Expression of Genes for Metabolism of Cyclic Adenosine 3':5'-Monophosphate in Somatic Cells

II. EFFECTS OF PROSTAGLANDIN E₁ AND THEOPHYLLINE ON PARENTAL AND HYBRID CELLS

JOHN D. MINNA AND ALFRED G. GILMAN

From the Laboratory of Biochemical Genetics, National Heart and Lung Institute, National Institutes of Health, Bethesda, Maryland 20014, and the Department of Pharmacology, School of Medicine, University of Virginia, Charlottesville, Virginia 22903

SUMMARY

Clonal differences in the response to prostaglandin E₁ (PGE₁) were found when changes in the intracellular concentration of cyclic adenosine 3':5'-monophosphate (cyclic AMP) were measured following exposure to this agent in a variety of cell lines cultured in vitro. When PGE₁ responses were present, the effect was nearly maximal after a 5-min exposure and was well maintained for 60 min. Dose-response curves for PGE₁ were complex and also exhibited clonal variation. The relative potencies of prostaglandins were: PGE₁ > PGE₂ > PGA₁. PGF₁₂ and PGB₁ were inactive at the concentration tested, indicating receptor specificity. When parental lines with no or small responses to PGE₁ were fused to cells with larger responses to PGE₁, the resulting hybrid cells expressed high levels of PGE₁ responsiveness in the majority of cases. Clonal differences in potentiation by theophylline of peak PGE₁ or catecholamine effects on intracellular cyclic AMP levels were also found. When theophylline-sensitive cells were fused to theophylline-insensitive cells, the resulting hybrids were responsive to theophylline. Thus, the inheritance of PGE₁ and theophylline responses in somatic cell hybrids is distinctly different from that seen for β-adrenergic responses, suggesting fundamental differences in their genetic regulation. These data could indicate different types of receptor mechanisms for catecholamines and prostaglandins.

In the preceding report we examined the effects of catecholamines on the intracellular accumulation of cyclic AMP in a variety of clonal cell lines cultured in vitro and somatic cell hybrids formed by mating such parental lines (1). When a β⁺ (catecholamine responsive) cell was fused to a β⁻ (catecholamine unresponsive) cell, the resulting hybrid cell lines in the severe crosses studied were β⁻. This was evidence for a heritable mechanism of negative control for catecholamine responsiveness. Other components of the system involved in metabolism and action of cyclic AMP also could have clonal differences in expression. It is reasonable to ask if these components are under similar types of negative control.

Prostaglandins have diverse biochemical and physiological actions, certain of which appear to result from their ability either to stimulate the accumulation of cyclic AMP in a large number of cell types or to inhibit such accumulation of the cyclic nucleotide in certain other cells (2-4). Effects of prostaglandins to stimulate cyclic AMP synthesis also have been studied in a variety of cultured cell lines. Cyclic AMP synthesis is stimulated by prostaglandins of the E series in many cells (5-8). Adenylate cyclase activity was unresponsive to prostaglandins in a few cell lines (7).

While virtually all cells have been found to contain one or more cyclic nucleotide phosphodiesterases, the responses of various cells to inhibitors of such activity are more complex. The usual pattern is for theophylline (an inhibitor of phosphodiesterase) to potentiate the stimulatory effects of a variety of hormones on cyclic AMP concentration, while having only a small effect alone on the basal level of the cyclic nucleotide (4). However, theophylline has little ability to potentiate the stimulatory effects of catecholamines and histamine on the accumulation of cyclic AMP in intact cell preparations derived from brain, despite the high activities of cyclic nucleotide phosphodiesterase in this tissue (9, 10).

In this report we extend the genetic analysis of cyclic AMP metabolism in cultured somatic cells and show that clonal differences exist for prostaglandin responsiveness and for potentiation of peak hormone effects by theophylline. Matings between parental cell lines that differ in phenotype have been analyzed to explore the nature of the genetic mechanisms controlling these phenomena. A preliminary report of this work has appeared (11).

METHODS AND MATERIALS

The methods used for production, isolation, growth, and characterization of parental and hybrid cell lines are described...
in the preceding report (1). In addition, conditions for experimental incubations and methods for relevant analyses are the same.

