ENHANCEMENT OF INTERFERON mRNA LEVELS IN BUTYRIC ACID-TREATED NAMALWA CELLS

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Received 29 January 1980

1. Introduction

Butyric acid (BA) has been shown to affect many cellular activities [1], including changes in the expression of genes [2], modifications in enzyme action [3] and alterations to cellular morphology [4]. The effects depend on the cell-type being investigated, but in all cases the extent of acetylation of histones is altered and DNA synthesis is inhibited [5–7]. It has been suggested that these changes are not linked to the increased expression of certain differentiated cell functions [6–9]. Here we have investigated the way in which BA enhances the yield of an inducible protein, interferon ([10,11] Johnston, personal communication).

2. Materials and methods

Namalwa cells were cultured, induced and the interferon was assayed on MDBK cells as in [12] and the titre expressed in reference research units. When cells were pretreated with BA they were transferred to medium containing the appropriate concentration of BA and 2% calf serum at 2×10^6/ml 48 h before induction. Fractionation of the cells and the subsequent RNA extractions were carried out as in [13]. RNA was microinjected into oocytes at 5 mg/ml and the interferon produced assayed [14].

3. Results

Namalwa cells were treated for 48 h with 1 mM BA, induced with Sendai virus, and the rate of accumulation of interferon in the medium was compared with that from untreated cells. Fig.1a shows that the interferon from both treated and untreated cells was produced over the same time period, but that the yield was increased ~5-fold at all times. RNA was extracted from these cells at different times after induction, microinjected into oocytes and the resultant interferon titres measured. The amount of interferon mRNA in both treated and untreated cells rose in parallel to a maximum at 10 h post-induction, subsequently decreasing together (fig.1b). No difference in the rate at which the interferon mRNA decayed in the treated and untreated Namalwa cells was observed suggesting that its half-life was unaltered. Thus at all times the specific activity of interferon mRNA was 5-fold greater in RNA extracted from treated cells than from control cells. This result could have been due to the RNA from BA-treated cells behaving differently from RNA from control cells in the oocyte assay. However measurement of the rate of synthesis of interferon in oocytes after microinjection of RNA from either treated or untreated cells showed that neither the stability of the mRNA in the oocyte nor the rate of secretion of interferon from the oocytes (fig.2) was changed. Therefore the increased levels of interferon mRNA detected by translation in oocytes is a reflection of enhanced levels of this mRNA in BA-treated Namalwa cells.

The increase in interferon titres in the incubation medium of Namalwa cells treated with BA was accompanied by an increase in interferon found in the cells homogenates and on the polysomes (table 1). Thus the BA treatment was not enhancing the secretion of interferon from the Namalwa cells.

The increase in concentration of interferon mRNA was not just found in the total RNA but also in the cytoplasmic RNA and polysomal RNA (table 1),

