7. P. A. Edwards and K. V. Shooter, *Biochem. J.* **114**, 227 (1969); A. B. Barclay and R. Eason, *Biochim. Biophys. Acta* **269**, 37 (1972).
8. L. Levine and H. Van Vunakis, *Methods Enzymol.* **11**, 928 (1967).
9. B. D. Stollar and M. Ward, *J. Biol. Chem.* **245**, 1261 (1970); E. Wasserman and L. Levine, *J. Immunol.* **87**, 290 (1960).
10. D. Goldblatt and M. Bustin, *Biochemistry* **14**, 1689 (1975).
11. L. Pothier, J. F. Gallagher, C. E. Wright, P. R. Libby, *Nature (London)* **255**, 350 (1975).
12. D. R. van der Westhuyzen and C. von Holt, *FEBS Lett.* **14**, 333 (1971).
13. V. G. Allfrey, V. C. Littau, A. E. Mirsky, *J. Cell Biol.* **21**, 213 (1964).
14. S. Panyim, D. Bilek, R. Chalkley, *J. Biol. Chem.* **246**, 4206 (1971).
15. E. Whittle, D. E. Bushnell, V. R. Potter, *Biochim. Biophys. Acta* **161**, 41 (1968).
16. K. Wakabayashi, S. Wang, L. S. Hmlica, *Biochemistry* **13**, 1027 (1974).
17. E. W. Johns, *Biochem. J.* **92**, 55 (1964).
18. J. Bartley and R. Chalkley, *J. Biol. Chem.* **245**, 4286 (1970).
19. J. L. Fahey, P. F. McCoy, M. Goulian, *J. Clin. Invest.* **37**, 272 (1958).
20. No. 282a, obtained from B. D. Stollar.
21. C. Dick and E. W. Johns, *Exp. Cell Res.* **51**, 626 (1968); D. E. Comings and E. Avelino, *ibid.* **86**, 202 (1974).
22. L. S. Desai, L. Pothier, G. E. Foley, R. A. Adams, *ibid.* **70**, 468 (1972).
23. R. Ziccardi and V. Schumaker, *Biochemistry* **12**, 3231 (1973); H. J. Li, C. Chang, M. Weiskopf, B. Brand, A. Rotter, *Biopolymers* **13**, 649 (1974).
24. S. Panyim and R. Chalkley, *Arch. Biochem. Biophys.* **130**, 337 (1969).
25. Supported by NIH grants AI-06860, GM-18153, and CA-12504 and by the National Foundation–March of Dimes. We thank G. Mihalakis and Dr. Giannis Stavrianopoulos for advice and assistance.

15 December 1975; revised 6 February 1976

## Neurotransmitter Regulation of Adenosine 3′,5′-Monophosphate in Clonal Nerve, Glia, and Muscle Cell Lines

Abstract. *Norepinephrine increases the intracellular level of adenosine 3′,5′-monophosphate (cyclic AMP) in clonal cell lines of nerve, glia, smooth muscle, and skeletal muscle. The largest response is in skeletal muscle, where the cyclic nucleotide concentration is elevated more than 500-fold. Glia and muscle cells, but not nerve cells, respond to dopamine with increased cyclic AMP accumulation. This response appears to be mediated through a beta-adrenoreceptor.*

The adenosine 3′,5′-monophosphate (cyclic AMP) content of many neural and muscle preparations is altered by exposure to putative neurotransmitters (*1–3*). However, because of the cellular heterogeneity in vivo or in tissue slices, it has proved difficult to characterize the responsive cells. To circumvent this difficulty, both primary cultures (*4, 5*) and clonal cell cultures (*5–9*) have been employed; only the latter can unambiguously define the responsive cell. Of the limited number of glia cell lines studied to date, some responded to catecholamines with the elevation of intracellular cyclic AMP, while the only neuronal cell line examined, the C1300 neuroblastoma, with one exception, did not (*5–10*). The recent availability of a larger collection of both nerve and glia cell lines from the rat central nervous system (*11*) and several skeletal (*12*) and smooth muscle (*13*) cell lines presents the opportunity to extend these studies to a larger number of cells. In this report we show that some nerve, glia, and muscle cells are capable of responding to catecholamines by elevating endogenous cyclic AMP; these cells appear to be much less sensitive to the other putative neurotransmitters.