Prostaglandins were stored at \(-20^\circ\text{C}\) as stock solution in ethanol. At the highest concentrations of prostaglandins utilized in these studies, the maximal possible ethanol concentration achieved was 0.5\%, a concentration that had no demonstrable effect on intracellular content of cyclic AMP. In the vast majority of experiments, the final ethanol concentration was at least 10-fold less.

Theophylline (1 mM) was either absent or present (as indicated) throughout the initial 30 min of incubation and during the subsequent indicated time of exposure to catecholamines or prostaglandins. The effect of the theophylline is expressed as the ratio of the cyclic AMP increment per mg of protein seen due to the addition of "hormone" in the presence of theophylline to that "hormone" response seen in its absence:

\[
\text{Theophylline ratio} = \frac{\text{cyclic AMP}_{\text{in presence of hormone}} - \text{cyclic AMP}_{\text{basal}}}{\text{cyclic AMP}_{\text{in presence of hormone}} - \text{cyclic AMP}_{\text{basal}}}
\]

A "theophylline ratio" of 1 thus indicates no observed effect of theophylline to the addition of "hormone" in the presence of theophylline to that "hormone" response seen in its absence.

All prostaglandins utilized were generously supplied by Dr. John Pike, The Upjohn Company, Kalamazoo, Mich.

RESULTS

Effect of Prostaglandins on Parental Lines—Clonal differences in accumulation of intracellular cyclic AMP in response to PGE\(_1\) were found among the eight parental cell lines. However, with one exception, the parental clones utilized in these studies show significant elevations of their intracellular content of cyclic AMP following exposure to PGE\(_1\). Six of the seven clones studied in detail (C6TG1A, B82, 3T3-4(C2), 3T3-4(E), N4TG1, and BRL30E) all may be classified phenotypically as PGE+ when this index of responsiveness is utilized. The response of BRL30E to PGE\(_1\) was considerably greater than that seen in any other clone.

In earlier experiments with C6TG1A and C6RC16, basal levels of cyclic AMP were relatively high and effects of PGE\(_1\) were more difficult to observe. In later experiments, for reasons that are not clear, basal levels have declined, while concentrations of cyclic AMP attained following exposure to PGE\(_1\) have remained relatively constant. This accounts for larger responses to PGE\(_1\) shown in certain figures when values are expressed relative to the control. RAG is the only clone in which significant elevations of cyclic AMP content were not detected and that is, therefore, designated as PGE-.

Further documentation of this negative result is supplied below.

Other clonal lines that have been tested and found to respond to PGE\(_1\) include a variety of other clones of murine neuroblastoma C-1300, a murine ganglioneuroblastoma developed and supplied by Dr. B. W. Ruffner, and the human fibroblasts WI38 and VA2. Thus, although prostaglandin responsiveness in cultured cell lines is not universal, it does appear to be rather prevalent.

It is clear from the data of Table I that the magnitudes of the effects of PGE\(_1\) among the responsive clones vary greatly, and comparison with the data of the accompanying report reveals no clear correlation between the ability of these clones to respond to PGE\(_1\) and similar responsiveness to catecholamines. For example, C6TG1A shows a 300-fold stimulation by catecholamines and only a 2.5-fold response with PGE\(_1\).

The responses of these parental lines have been studied as a function of concentration and length of exposure to PGE\(_1\), and the relative abilities of other available prostaglandins to elicit this effect also have been determined.

The dose-response curves for PGE\(_1\) clones were quite variable (Fig. 1). With L82, for example, a PGE\(_1\) concentration of 0.1 \(\mu\)g per ml gave a maximal response, while the half maximal concentration appeared to be less than 10 ng per ml. In contrast, C6TG1A and 3T3-4(E) were relatively insensitive to stimulation by PGE\(_1\). With these clones it is not apparent whether the highest concentration tested (10 \(\mu\)g per ml) was a maximally effective one. The other responsive clones appear to show intermediate sensitivity, and the curves generated are perhaps suggestive of multiple affinities within each line for PGE\(_1\).