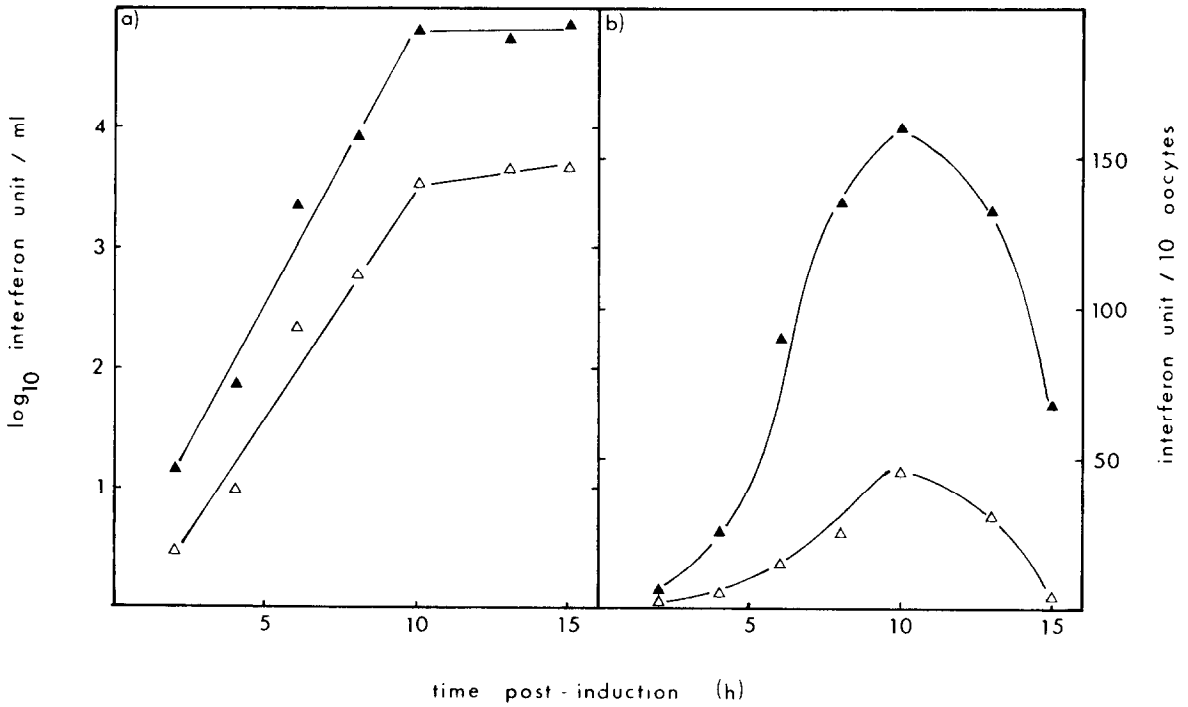

Fig.1. (a) Shows the accumulated yields of interferon in cells pretreated with BA (▲——▲) or in control cells treated identically (△——△). RNA was extracted from these cells. (1b) Shows the interferon secreted from oocytes 24 h after microinjection with RNA (5 mg/ml) from cells pretreated with BA (▲——▲) or control cells (△——△).

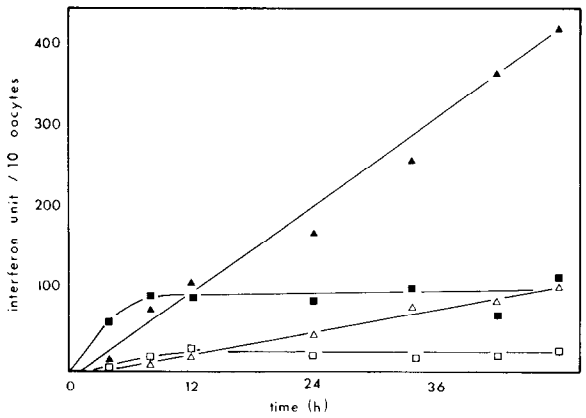

Fig.2. RNA was extracted from untreated and BA-treated Namalwa cells 9 h after induction, injected into oocytes and the interferon measured after different times of incubation. The interferon yields present (i) in oocytes injected with RNA from untreated cells (□——□), their incubation medium (△——△) or (ii) in oocytes injected with RNA from BA-treated cells (■——■) or their incubation medium are shown (▲——▲).

although the yield of polysomes was lower from BA-treated cells because they are more difficult to lyse (A. M., unpublished). Therefore the enhanced yields of interferon produced by BA-treated cells were not caused by a higher proportion of interferon mRNA being recruited onto the polysomes, but by a general increase in the level of the interferon mRNA.

When the poly(A^+) RNA was isolated from the total RNA, the specific activity of interferon mRNA was found to be 5-fold higher in the RNA from treated cells. Thus BA-treatment did not cause a rise in the total amount of poly(A^+) RNA because the amount of RNA binding to oligo(dT) cellulose did not alter (2.3% of total RNA from untreated cells and 2.4% of total RNA from treated cells, both values means of 4 determinations). Neither was the mRNA from treated cells more active in a cell-free system because its ability to stimulate the mRNA-dependent reticulocyte lysate protein synthesising system [15] was the same as the mRNA from untreated cells. Thus, these results show that the increase in specific activity of interferon