Of the cell lines employed, B35 Cl3, B50 Cl5, B65 Cl27, B103 Cl4, and B104 Cl7 are neuronal, as defined by electrical excitability and neurotransmitter synthesis, while B11 Cl4, B12 Cl1, B19 Cl4, B23 Cl8, B27 Cl1, B28 Cl6, B49 Cl1, B90 Cl2, B92 Cl5, B111 Cl1, C6B, and RN2 are of glial origin (*6, 11, 14*). Line L6 is skeletal muscle (*12*) and $BC_3H1$ Cl9 is smooth muscle (*13*). In all cases early passages of the individual clones were used. All cells were grown in Vogt's modified Eagle's medium (*15*) containing 10 percent fetal calf serum, as described previously (*11*). The assay conditions were the same as those described for clonal glia cells (*6*). Cells were incubated for 60 minutes with $10^{-3}M$ theophylline, followed by the addition of the freshly prepared putative neurotransmitters, analogs, or drugs at the indicated concentrations. After 15 minutes, the cells were extracted with trichloroacetic acid and the cyclic nucleotides assayed (*16*). The data are expressed both as the actual amount of cyclic nucleotide and as the percentage change relative to control cultures, which were treated identically except for the addition of the test compound. Unless otherwise indicated, stationary phase cells were used since these cells usually express a more highly differentiated phenotype than exponentially growing cells (*11, 13*).

Table 1 shows that of the seven neurotransmitters or neurotransmitter analogs examined, only dopamine and norepinephrine had a stimulatory effect on cyclic AMP; dopa, γ-aminobutyric acid, histamine, carbamylcholine, and 5-hydroxytryptamine had no significant effects. Two of the five neuronal lines responded to norepinephrine with an increase in endogenous cyclic AMP, while nine of the eleven glial lines responded; the response was larger in the majority of the glial lines than in the nerve cells. The only qualitative difference between the nerve and glia cells was the dopamine response associated with glia cells. This may, however, be due to the generally higher catecholamine sensitivity of the glia cells. The basal cyclic AMP levels in nerve (16.6 ± 8.3 pmole per milligram of protein, $N = 5$) and glia (15.1 ± 8.1 pmole/mg, $N = 11$) were not significantly different.

The L6 skeletal muscle line responded to both norepinephrine and dopamine with increases in intracellular cyclic AMP, and the fused fibers were less responsive to norepinephrine than the exponentially dividing myoblasts. In contrast, the magnitude of the response to catecholamines was less in the $BC_3Hl$ smooth muscle cells, and the dividing $BC_3H1$ myoblasts responded less to norepinephrine than did stationary phase cultures.

Both norepinephrine- and dopamine-responsive adenylate cyclase systems have been described in the mammalian central nervous system (*1, 2, 17*). These classes of receptors can be distinguished on the basis of their pharmacological properties. Thus the dopamine system is not activated by the potent β-adrenoreceptor stimulant isoproterenol, nor is the dopamine response blocked by β-receptor antagonists such as propanolol (*17*). The dopamine response is, however, blocked by high concentrations of α-adrenoreceptor antagonist drugs and by a defined sequence of antipsychotic drugs (*17*). When examined by these criteria, catecholamine-induced cyclic AMP responses in the cell lines appeared to function through a β-adrenoreceptor. In the cell lines where dopamine elicited a cyclic AMP increase greater than 1.5-fold, a similar increase was generated by at least a 10-fold lower concentration of isoproterenol or norepinephrine. In addition, the responses are inhibited by β-blocking agent propanolol but not as well by phentolamine, an α-blocking agent (Table 2). The tricyclic antidepressant imipramine and various antipsychotic drugs block the dopamine response in both the myoblast and glia cells to varying extents, but not in the order characteristic of the previously defined dopamine receptors (*17*).

