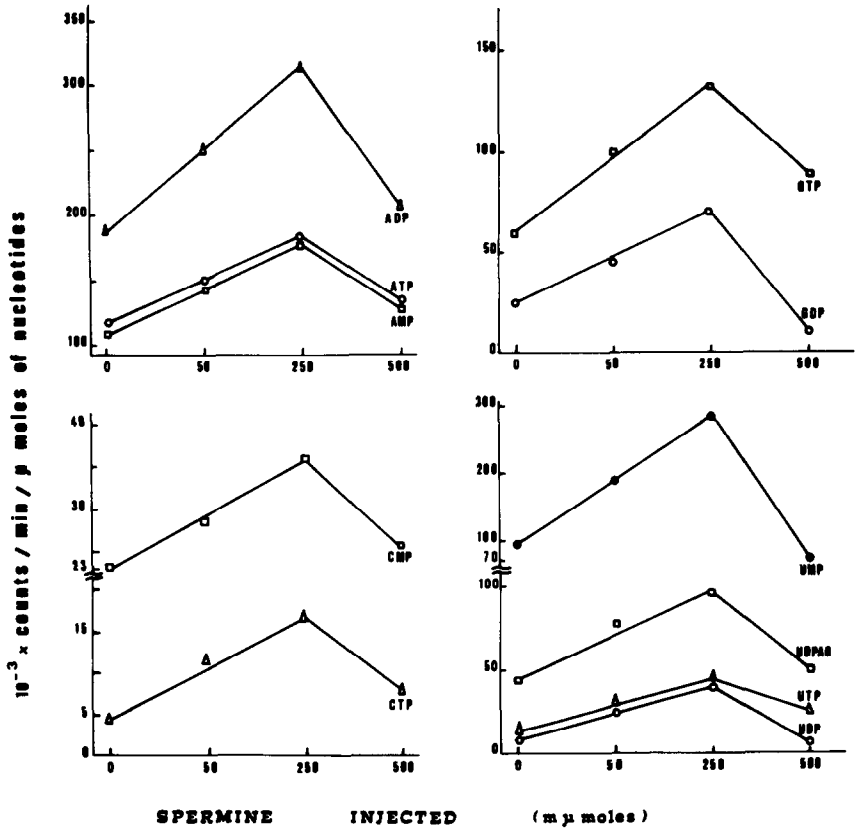

Fig. 2. Effect of different doses of spermine on the incorporation rate of ^{14}C-orotic acid and ^{3}H-formate into free nucleotides of the chick embryo.

Table 1

Effect of spermine on the specific activity of free nucleotides, measured as percentage of control (control = 100).

Nucleotides	Time after spermine injection		
	45 min	1½ hr	3 hr
CMP	113*	117	104
AMP	153	100	105
UMP	318	126	215
ADP	170	162	131
UDPAG	216	143	144
UDPG	220	161	127
GDP	305	295	307
UDP	350	150	109
CTP	416	209	264
ATP	169	125	125
GTP	226	218	190
UTP	410	360	356

The spermine (250 nmoles) was injected into the air space of the eggs, at 8th day of incubation, 90 min after the labelled precursors (2.5 μCi of ^{14}C-orotate and 20 μCi of ^{3}H-formate for each embryo).

* The results represent the mean of two determinations of pooled embryos.

increased rapidly and therefore the higher level of aminoaldehydes slows down the biosynthesis of nucleotides. Nevertheless, it is also possible that the decreased concentration of polyamines is unable to stimulate incorporation of labelled precursors into nucleotides.

All these results led us to think that the action of spermine on the stimulation of the nucleic acid synthesis observed by us and other authors [9, 10], is related to a higher rate of synthesis of free nucleotides. This phenomenon may be a consequence both of an increased availability of precursors of the nucleotide pool or an action on the enzymes involved in nucleotide biosynthesis. We are further investigating the exact mechanism of this action.

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