Table 1

	Control	BA-treated	Ratio
Interferon formed by Namalwa cells:			
in medium (unit/ml/10^6 cells)	5623	31 622	5.6:1
in homogenate (unit/10^6 cells)	1.2	6.4	5.3:1
on polysomes (unit/10 A_{260})	64	267	4.0:1
Interferon formed by microinjection of RNA into oocytes:			
total RNA (unit/10 oocytes)	40	156	3.9:1
cytoplasmic RNA (unit/10 oocytes)	40	200	5.0:1
polysomal RNA (unit/10 oocytes)	80	390	4.9:1

mRNA from treated cells is not due to a general increase in the level of mRNA or its activity.

Finally, the effect of varying the concentration of BA on the interferon yields of Namalwa cells and on the specific activity of interferon mRNA was investigated. Fig.3 shows that the enhancement of yield in tissue culture correlates exactly with the increase in the amount of interferon mRNA. This was true whether the interferon inside the oocytes or the material that they had secreted was measured. Therefore the BA-treatment of Namalwa cells leads to an increased production of interferon because the specific activity of the interferon mRNA has risen by an amount sufficient to account for it.

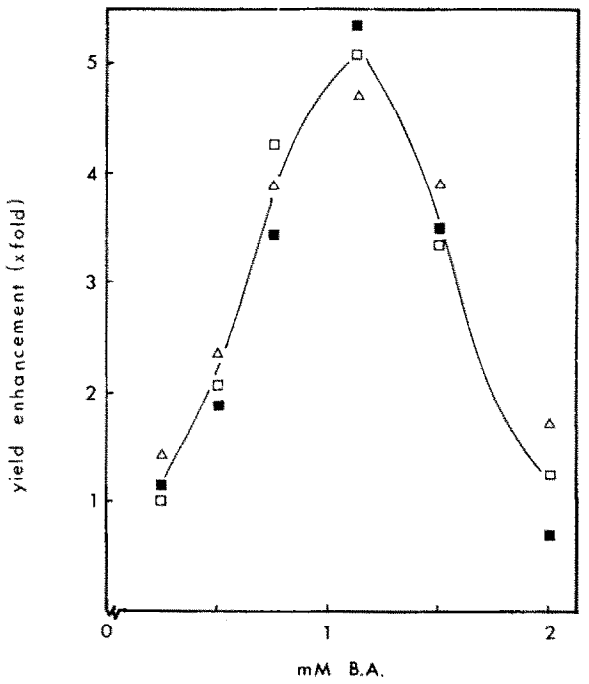

Fig.3. Cells were pretreated with varying concentrations of BA, induced and the RNA extracted 9 h after induction as in fig.1. The interferon titres produced (i) by untreated Namalwa cells (△——△) was 5018 unit/ml, or (ii) by oocytes 24 h after microinjection with RNA from untreated cells were 64 unit/10 oocytes from homogenised oocytes (■——■) or 32 unit/10 oocytes secreted from the oocytes (□——□).

4. Discussion

We have shown that treatment of Namalwa cells with BA increases interferon yields when they are subsequently induced, and that the concentration of interferon mRNA is greater in treated than in untreated cells, by an amount sufficient to account for the enhanced yields.

The system described here for increasing interferon yields using pretreatment of cells with BA can be compared with the superinduction system [16]. When cells are superinduced, the interferon mRNA concentration is also increased, but that increase is caused by an increase in the half-life of the interferon mRNA [17]. Thus synthesis of interferon is prolonged and it appears that this accounts for the enhanced yields. In contrast, cells treated with BA did not synthesise interferon for longer than control cells, indicating that the half-life of the interferon mRNA was unaltered. Instead an increase in the specific activity of interferon mRNA was found, suggesting that the transcription or processing (or both) of the interferon mRNA was enhanced in treated cells.

Acknowledgements

The authors wish to thank Mrs J. G. Flint for excellent technical assistance, Dr N. B. Finter and Dr M. D. Johnston for making their materials and results available and Professor D. C. Burke for advice and encouragement. We thank the MRC for support as a programme grant to Professor D. C. Burke and the CRC for support of Alan Colman.

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