On the basis of these data, it can be concluded that nerve, glia, and both smooth and skeletal muscle cells can respond to norepinephrine by accumulating intracellular cyclic AMP. The catecholamine response has been well studied in certain types of smooth muscle,

where it may be involved in membrane resting potential changes (*2, 3*). The role of cyclic AMP in mediating the catecholamine effect on glycogen metabolism in skeletal muscle is well documented (*18*). The glial response to catecholamines has been examined in a few clonal cell lines; they are qualitatively similar to those reported here (*5, 6, 9*). These data also indicate that some of the nerve cells studied respond to norepinephrine by accumulating cyclic AMP. Experiments have been presented which suggest that cyclic AMP may mediate the catecholamine response in Purkinje cells (*19*), and a dopamine response has been reported in one clone of the Cl300 neuroblastoma (*10*). Finally, the cyclic AMP responses to catecholamines seen in nerve, muscle, and glia cells seem to be cell type–specific, as suggested by the selective response of glia cells to dopamine and the fact that not all nerve and glia cells are responsive to norepinephrine.

Little is known about the role of catecholamine-stimulated cyclic AMP synthesis in the central nervous system. This phenomenon may be involved in some form of specific intercellular communication (*6*), or it may have a critical role in metabolic regulation (*2, 20*). A third alternative, perhaps incorporating both of the above, is that the neurotransmitter-mediated cyclic AMP fluctuations may mediate the differentiation of nerve and glia cells (*21*).

Table 1. Effect of putative neurotransmitters on cyclic AMP levels in clonal cell lines. Cells were incubated for 60 minutes with $1 \times 10^{-3}M$ theophylline. Either $1 \times 10^{-4}M$ norepinephrine, dopa, or dopamine was added for 15 minutes, and the cyclic AMP levels were determined. Data are presented as picomoles of cyclic AMP per milligram of total cellular protein ± the standard deviation of six determinations. Numbers in parentheses give the increase relative to control cultures to which medium alone was added, expressed as the ratio of experimental to control values. The following compounds were tested at $1 \times 10^{-4}M$ and produced no detectable (< 1.1-fold) alteration in cyclic AMP levels: γ-aminobutyric acid, carbamylcholine, histamine, and 5-hydroxytryptamine.