Significant responses to PGE\(_1\) were not seen with clone RAG at any concentration of the prostaglandin utilized.

The clonal differences in PGE\(_1\) dose-response curves stand in contrast to those seen with isoproterenol, where the half-maximal concentrations to stimulate the three responsive parental clones were very similar (1). While different sensitivities could indicate molecular heterogeneity of prostaglandin receptors, they could also reflect factors such as differential permeability or cellular metabolism of prostaglandins.

The responses of the parental cell lines to PGE\(_1\) as a function of time were more similar (Fig. 2). Peak effects were usually observed by 5 min or slightly later. Maximal levels were well maintained throughout the 1-hour period of observation for B82 and N4TG1. Clones BRL30E and 3T3-4(E) showed declining cyclic AMP concentrations after peak levels had been attained, and the rates of these declines were quite similar to those observed when the intracellular content of cyclic AMP was elevated.

### Table I

| Parental cell line | Tissue source and species* | Cyclic AMPa | Ratio of PGE to control | Symbol for phenotype |
|-------------------|--------------------------|-------------|------------------------|---------------------|
| RAG .............. | Renal adenocarcinoma (M) | 9 5 1       | PGE-                    |                     |
| C6TG1A ........... | Glioma (R)               | 10 25 2.5  | PGE+                   |                     |
| C6RC16 ........... | Glioma (R)               | 18 27 1.5  | PGE+                   |                     |
| B82; (A9) ....... | Subcutaneous (M)         | 14 48 3.4  | PGE+                   |                     |
| 3T3-4(C2) ....... | Whole embryo (M)         | 6 52 8.7   | PGE+                   |                     |
| 3T3-4(E) ......... | Whole embryo (M)         | 17 110 6.5 | PGE+                   |                     |
| N4TG1 .........   | Neuroblastoma (M)        | 11 179 16  | PGE+                   |                     |
| BRL30E ........... | Liver (R)                | 15 1110 60 | PGE+                   |                     |

* Letter in parentheses designates rat (R) or mouse (M) origin.

a Incubations were performed in the presence of 1 mM theophylline. PGE was present during a 10-min incubation. Values represent averages of three to nine experiments with at least duplicate incubations per experiment in all cases.
in these clones with isoproterenol. No alteration of cyclic AMP concentration was observed with RAG at any time interval examined. Incubations of 10 min were chosen for other experimental situations.

A general pattern of effectiveness is apparent when the relative abilities of other prostaglandins to alter cyclic AMP metabolism was tested (Fig. 3). Thus, the largest effects are elicited in each clone with PGE₁. In most cases, PGE₂ and PGA₁ also show at least limited ability to augment cyclic AMP concentrations, while PGE₆ and PGB₁ are essentially ineffective at this concentration. The apparently greater relative effectiveness of PGE₁ in some clones (e.g. B82) is probably at least in part due to the concentrations of prostaglandins utilized in these experiments (1 μg per ml) and to the relative sensitivities of the various clones. Thus, 1 μg per ml of PGE₁ is a supramaximally effective concentration for B82, and the greater apparent effectiveness of PGE₂ and PGA₁ should be viewed in this light. Other prostaglandins were not capable of eliciting responses in RAG. These patterns of response are thus consistent with the hypothesis that all of the PGE₁⁺ parental lines have “receptors” for prostaglandins with some degree of specificity that are ultimately responsible for elevated intracellular concentrations of cyclic AMP.

