NRK CELLS SYNCHRONIZED IN G_1 BY PICOLINIC ACID ARE SUPER-SENSITIVE TO PROSTAGLANDIN E_1 STIMULATION

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1. Introduction

We have previously demonstrated that addition of the pyridine derivative, α-picolinic acid, to the culture medium reversibly arrests NRK cell growth in the G_1 phase of the cell-cycle [1]. Research in two areas suggested to us that α-picolinic acid could act through alterations in cyclic AMP levels. Cyclic AMP has been postulated to be important for growth control (reviewed in [2]) and certain pyridine derivatives influence cyclic AMP metabolism in some cells. Nicotinic acid prevents the ACTH-, glucagon- or epinephrine-induced elevation of cyclic AMP levels in fat cells [3] and NAD^+ is required for the activation of adenylate cyclase by cholera toxin in erythrocytes and certain tumor cells [4,5]. A preliminary report of these results has been presented [6].

2. Methods and materials

NRK cells were grown as previously described [1]. Cells were planted in most experiments at 4000 cells/cm^2 in 50 cm^2 tissue culture dishes (Falcon Plastics). Unless otherwise indicated, the media were replaced the next day with control medium or medium containing 3 mM α-picolinic acid (Sigma). The α-picolinic acid was dissolved 10 min before addition. After 42 h the cells were treated for cyclic AMP analysis as previously described [7]. The cyclic AMP was assayed using the acetylation modification [8] of the radioimmunoassay [9]. Labelled antigen was obtained from Collaborative Research. Antibody was prepared as previously described [10]. Prostaglandin E_1 (PGE_1 was dissolved in ethanol (10 mg/ml) and aliquots were added to the medium (final concentration 0.05% v/v). This concentration of ethanol did not affect cyclic AMP levels. Greater than 99% of the immunoreactive material was hydrolyzed by treatment with cyclic nucleotide phosphodiesterase.

3. Results

NRK cells treated for 42 h with 3 mM α-picolinic acid are 90% synchronized in G_1 [1]. The basal cyclic AMP levels in cells treated in this manner are approximately 50% increased; moreover, the ability of PGE_1 to elevate cyclic AMP levels in these cells [11] is potentiated 5-fold (table 1). These effects are specific for α-picolinic acid since nicotinic acid does not synchronize the cells [1] or significantly alter cyclic AMP levels (table 1). The potentiation is also observed with larger doses of PGE_1 (fig.1). The increased PGE_1

Table 1
Effect of pyridine derivatives on cyclic AMP levels

Treatment	Cyclic AMP (pmol/10^6 cells)	
	Basal	+ PGE_1
Control	2.40 ± 0.06 (8)	535 ± 16 (9)
α-Picolinic acid (3 mM)	3.39 ± 0.08 (6)	2570 ± 67 (9)
Nicotinic acid	2.46 ± 0.07 (9)	448 ± 12 (9)

Cells were grown as described in Methods and materials. Cyclic AMP was extracted after 42 h treatment with the indicated compound plus 15 min with PGE_1 (5 μg/ml). The values are the mean and standard error of the mean from 2 experiments with the number of assays in parentheses.

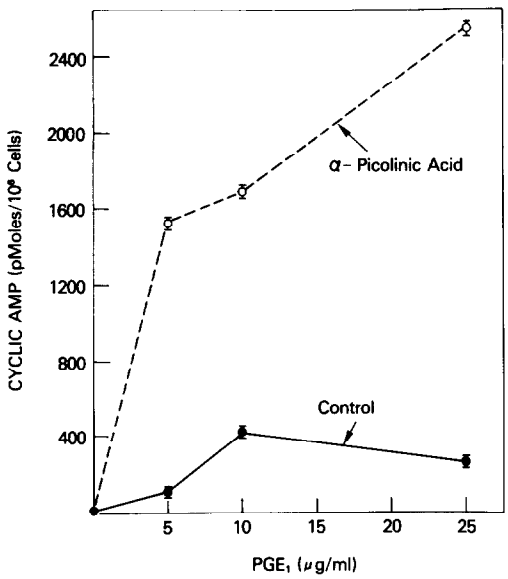

Fig.1. Effect of PGE_1 concentration on cyclic AMP levels. Cells were grown as described in Methods and materials. After 42 h PGE_1 was added at the indicated concentration for 15 min.