| Cell line | Cell type | Cyclic AMP (pmole/mg) | | | |
|---|---|---|---|---|---|
| | | Control | Norepinephrine | Dopa | Dopamine |
| B35 Cl3 | Nerve | 22.7±2.1 | 23.8±3.6 | 24.5±0.9 | 25.0±3.0 |
| B50 Cl5 | Nerve | 24.6±3.1 | 71.8±1.3 (3.0) | 21.7±3.0 | 28.1±0.9 |
| B65 Cl27 | Nerve | 4.1±0.3 | 4.3±1.0 | 3.8±0.2 | 3.7±0.7 |
| B103 Cl4 | Nerve | 18.5±2.1 | 25.5±1.1 (1.4) | 17.2±1.0 | 19.8±2.3 |
| B104 Cl7 | Nerve | 13.0±1.3 | 13.5±0.9 | 11.5±1.1 | 14.4±2.5 |
| B12 Cl1 | Glia | 8.3±0.9 | 33.2±1.5 (4.0) | 8.2±1.0 | 9.7±0.5 (1.2) |
| B19 Cl4 | Glia | 10.7±0.8 | 139±10 (13) | 7.4±0.7 | 23.9±1.3 (2.2) |
| B23 C18 | Glia | 12.0±1.4 | 1587±10 (132) | 12.9±0.9 | 41.3±5.6 (3.4) |
| B27 Cl1 | Glia | 22.0±3.0 | 56.1±3.0 (2.5) | 21.7±0.9 | 28.1±0.9 (1.3) |
| B28 Cl6 | Glia | 26.3±0.9 | 27.3±1.4 | 20.5±1.3 | 26.7±1.5 |
| B49 Cl1 | Glia | 9.5±1.1 | 82.5±3.9 (8.6) | | 20.3±0.8 (2.1) |
| B90 Cl2 | Glia | 27.8±4.4 | 27.5±3.0 | 26.3±0.5 | 34.2±1.0 (1.2) |
| B92 Cl5 | Glia | 14.4±4.9 | 915±62 (64) | 6.7±2.8 | 451±48 (31) |
| B111 Cl1 | Glia | 5.6±0.8 | >1300 (>235) | | 21.9±6.0 (3.9) |
| C6B | Glia | 6.8±0.6 | 15.0±1.0 (2.2) | 7.7±0.4 | 11.3±0.7 (1.7) |
| RN2 | Glia | 22.3±3.8 | 268±5 (12) | 24.4±0.9 | 46.1±6.1 (2.1) |
| L6 | Skeletal muscle myoblast | 15.6±1.1 | 8074±500 (518) | 14.9±1.7 | 257±20 (16) |
| L6 | Skeletal muscle myotubes | 19.4±1.7 | 2951±219 (152) | 21.3±0.6 | >194 (>9.6) |
| $BC_3H1$ Cl9 | Smooth muscle undifferentiated cells | 40.2±4.4 | 636±24 (16) | 27.3±4.5 | 100±9.1 (2.5) |
| $BC_3H1$ Cl9 | Smooth muscle differentiated cells | 13.6±0.4 | 1328±77 (92) | 12.4±1.0 | 55.0±4.7 (4.0) |

Table 2. Inhibition of dopamine response by various drugs. Drugs were tested in triplicate at each of six to ten concentrations between $10^{-4}$ and $10^{-7}M$, with a constant dopamine concentration of $10^{-4}M$. Data are presented as the concentration of drug which gave 50 percent inhibition of the cyclic AMP response to dopamine.

| Drug | Concentration ($\mu M$) | | | |
|---|---|---|---|---|
| | L6 (myoblast) | B19 Cl4 (glia) | B92 Cl5 (glia) | B111 Cl1 (glia) |
| Propanolol | <0.1 | <0.1 | <0.1 | <0.1 |
| Phentolamine | 10 | >100 | >100 | >100 |
| Imipramine | 5 | | 8 | 40 |
| Promethazine | 17 | 10 | 1 | 8 |
| Fluphenazine | 15 | 10 | 10 | |
| Triflupromazine | 3 | | 3 | 30 |
| Thioridazine | 18 | | 3 | 10 |
| Haloperidol | >100 | 30 | >100 | >50 |
| Benztropine | 16 | >100 | 4 | >100 |


DAVID SCHUBERT
HELGI TARIKAS
MONIQUE LACORBIERE
*Department of Neurobiology, Salk Institute, San Diego, California 92112*


