The Effects of Adenine Nucleotides and Guanine Nucleotides on Urate Synthesis *de novo* by Isolated Chick Liver and Kidney Cells

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(Received 6 March 1975)

1. Isolated chick liver and kidney cells produce urate *de novo* from glycine, and this is partially inhibited by 1 mM-AMP and by 1 mM-GMP in liver cells but not in kidney cells.
2. Azaserine fully inhibits this synthesis *de novo*, but attempts to isolate formylglycine amide ribonucleotide from azaserine-blocked cells were unsuccessful.

The biosynthetic pathways of uric acid and the purine nucleotides in chick are thought to be common through to inosinic acid, which is then metabolized via different pathways to the end products (Hartman, 1970). In mammalian cells purine nucleotide synthesis is under feedback control; the end products, principally AMP and GMP, inhibit the first enzyme of the pathway, glutamine phosphoribosyl pyrophosphate amidotransferase (McFall & Magasanik, 1960; Henderson, 1962). As uric acid is the main nitrogenous excretory product in chicks, it might be expected that this mechanism would be of minor importance, and raises the question as to the factors controlling urate and purine nucleotide synthesis.

It has, however, been found that this amidotransferase is inhibited by AMP and GMP in both rat and chicken liver homogenates, although to a smaller extent in the latter (Katunuma *et al.*, 1969). Several authors have observed an inhibitory effect of AMP and GMP on purified amidotransferase from chick or pigeon liver (Caskey *et al.*, 1964; Wyngaarden & Ashton, 1959). Some reports of a lack of inhibition have been ascribed to destruction of the enzyme regulatory site during purification (Buchanan, 1973).

Isolated chick liver cells have previously been shown to synthesize urate (Badenoch-Jones & Buttery, 1975), and we have therefore examined the capacity of these and chick kidney cells to synthesize urate from glycine and the effect of AMP and GMP on this.

Materials and methods

Azaserine (grade A) was obtained from Calbiochem, London W.1, U.K. [$1\text{-}^{14}\text{C}$]Glycine ($53.8\text{ }\mu\text{Ci}/\mu\text{mol}$) and [$2\text{-}^{14}\text{C}$]uric acid ($56.7\text{ }\mu\text{Ci}/\mu\text{mol}$) were obtained from The Radiochemical Centre, Amersham, Bucks., U.K. AMP and GMP were obtained from the Sigma (London) Chemical Co., Kingston-upon-Thames, Surrey, U.K., and Ficoll (mol.wt. 70000) was from Pharmacia, Uppsala, Sweden. Other chemicals and the media used were as

described previously (Badenoch-Jones & Buttery, 1975).

Chick liver cells were prepared as previously described (Badenoch-Jones & Buttery, 1975); kidney cells were prepared by the same method and were obtained in a similar yield and viability.

Urate synthesis *de novo* was measured by precipitation of the mercuric salt by the method of Bergmann & Dikstein (1954). Cell suspensions (10 ml at 30×10^6 cells/ml) in Hepes [$2\text{-}(N\text{-}2\text{-hydroxyethylpiperazin-}N'\text{-yl)ethanesulphonate}$]-buffered Hanks medium were incubated in the presence of 10 mM -glycine ($2\text{ }\mu\text{Ci}$ of [$1\text{-}^{14}\text{C}$]glycine), and $0.2\text{ }\mu\text{g}$ of insulin was added every 20 min to facilitate uptake of the glycine (Barratt *et al.*, 1974). Conditions of incubation and the medium used were as described previously (Badenoch-Jones & Buttery, 1975). At intervals 1 ml portions were removed and the radioactively labelled urate formed was precipitated as the mercuric salt; the precipitate was washed three times and the radioactivity was counted with the use of the scintillation mixture described by Barratt *et al.* (1974). The recovery of added [$2\text{-}^{14}\text{C}$]uric acid was $89\pm 5\%$.

Formylglycine amide ribonucleotide was measured as described by Moore & LePage (1957). The cell suspension was pretreated with azaserine (final concentration $20\text{--}300\text{ }\mu\text{M}$) for 30 min , then incubated as above. At intervals between 30 min and 3 h , 1 ml portions were removed and each was added to 1 ml of ice-cold 6% (w/v) HClO_4 . After removal of the precipitated protein by centrifugation, the clear supernatant was (a) applied to and eluted from a Dowex 1 (formate form) column (3.0 ml volume) as described by Moore & LePage (1957) or (b) separated by thin-layer chromatography as described by Reem (1974) or (c) separated by paper chromatography with the solvent system no. 4 [propan-2-ol-water (7:3, v/v) with the addition of 0.35 ml of NH_3 solution (sp.gr. 0.88)/litre jar volume] of Goldthwait *et al.* (1956). Authentic samples of formyl[$1\text{-}^{14}\text{C}$]glycine amide ribonucleotide, kindly supplied by

Professor J. F. Henderson, University of Alberta, were run in parallel with the cell extracts.