Effect of PGE₁ on Hybrid Lines—A large number of hybrid clones formed as a result of fusion of B82 and N4TG1 were available that have been studied previously for expression of differentiated neuronal properties found in the neuroblastoma parent (12, 13). Since a number of well characterized clones were available that show varying degrees of chromosomal loss, these hybrids were studied in a control series of experiments to ascertain whether they would show expected responsiveness to PGE₁. These hybrids were studied at extended times after fusion (>80 generations) and have been recloned. There is broad variation in both their chromosomal content and their magnitude of response to PGE₁ (Table II). However, all clones retain the ability to respond to PGE₁ after virus-induced cell fusion, selection in D5HAT medium (Dulbecco’s modification of Eagle’s medium containing 5% fetal calf serum, supplemented by 100 μM hypoxanthine, 0.4 μM aminopterine, and 16 μM thymidine), and cloning. The two clones with the greatest complement of chromosomes (NL1A, NL1) show responses of magnitudes that could be consistent at least with addition of the two parental responses. The other hybrid clones show intermediate and lower levels of response. There is positive correlation of 0.8 between the mean chromosome number of these clones and the magnitude of the elevation of cyclic AMP concentration seen following exposure to PGE₁. While this suggests a causal quantitative relationship between gene dosage and the level of PGE₁ response, clonal variability of the parental lines could also

### Table II

| Cell line      | Cyclic AMP* | Mean chromosome number (range) |
|----------------|-------------|--------------------------------|
|                | Control     | + PGE₁ (2 μg/ml)               |
|                | ng/mg protein |                                      |
| Parent         |             |                                |
| N4TG1          | 11          | 179                             | 142 (129-150) |
| B82            | 14          | 48                              | 52 (48-56)    |
|                | Exp.*       |                                  | 194           |
| Hybrid         |             |                                |
| NL1A           | 10          | 455                             | 183 (166-190) |
| NL1            | 19          | 271                             | 180 (162-194) |
| NL14J          | 15          | 147                             | 124 (120-134) |
| NL1F⁺          | 12          | 101                             | 101 (101-101) |
| NL2N           | 14          | 83                              | 85 (79-93)    |
| NL3H           | 19          | 84                              | 138 (130-145) |
| NL13C          | 13          | 56                              | 111 (95-119)  |
| NL7AC          | 23          | 64                              | 120 (118-127) |
| NL30           | 8           | 31                              | 135 (133-143) |

* Incubations for 10 min in the presence of 1 mM theophylline.
* Exp., expected number of chromosomes obtained from fusion of mean parental cells.
* NL1F is a subclone of NL1.
be in part responsible. Other neuroblastoma × L cell hybrids resulting from the mating N18TG2 × B82 (12, 13) also have been studied for the PGE\(_i\) response (data not shown). There was no correlation between the level of PGE\(_i\) response and the expression of genes for acetylcholinesterase, membrane electrical excitability, or neurite formation in either of these series of NL hybrids.

Since hybrids formed between clones with large quantitative differences in their responses to catecholamines showed responses more related to that of the lower responding parent, a study of hybrids formed by mating parents with large quantitative differences in their level of response to PGE\(_i\) was undertaken. First, interspecific (rat × rat and mouse × mouse) matings with quantitative differences in their responses were examined.

The mating C6RC16 × BRL30E represents a cross between parental lines with 2- and 70-fold responses to PGE\(_i\), respectively. In four of the five hybrid lines examined 20 to 30 generations after fusion, the response to PGE\(_i\) was at least as great as that seen with BRL30E, and in one of these clones the effect may be significantly greater, although this has not been evaluated in detail (Table III). In one clone (C6BR3A) only a small response to PGE\(_i\) was seen. This hybrid line shows a large response to isoproterenol (1) and little response to PGE\(_i\), and thus resembles the C6 parent. However, chromosomal composition and morphology strongly suggest that it is not a revertant of C6. The capability of the parental clones to respond to PGF\(_i\) and PGE\(_i\) is nearly identical to that of BRL30E and is distinctly different from C6RC16. The C6RC16 × BRL30E hybrids have almost exactly the total number of chromosomes expected from fusion of the two parental cells. Only one to two chromosomes (average) are not present. Since four of the C6BR hybrid lines support the notion that PGE\(_i\) responsiveness is inherited in hybrid cells, the novel line (C6BR3A) may have lost this response as the result of a segregation of one or a few chromosomes. This line, therefore, should be particularly useful for examinations of functions of PGE\(_i\).