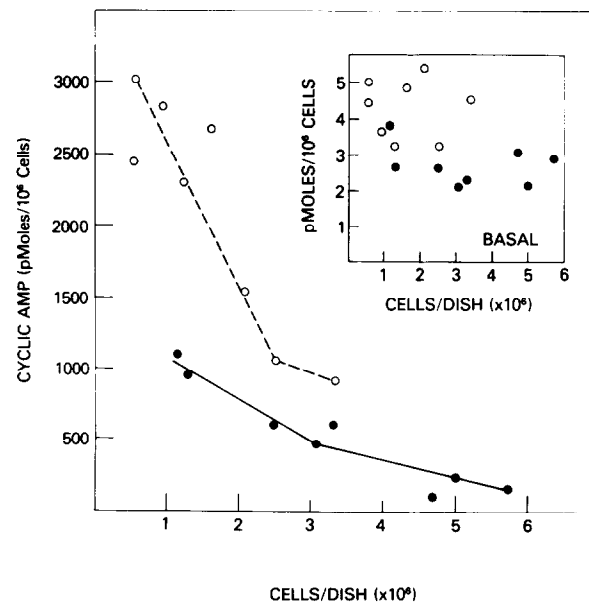

Fig.2. Effect of cell density on basal and PGE_1-stimulated cyclic AMP levels. Cells were planted at densities varying from 1500–10 000 cells/cm² and grown as described in Methods and materials. After 42 h the cells were analyzed for cyclic AMP levels (insert) or after an additional 15 min with PGE_1 (5 μg/ml). Each point is the average from 3 assays. The cells/dish is the cell density at the time of harvest. Open circles, cells treated with 3 mM picolinic acid. Closed circles, control cells.

response is not observed at early times after the addition of α-picolinic acid. At 6 h there is even a decrease in the elevation of cyclic AMP by PGE_1 (table 2).

The response of both control and treated cells to

Table 2
Time-dependence for α-picolinic acid treatment

Experiment	Time	Cyclic AMP (pmol/10^6 cells)			
		Control		+ α-Picolinic acid	
		Basal	+ PGE_1	Basal	+ PGE_1
A	6 h	5.91 ± 0.31	193 ± 4	2.62 ± 0.06	144 ± 1
	42 h	3.86 ± 0.08	1110 ± 70	5.02 ± 0.31	3030 ± 60
B	6 h	3.28 ± 0.10	262 ± 7	3.47 ± 0.05	143 ± 2
	42 h	2.94 ± 0.07	184 ± 1	4.55 ± 0.31	905 ± 20

Cells were grown as described in Methods and materials. Twenty four h (B) or 42 h (A) after the first medium change the medium was removed and replaced by medium with or without 3 mM α-picolinic acid. The cyclic AMP was extracted after 6 h. Each value is the mean and standard error of the mean from 3 assays. The difference in response at 42 h in the 2 experiments was due to differences in cell density (see fig.1). The cells/dish in the control culture was 1.15×10^6 in A and 5.71×10^6 in B. The cells/dish in α-picolinic acid treated cultures (42 h) was 0.55×10^6 in A and 3.37×10^6 in B.

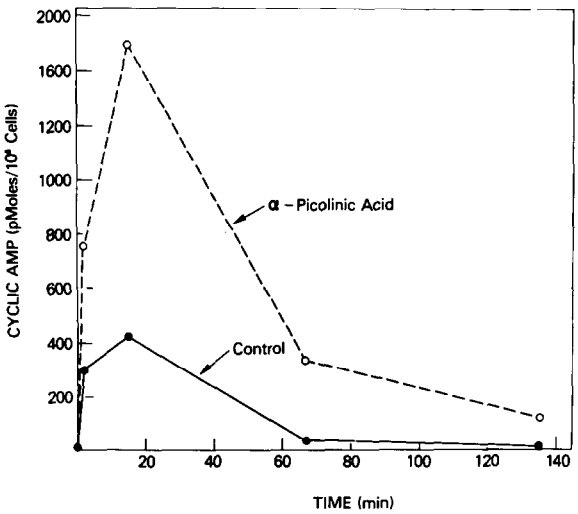

Fig.3. Effect of time on cyclic AMP levels after addition of PGE_1. Cells were grown as described in Methods and materials. After 42 h PGE_1 (10 μg/ml) was added and cyclic AMP was extracted at the indicated times. Each point is the average from 3 assays.