Glycine uptake was measured by removing 1 ml portions of the cell suspension, incubated as above with 10 mM-glycine (2 μ Ci of [1- 14 C]glycine) and 0.2 μ g of insulin, and layering on to 4 ml of Hepes-buffered Hanks medium containing 5% (w/v) of Ficoll in conical glass centrifuge tubes. The samples were centrifuged at 500 $g_{\max}$ for 3 min, the supernatants were removed and radioactivity in the pellets was counted.

Results and discussion

Purine synthesis *de novo* is usually measured by following the accumulation of formyl[1- 14 C]glycine amide ribonucleotide in azaserine-blocked cells incubated with [1- 14 C]glycine or [1- 14 C]formate (Semple *et al.*, 1974; Moore & LePage, 1957; LePage & Jones, 1961). Azaserine in low concentrations inhibits formylglycine amide ribonucleotide amidotransferase and hence causes an accumulation of formylglycine amide ribonucleotide.

Fig. 1. Synthesis of [14 C]urate from [1- 14 C]glycine by liver (●) and kidney (■) cells

Cells (30×10^6 /ml) were incubated at 40°C with [1- 14 C]glycine (10 mM-glycine), and 0.2 μ g of insulin was added every 20 min. Portions were removed, the urate formed was precipitated as the mercuric salt and its radioactivity was counted. Results are plotted as the difference in c.p.m. for each sample and the c.p.m. at time zero. The counting efficiencies were the same for all samples. Each point shows the mean of three experiments. Individual results are within $\pm 8\%$ of the mean.

In our system, with either liver or kidney cells, no accumulation of formylglycine amide ribonucleotide was observed, although the formyl[1- 14 C]glycine amide ribonucleotide standard was easily quantified by using the separation systems described above under 'Materials and methods'.

In view of this, urate synthesis *de novo* was measured by following the incorporation of [1- 14 C]glycine into urate. As shown in Fig. 1, urate production was sigmoid with time; the initial lag was not due to a slow uptake of glycine, as this was initially taken up very rapidly by the cells and reached an equilibrium after some 15–30 min. Possibly the cells are recovering from the dispersal process or accumulating some required critical intermediate, resulting in an initial low rate of synthesis.

Liver is thought to be the main site of urate production in chick (Edson *et al.*, 1936), although several extrahepatic sites of synthesis have been suggested, including kidney, pancreas (Chou, 1971, 1972) and intestine (Karasawa *et al.*, 1973). There is, however, no direct evidence of urate synthesis *de novo* in these tissues. Kidney cells were indeed found to synthesize [14 C]urate (Fig. 1), although at a rate approximately fivefold lower than liver cells. Hence, in agreement with a suggestion by Chou (1972), based on indirect experiments, the kidneys must make a minor but significant contribution to the total urate production by the whole chick.

Azaserine blocks urate synthesis *de novo* by almost 100% at a final concentration of 100 μ M in both liver and kidney. We have also found that the

Fig. 2. Effects of AMP (●) and GMP (■) on urate synthesis by liver and kidney cells

Results are expressed as the percentage of the urate production in the absence of added nucleotides, measured between 60 and 120 min. Each point shows the mean of three experiments. Individual results are within $\pm 8\%$ of the mean.

total endogenous urate production in the absence of added substrates, measured as described previously (Badenoch-Jones & Buttery, 1975), is inhibited by some 30% by azaserine at this concentration. Presumably the remainder of the total urate production originates from preformed purine nucleotides via the salvage pathway.

The effects of AMP and GMP on urate production *de novo* are shown in Fig. 2, which indicates that urate production is substantially inhibited in liver but not in kidney. This observed inhibition is probably due to inhibition of glutamine phosphoribosyl pyrophosphate amidotransferase, although an alternative site of inhibition cannot be excluded.

Hence urate synthesis *de novo* has been inhibited in structurally intact liver cells. The precise significance of this inhibition in relation to the overall control of urate and purine nucleotide synthesis must remain a matter for speculation at the present time. It is, however, clear that under conditions of high intracellular nucleotide concentrations urate synthesis will be partially inhibited in liver, but not in kidney.

The support of the Wellcome Foundation is gratefully acknowledged. We thank Professor J. F. Henderson, University of Alberta, for the formyl[1-¹⁴C]glycine amide ribonucleotide standard.

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