Fig. 1. The Benjamin Franklin and Timothy Folger chart of the Gulf Stream printed by Mount and Page in London in about 1769–1770. Two prints of this chart were found in the Bibliothèque Nationale in Paris. [Courtesy Bibliothèque Nationale, Paris]

occurred to me that a copy of the chart might have been saved by the French because Franklin was envoy to France from 1776 to 1785 and both Franklin and his ideas were highly regarded by the French. It was clear that the two charts that I found were examples of Franklin and Folger's 1769–1770 chart for three reasons. First, the Gulf Stream has the same characteristic configuration seen in later versions. Second, the legend, the speeds of the Gulf Stream, and the printed remarks agree with Franklin's 1768 correspondence concerning the chart. Third, a later version of the chart printed by Le Rouge in Paris, which was also in the Bibliothèque Nationale, matches the first chart perfectly and therefore is a direct copy of it. The 1786 American version, frequently referred to as the 1769–1770 chart, is not a direct copy of the original chart; the projection is different, the Gulf Stream has been modified, and there are inaccuracies such as the position of Bermuda.

The Franklin-Folger chart measures 86 by 96 cm; it consists of four separate subcharts joined together along 16°N and 32.5°W. The prime meridian falls through Lizard Point on the southwest coast of England, 5.2° west of Greenwich. The legend on the chart is as follows:

A New and Exact Chart of
Mr. E. Wrights projection, rut: Mercators
Chart, con: the Sea Coast of
EUROPE, AFRICA, &
AMERICA, from the Isles of Orkney to Cape
Bona Esperance, & Hudsons Bay to the
Straits of Magellan
According to the Observations of
Capt. E. Halley, fellow of the R: S.
To the Rt. Honorable and Principle Officers &
Commissioners of his Majesties
Navy, This chart is most humbly dedicated
and presented by
their most obedient faithfull servants
John Mount & Th. Page.
Sold by
Jno. Mount and Tho. Page at
The Postern on Great Tower Hill London

Located east of Newfoundland on the chart are Folger's instructions on how to avoid the Gulf Stream and banks and shoals when sailing westward. These remarks were clarified and amplified by Franklin and were included with his 1786 chart.

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**References and Notes**

1. For a description of the history and significance of the Franklin-Folger chart see: F. Bache, *Proc. Am. Philos. Soc.* **76**, 731 (1936); L. Brown (*2*); L. De Vorsey (*3*).
2. L. Brown, in *Essays Honoring Lawrence C. Wroth* (Anthoensen, Portland, Maine, 1951), p. 69.
3. L. DeVorsey, *Imago Mundi* **28**, 105 (1976).
4. B. Franklin, *Trans. Am. Philos. Soc.* **2**, 294 (1786).
5. W. G. DeBrahm, *The Atlantic Pilot* (T. Spilsbury, London, 1772). See (*3*).
6. H. Stommel, *The Gulf Stream: A Physical and Dynamical Description* (Univ. of California Press, Berkeley, 1958); F. C. Fuglister, in *Progress in Oceanography* (Pergamon, London, 1963), vol. 1; L. V. Worthington, in *On the North Atlantic Circulation* (vol. 6 of the Johns Hopkins Oceanographic Series, Johns Hopkins Univ. Press, Baltimore, 1976); C. Mann, *Deep-Sea Res.* **14**, 337 (1967).
7. The address of the Bibliothèque National is 58 rue Richelieu, 75084 Paris, Cedex 02, France. The charts are located in the Department des Cartes et Plans and are identified as follows: Portefeuille 117, Division O, Pièces 7 and 7′, Mount and Page: A New and Exact Chart. Photographic copies of the chart may be obtained from the Service Photographique of the Bibliothèque Nationale, which provided several copies for me, one of which is shown in Fig. 1. Since this report was written, L. DeVorsey has identified a third copy of the Franklin-Folger chart in the Naval Library, Ministry of Defence, Empress State Building, Lillie Road, London, S.W. 6, England.
8. I thank F. C. Fuglister, who suggested to me that the Franklin-Folger map of the Gulf Stream was a very good summary of the Gulf Stream and who spent many hours discussing various aspects of this body of water with me. I thank the librarians at the Bibliothèque Nationale who were very helpful in my search for the Mount and Page chart and the Le Rouge chart. Funds for this work were provided by the Office of Naval Research under contract N00014-74-C-0262, NR 083-004, and the United States–France Exchange of Scientists Program, which is funded by the National Science Foundation and the Centre National de la Recherche Scientifique. This report was written during a year that I spent at the Muséum National d'Histoire Naturelle in Paris. I thank H. Lacombe and J. Gonella who helped arrange my visit and all the members of the Laboratoire d'Océanographie Physique who made my stay a pleasant one. Contribution 4421 from the Woods Hole Oceanographic Institution.