In several clones (e.g. C6BR2A, C6BR3B, C6BR3C) the response appeared to be significantly larger than that of B82. It is possible that RAG supplies some factor(s) used in the PGE\(_i\) response that is limiting in B82 or that cyclic AMP degradation is slowed. These hybrids have 69 to 91% of the total chromosome number expected from the fusion of two average parental cells. While large numbers of marker chromosomes are not available in this mating, the hybrid clone(s) appear to retain the large metacentric chromosomes from both parental lines (Table IV). Thus, the data from the C6RC16 × BRL30E and RAG × B82 matings show that the PGE\(_i\) response pattern of the higher responding parent was preserved in each of the nine clones studied.

A variety of matings of the rat-derived C6TG1A and C6RC16 with mouse parental lines were studied to evaluate the results of interspecific crosses and to test further the hypothesis of full inheritance of the PGE\(_i\) phenotype in matings between parents with quantitative differences in their responses.

Experiments were performed with 12 hybrid clones and two multi-clone pools (approximately 100 clones each) formed by the fusion of C6TG1A and B82 (Table V). In all cases the magnitude of the change in cyclic AMP concentration is at least as great as that seen in the greater responding B82 parental clone. In several clones (e.g. C6BR2B, C6BR3A, C6BR3C, C6BR3D, C6BR3E), the response appeared to be significantly larger than that of B82. The average increment of cyclic AMP concentration in the 14 lines studied was 80 pmoles per mg of protein, a response that is at least as large as that which could be expected by addition of the capabilities of the parental clones.

Hybrid clones generated from the matings C6RC16 × 3T3-4(C2) and C6RC16 × 3T3-4(E) were also analysed for PGE\(_i\) responsiveness (Tables VI and VII). At the time of analysis the hybrid cells had nearly a complete complement of chromosomes expected from the fusion of two average cells. In addition, all of the C6RC16 marker chromosomes were present in the majority of cell lines. Large quantitative differences in the PGE\(_i\) response were noted 30 to 40 generations after fusion. Ten of the 15 lines had large responses consistent with the inheritance of the PGE\(_i\) response in hybrid cells. However, five

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**Table III**

| Cell line | Cyclic AMP* | Mean chromosome numberb (range) |
|-----------|-------------|--------------------------------|
|           | Control     | + PGE\(_i\) (2 μg/ml)           |
| Parents   |             |                                |
| C6RC16    | 16          | 27                             |
| BRL30E    | 16          | 1110                           |
| Exp.      |             |                                |
| Hybrids   |             |                                |
| C6BR3B    | 13          | 2660                           |
| C6BR4A    | 13          | 1130                           |
| C6BR1A    | 10          | 1050                           |
| C6BR2A    | 11          | 800                            |
| C6BR3A    | 8           | 35                             |

* Incubations for 10 min in the presence of 1 mM theophylline.  
* Data for marker chromosomes in Reference 1.

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**Table IV**

| Cell line | Cyclic AMP* | Chromosome number (clone) |
|-----------|-------------|---------------------------|
|           | Control     | + PGE\(_i\) (2 μg/ml)     |
| Parents   |             |                           |
| RAG       | 5           | 5                         |
| B82       | 14          | 48                        |
| Exp.      |             |                           |
| Hybrids   |             |                           |
| RB4B      | 31          | 110                       |
| RB1B      | 17          | 83                        |
| RB10      | 25          | 74                        |
| RB2A      | 12          | 57                        |

* Incubations for 10 min in the presence of 1 mM theophylline.  
* Exp., expected number of chromosomes obtained from fusion of mean parental cells.
Hybrids and 6B arose on a single culture dish.

Parents

| Cell line   | Cyclic AMP* | Mean chromosome number* (range) |
|-------------|-------------|---------------------------------|
|             | pmol/mg protein |                                  |
|             | Control + PGE1 (2 µg/ml) |                                  |
| Parents     |              |                                 |
| C6TC1A      | 10           | 35                              |
| BS2         | 14           | 48                              |
| Exp.*       |              | 94                              |
| Hybrids     |              |                                 |
| CB1        | 39           | 134                             |
| CB4        | 18           | 121                             |
| Pool 2     | 20           | 122                             |
| CB2        | 20           | 120                             |
| CB10       | 17           | 115                             |
| CB9        | 9            | 98                              |
| CB3        | 15           | 96                              |
| CB1        | 17           | 90                              |
| CB12       | 17           | 84                              |
| Pool 1     | 16           | 81                              |
| CB8        | 10           | 74                              |
| CB5        | 17           | 79                              |
| CB7        | 12           | 66                              |
| CB6        | 17           | 90                              |

* Incubations for 10 min in the presence of 1 mM theophylline.