### References and Notes


1. M. Chasin, I. Rivkin, F. Mamrak, S. G. Samaniego, S. M. Itless, *J. Biol. Chem.* **246**, 3037 (1971); J. W. Kebabian and P. Greengard, *Science* **174**, 1346 (1971); T. W. Rall and A. Sattin, *Psycopharmacology* **3**, 113 (1970); W. P. Burkard, *J. Neurochem.* **19**, 2615 (1972); T. P. Lee, J. F. Kuo, P. Greengard. *Proc. Natl. Acad. Sci. U.S.A.* **69**, 3287 (1972); K. V. Hungen and S. Roberts, *Eur. J. Biochem.* **36**, 391 (1973); J. W. Daly, M. Huang, H. Shimizu, in *Advances in Cyclic Nucleotide Research*, P. Greengard and G. A. Robison, Eds. (Raven, New York, 1972), vol. 1, pp. 375–395.
2. R. Andersson, L. Lundholm, E. Mohme-Lundholm, K. Nilsson, in *Advances in Cyclic Nucleotide Research*, P. Greengard and G. A. Robison, Eds. (Raven, New York, 1972), vol. 1, pp. 213–229.
3. A. P. Somlyo, A. V. Somlyo, V. Smiesko, in *ibid.*, p. 175.
4. A. G. Gilman and B. K. Schrier, *Mol. Pharmacol.* **8**, 410 (1972).
5. A. G. Gilman, in *Advances in Cyclic Nucleotide Research*, P. Greengard and G. A. Robison, Eds. (Raven, New York, 1972), vol. 1, pp. 389–410.
6. ——— and M. Nirenberg, *Proc. Natl. Acad. Sci. U.S.A.* **68**, 2165 (1971).
7. ———, *Nature (London)* **234**, 356 (1971).
8. J. Schultz, B. Hamprecht, J. W. Daly, *Proc. Natl. Acad. Sci. U.S.A.* **69**, 1266 (1972).
9. R. B. Clark, R. Gross, Y. F. Su, J. P. Perkins, *J. Biol. Chem.* **249**, 5296 (1974).
10. K. N. Prasad and K. N. Gilmer, *Proc. Natl. Acad. Sci. U.S.A.* **71**, 2525 (1974).
11. D. Schubert, S. Heinemann, W. Carlisle, H. Tarikas, B. Kimes, J. Patrick, J. H. Steinbach, W. Culp, B. L. Brandt, *Nature (London)* **249**, 224 (1974).
12. D. Yaffe, *Proc. Natl. Acad. Sci. U.S.A.* **61**, 477 (1968).
13. D. Schubert, A. J. Harris, C. E. Devine, S. Heinemann, *J. Cell Biol.* **61**, 398 (1973).
14. S. Pfeiffer and W. Wechsler, *Proc. Natl. Acad. Sci. U.S.A.* **69**, 2885 (1972).
15. M. Vogt and R. Dulbecco, *ibid.* **49**, 171 (1963).
16. A. L. Steiner, C. W. Parker, D. M. Kipnis, *J. Biol. Chem.* **247**, 1106 (1972); J. Watson, *J. Exp. Med.* **141**, 97 (1975).
17. L. L. Iversen, *Science* **188**, 1084 (1975); M. Karobath and H. Leitich, *Proc. Natl. Acad. Sci. U.S.A.* **71**, 2915 (19 Y. C. Clement-Cormier, J. W. Kebabian, G. L. Petzold, P. Greengard, *ibid.*, p. 1113.
18. S. E. Mayer and J. T. Stull, *Ann. N.Y. Acad. Sci.* **185**, 433 (1971).
19. B. J. Hoffer, G. R. Siggins, F. E. Bloom, in *Role of Cyclic AMP in Cell Function*, P. Greengard and E. Costa, Eds. (Raven, New York, 1970).
20. J. DeVellis and G. Kukes, *Texas Rep. Biol. Med.* **31**, 271 (1973); R. W. Newburgh and R. N. Rosenberg, *Proc. Natl. Acad. Sci. U.S.A.* **69**, 1677 (1972).
21. D. McMahon, *Science* **185**, 1012 (1974); P. Furmanski, D. S. Silverman, M. Lubin, *Nature (London)* **233**, 413 (1971); D. Schubert, *Neurobiology* **4**, 376 (1974).


22. Supported by grants from the National Institutes of Health, the National Foundation, and the Muscular Dystrophy Associations of America. D.S. is supported by a faculty research award from the American Cancer Society. We thank S. Snyder for the antipsychotic drugs.

14 May 1975, revised 30 January 1976