PGE_1 is dependent upon the cell density; the greater the cell density, the less the elevation of cyclic AMP (fig.2). The basal cyclic AMP levels are independent of cell density. The average of the values shown are 58% higher in the treated cells (fig.2, insert). We have previously reported that cyclic AMP levels increase at high cell densities in a different clone of NRK cells [12]. The reasons for this difference are not understood.

It is well known that when activators of adenylate cyclase such as PGE_1 are added to cells, cyclic AMP levels do not remain elevated. Rather they begin to fall after a few minutes even though a sufficient amount of the agent to fully maintain high cyclic AMP levels is available to the cell [13,14]. We reasoned that α-picolinic acid could prevent this fall and this would explain the increased cyclic AMP levels. This does not appear to be the explanation since treated cells have higher cyclic AMP levels than do the control cells at all times tested (fig.3).

4. Discussion

NRK cells treated with α-picolinic acid are reversibly arrested in G_1 and accompanying this growth inhibition is a potentiation of the ability of PGE_1 to elevate cyclic AMP levels. These observations raise three interesting questions.

Does α-picolinic acid act directly? The potentiation of the PGE_1 response is not observed at 6 h. This suggests that it is not a result of direct inhibition of phosphodiesterase or activation of adenylate cyclase and that α-picolinic acid may be converted into another molecule. Numerous pyridine derivatives undergo an exchange reaction catalyzed by NAD^+ glycohydrolase (EC 3.2.2.5) with the nicotinamide moiety of NAD^+ to form the corresponding NAD^+ analog [15]. A similar derivative of α-picolinic acid may be the active species.

How does α-picolinic acid or a metabolite increase the PGE_1 response? It is known that NAD^+ is required for the stimulation of adenylate cyclase by cholera toxin [4,5]. Picolinic acid or a metabolite of it could participate in the activation of adenylate cyclase by PGE_1. Another possibility is that the increased response to PGE_1 is a result of synchronization at a specific point in G_1 where the cells are highly responsive to PGE_1. Melanocyte-stimulating hormone activates adenylate cyclase in melanoma cells at a specific point in the cell-cycle (G_2) [16]. The decreased response to PGE_1 at higher cell densities in the control cells is somewhat surprising since we have found by using flow microfluorograph analysis (J. A. Fernandez-Pol, V. Bono, and G. S. Johnson, unpublished results) that dense NRK cells are arrested in G_1. This indicates that perhaps other factors are involved; however, α-picolinic acid could arrest the cells at a different point in G_1.

What is the significance of concomitant G_1 growth arrest and changes in cyclic AMP metabolism? Cyclic AMP has previously been postulated to be an important factor in growth arrest in confluent normal cells (reviewed in [2]). Basal cyclic AMP levels are 50–60% elevated in α-picolinic acid treated cells. This suggests that the cessation of growth does not require a large increase in cyclic AMP. It is also possible that there is a large accumulation of cyclic AMP in a small portion of the cell. Functions for PGE_1 in growth control are not clear; therefore, it is difficult to speculate on the significance of the increased ability of PGE_1 to raise cyclic AMP levels. This may be representative of altered responses to several hormones and agents

which influence cellular behavior. The growth arrest could be caused by an abnormal cellular response to growth factors in the culture medium.

Picolinic acid should be a useful tool to study cyclic AMP metabolism and growth control. Since it is a naturally occurring compound in at least some cell-types (see ref. [1]), the possibility should be considered that it is part of a normal control mechanism.

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