TABLE VII

Effect of PGE1 on C6RC16 X 3T3-4(E) hybrid cells

| Cell line   | Cyclic AMP* | Mean chromosome number* (range) |
|-------------|-------------|---------------------------------|
|             | pmol/mg protein |                                  |
|             | Control + PGE1 (2 µg/ml) |                                  |
| Parents     |              |                                 |
| C6RC16      | 18           | 27                              |
| 3T3-4(E)    | 17           | 110                             |
| Exp.*       |              | 107                             |

* Incubations for 10 min in the presence of 1 mM theophylline.

TABLE VI

Effect of PGE1 on C6RC16 X 3T3-4(C2) hybrid cells

| Cell line   | Cyclic AMP* | Mean chromosome number* (range) |
|-------------|-------------|---------------------------------|
|             | pmol/mg protein |                                  |
|             | Control + PGE1 (2 µg/ml) |                                  |
| Parents     |              |                                 |
| C6RC16      | 18           | 27                              |
| 3T3-4(C2)   | 6            | 52                              |
| Exp.*       |              | 106                             |
| Hybrids     |              |                                 |
| C6C25B      | 14           | 107                             |
| C6C25A      | 14           | 101                             |
| C6C24A      | 9            | 73                              |
| C6C21A      | 15           | 66                              |
| C6C20A      | 13           | 59                              |
| C6C26A      | 21           | 58                              |
| C6C26B      | 12           | 25                              |
| C6C26B      | 14           | 27                              |

* Incubations for 10 min in the presence of 1 mM theophylline.

TABLE VIII

Effect of theophylline* on responses to PGE1 and isoproterenol in parental cell lines

| Phenotype | Parental line | Theo effect |
|-----------|---------------|-------------|
| Theo+     | BS2 (P)       | 1.0         |
| PGE1 (I)  |               |             |
| C6TC1A (I)| 0.9           |
| C6RC16 (I)| 0.9           |
| BRL30E (P)| 1.1           |
| BRL30E (I)| 1.1           |
| RAG       |               |             |
| N4TG1 (P) | 2.4           |
| 3T3-4(C2) (P) | 2.0       |
| Theo+     | 3T3-4(E) (P)  | 2.4         |

* There is no known stimulating hormone to test this response.

Thus, in N4TG1 and in the 3T3 clones, responses due to addition of the lines had only minimal responses: an increment of 9 to 14 pmol of cyclic AMP per mg of protein over a control level of 9 to 20 pmol of per mg of protein. These hybrid clones are not different in total chromosome number or C6CR16 marker chromosome from hybrid clones with much larger PGE1 response. Possible mechanisms responsible for such phenotypic variation are discussed in detail below and have been mentioned previously for the hybrid clone C6B3RA.

Effect of Theophylline on Parental Clones—Parental clones differed in the ability of theophylline to augment the peak levels of cyclic AMP. Some clones showed little or no effect of theophylline on peak levels of cyclic AMP, while others showed a marked increase in cyclic AMP levels. However, the mechanism by which theophylline potentiates the peak effect of PGE1 is not yet understood.
of PGE1 were doubled by exposure of cells to 1 μM theophylline (Table VIII). The size of the theophylline effect was similar at all concentrations of PGE1 utilized (10 ng per ml to 10 μg per ml) and at all times of exposure to PGE1 examined (5 to 60 min). By contrast, B82, the C6 clones, and BRL30E failed to show significant potentiation of peak accumulation in the presence of the methyl xanthine. This is true in a clone where the effects of PGE1 are relatively small (B82) and is also the case in BRL30E, where the stimulatory effects on cyclic AMP accumulation are large with both PGE1 (70-fold) and isoproterenol (150-fold). Theophylline has a limited ability to augment cyclic AMP concentration in the Theo- lines if extended times of incubation are examined. While all of the clones have detectable cyclic nucleotide phosphodiesterase activity, a more detailed study of the specific activities and properties of these enzymatic activities has not yet been done.

Effect of Theophylline on Hybrid Clones—A number of the matings described above have been between clones showing no potentiation of peak accumulation of cyclic AMP by theophylline (Theo- × Theo-) and between Theo- and Theo+ parental clones.

Examples of the data obtained with the two types of matings are shown in Table IX. The fusion of two clones insensitive to PGE1 resulted in a series of hybrid clones (Table VIII). The fusion of two clones insensitive to PGE1, and at all times of exposure to PGE1 examined (5 to 60 min). The difference between the clonal cell lines with different phenotypes. Somewhat different results were obtained from PGE1 × Theo+ matings, theophylline ratios were 2.8 (B82 × N4TGl), 2.4 (C6RC16 × 3T3-4(E) stimulated by PGE1), and 2.2 (C6RC16 × 3T3-4(E) stimulated by isoproterenol).

| Parental phenotypes | Matings | Number of hybrid clones showing |
|---------------------|---------|-------------------------------|
| Theo+ × Theo+       | C6RC16 × BRL30E | 5 | 0 |
|                     | C6RC16 × 3T3-4(E) | 7 | 0 |
| Theo+ × Theo-       | C6TGlA × B82 | 14 | 0 |
|                     | C6RC16 × 3T3-4(C2) | 8 | 0 |
| PGE+ × PGE-         | N4TGl × B82 | 1 | 0 |
|                     | C6TGlA × B82 | 0 | 0 |
|                     | C6RC16 × BRL30E | 1 | 0 |
|                     | C6RC16 × 3T3-4(C2) | 2 | 0 |
|                     | C6RC16 × 3T3-4(E) | 3 | 2 |
| PGE- × PGE+         | RAG × B82 | 0 | 0 |
| Theo- × N4TGl × B82 | 0 | 2 |
| Theo- × 3T3-4(C2) × C6RC16 | 0 | 0 |
| Theo- × 3T3-4(E) × C6RC16 | 0 | 0 |
| Theo- × C6TGlA × B82 | 12 | 1 |
| Theo- × C6RC16 × BRL30E | 5 | 0 |

*See footnotes to Table VIII.

Summary of mating studies performed to determine nature of genetic control of cyclic AMP metabolism.

DISCUSSION

The results presented in this and the preceding paper show that it is possible to undertake a genetic analysis of cyclic AMP metabolism in mammalian somatic cells in vitro. Clonal differences with respect to β-adrenergic and PGE1 responses were found in parental and hybrid cells. These were inherited mitotically from one cell generation to the next, and thus there must exist heritable differences in gene content or gene expression between the clonal cell lines with different phenotypes.

Current hypotheses describing mechanisms regulating cyclic AMP synthesis envision the ubiquitous occurrence of adenylyl cyclase in cells, with the cellular specificity of response to activating agents dictated by the presence or absence of a variety of specific receptors for these agents. Most tissue specific or differentiated functions are not expressed in hybrid somatic cells formed by mating clones differing qualitatively in this regard. Therefore, one might expect that cellular differences in ability to regulate cyclic AMP accumulation might be extinguished in hybrid cells.

A summary of the mating studies performed to determine the nature of inheritance of the parental phenotypes with respect to cyclic AMP metabolism is given in Table X. The responses of cells to β-adrenergic stimulators was not inherited in hybrid cells from β+ × β- matings, while stimulation by PGE1 and theophylline potentiation provide a striking contrast and were expressed.
in the majority of hybrid lines arising from crosses between cell lines differing in their abilities to respond to these agents.

These differences in inheritance suggest differences in genetic mechanisms controlling the synthesis of specific receptors for catecholamines and prostaglandins. This could imply essential differences in the basic mechanisms by which these agents augment cyclic AMP accumulation. Since hybrid cell lines can be constructed that contain the vast majority of genetic information from both parents, but that differ in the pattern of gene expression from at least one of the parents, they should provide biologically unique material for such biochemical analysis.

It is of interest that others have hypothesized an action of prostaglandins at a site that could be more proximal to adenylyl cyclase than is the position assumed to be occupied by the usual hormonal receptor. Thus Kuehl and co-workers (15) have found that a prostaglandin antagonist, 7-oxa-13-prostanoic acid, is capable of inhibiting the stimulatory effects of luteinizing hormone on cyclic AMP accumulation in the corpus luteum, a tissue in which PGE, is also capable of augmenting the accumulation of cyclic AMP. On the basis of these and other data, they have proposed that prostaglandins are essential intermediates in the action of luteinizing hormone (16). Other possible interpretations are also discussed. The prostaglandin could, in this model, act directly on the adenylyl cyclase or at some unknown intermediate point in the hypothetical reaction scheme. Binding activity for prostaglandins, with specificity for those of the E series, has recently been detected in adipocytes by Kuehl and Humes (17).

The prevalence of positive responses to PGE, among the cultured cell lines studied also requires comment. While the sample is small, all lines but one were responsive. We are particularly surprised by the fact that large responses to PGE, are seen with lines of neural and hepatic origin. Preparations derived from normal brain and liver have not been described that show such effects, and it is possible that such responses are related to the "less differentiated status" of those cells. If these speculations have any validity, studies of the effect of prostaglandins on the accumulation of cyclic AMP during tissue development could be revealing. Such data have not, to our knowledge, been obtained.

Mechanisms underlying the differences seen with regard to the effect of theophylline are also unclear, although susceptible to experimentation. It is very possible that the effects seen could be related primarily to the relative specific activities of cyclic nucleotide phosphodiesterases in the various parental cell lines, and experiments to determine this are being initiated. However, the existence of multiple forms of such phosphodiesterase activity and the fact that preparations derived from brain also fail to show effects of theophylline to potentiate neurohormonal stimulation of cyclic AMP accumulation indicate that the phenomenon could be much more complex. It is hoped that the availability of series of hybrid cell lines, in addition to the various parental clones, will be of aid in the elucidation of the biochemical mechanisms involved in the control of cyclic AMP hydrolysis.

The assignment of certain isozymes to individual chromosomes is progressing rapidly (18, 19). Although detailed assignment of functions related to cyclic AMP metabolism to particular chromosomes has not as yet been made, it should be possible, using these or other interspecific hybrids, to make such assignment of tissue specific functions. In this regard, it is of particular interest to note matings where several phenotypic classes occurred in the hybrid cell progeny. In the C6RCl × 3T3-4(C2), C6RCl × 3T3-4(E), and C6RCl × BRL30E crosses, a few hybrid clones were found with PGE responses different from the majority of the hybrids. Because the vast majority of the chromosomes are retained in these cells, it is reasonable to presume that the PGE response is localized to one or a few chromosomes.

The different PGE phenotypes found in these few hybrid cells also could have other explanations. Since populations of cells were studied biochemically in phenotyping the parental lines, it is possible these clones have become heterogeneous with respect to gene content or gene regulation. Thus it will be important to subclone parental lines, follow the phenotype of the subclones, and use these as parents in matings.

Characterized parental cell lines like those reported here represent test reagents for a genetic classification of the mechanisms regulating cyclic AMP metabolism. We have found β4 responses extinguished, while PGE and Theo responses were retained. It would be of great interest to discover crosses where different results obtain. The parental cell lines involved in these matings would be genetically distinguishable from those reported here, despite their phenotypic similarity.

Another feature that should be examined is the role of intracellular cyclic AMP in regulating cell proliferation and contact inhibition in vitro. Information is available that suggests that high endogenous levels of cyclic AMP could slow cell growth and locomotion and inhibit growth to higher densities of some cell lines (20–23). Thus there could be a selective advantage, under permissive culture conditions, for those cells that had lost the ability to elevate cyclic AMP levels. It should be possible to take advantage of such control mechanisms to select for hybrid cells or their progeny that have lost specific receptors.

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John D. Minna and Alfred G. Gilman

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