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# β-Adrenergic Regulation of $\alpha_2$-Adrenergic Receptors in the Central Nervous System

Abstract. *Incubation of rat cerebral cortical slices with the β-adrenergic agonist isoproterenol causes an increase in $\alpha_2$-adrenergic receptor binding in addition to a decrease in β-adrenergic receptor binding. The effects are rapid and reversible, show a parallel time course, and are blocked by sotalol, a β-adrenergic receptor antagonist. The β-mediated regulation of $\alpha_2$-receptor sensitivity at brain norepinephrine synapses may be a mechanism for the homeostatic control of central noradrenergic activity.*

Modification of receptor sensitivity appears to be one of the methods by which hormone action, or synaptic chemical transmission, is regulated. At norepinephrine (NE) synapses, long-term receptor stimulation in vivo desensitizes receptors, whereas transmitter depletion, NE terminal destruction, or long-term receptor blockade supersensitizes both α- and β-receptors (*1–3*). Furthermore, a rapid and reversible β-receptor desensitization occurs in rat brain tissue in vitro after it is exposed to agonist (*4*). With regard to localization and function, α-adrenergic receptors have been subdivided into those mediating the postsynaptic or postjunctional response to NE ($\alpha_1$-receptors), and those controlling the release of NE from presynaptic NE terminals at peripheral sympathetic junctions and at central noradrenergic synapses ($\alpha_2$-receptors) (*5*). We have studied alterations in brain $\alpha_2$-receptor sensitivity in response to in vitro pharmacological manipulation of central noradrenergic synaptic function. The results indicate that long-term activation of the β-adrenergic system, while decreasing the number of β-receptors, rapidly and reversibly increases the number of $\alpha_2$-receptors at central noradrenergic synapses.

Cerebral cortical slices (0.26 by 0.26 by 2.0 mm) from adult male Sprague-Dawley rats were transferred in gauze bags from ice-cold oxygen-saturated buffer (*6*) to vials containing fresh buffer at 37°C. After initial incubation (with one change of buffer) in drug-free medium for 40 minutes (in an atmosphere of 95 percent $O_2$ and 5 percent $CO_2$) in a shaking bath, 100 $\mu M$ (−)-isoproterenol was added to the medium, and incubation was maintained at 37°C. At various times after the addition of isoproterenol, slices were transferred to ice-cold tris-saline buffer and centrifuged three times at 1200*g* for 30 seconds at 4°C, with intermediate resuspension in fresh cold buffer. The slices were then resuspended in cold 0.05*M* tris-HCl buffer (*p*H 7.7 at 25°C), disrupted with a Brinkmann Polytron, and the resulting membranes washed twice by centrifugation.

Assays for β-receptor binding, using [$^3$H]dihydroalprenolol (New England Nuclear, 58.5 Ci/mmole) as a ligand, and $\alpha_2$-receptor binding, using *p*-[$^3$H]aminoclonidine (New England Nuclear, 46 Ci/mmole) as a ligand, were performed (*2, 7*). Membranes derived from slices were incubated at 25°C in triplicate with various concentrations of [$^3$H]-dihydroalprenolol for 20 minutes or


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Table 1. Prevention by sotalol of isoproterenol-induced changes in rat cerebral cortex binding of [$^3$H]dihydroalprenolol to $\beta$-adrenergic receptors and of *p*-[$^3$H]aminoclonidine to $\alpha_2$-adrenergic receptors. Slices of cortex were incubated for 120 minutes with 100 $\mu M$ (−)-isoproterenol, 20 $\mu M$ sotalol, or 100 $\mu M$ (−)-isoproterenol plus 20 $\mu M$ sotalol. Control slices were incubated with an equal volume of 0.1 percent ascorbic acid vehicle. Membranes were subsequently prepared from the slices and incubated under standard assay conditions with four to five concentrations of [$^3$H]dihydroalprenolol (0.1 to 30 n$M$) and five concentrations of *p*-[$^3$H]aminoclonidine (0.1 to 10 n$M$), with appropriate blanks to determine specific binding. Values for $B_{max}$ and $K_d$ are means ± standard errors (S.E.) for six experiments. Significant differences from control were determined by two-tailed *t*-tests.

| Treatment | [$^3$H]Dihydroalprenolol | | *p*-[$^3$H]Aminoclonidine | |
|---|---|---|---|---|
| | $B_{max}$ (pmole per milligram of protein) | $K_d$ (n$M$) | $B_{max}$ (pmole per milligram of protein) | $K_d$ (n$M$) |
| Control | 0.55 ± 0.05 | 2.4 ± 0.2 | 1.2 ± 0.15 | 1.6 ± 0.2 |
| Isoproterenol | 0.23 ± 0.03** | 2.3 ± 0.2 | 1.8 ± 0.2* | 1.5 ± 0.2 |
| Sotalol | 0.62 ± 0.08 | 3.7 ± 0.5* | 1.3 ± 0.1 | 1.5 ± 0.3 |
| Sotalol + isoproterenol | 0.50 ± 0.07 | 4.9 ± 0.4** | 1.2 ± 0.2 | 1.3 ± 0.2 |

*$P < .05$. **$P < .01$.

*p*-[$^3$H]aminoclonidine for 30 minutes. Binding assays were terminated by rapid filtration under vacuum over filters (Whatman GF/B) with three 5-ml rinses of cold buffer. The filters were counted by liquid scintillation spectrometry. Concurrent incubation with 1.0 $\mu M$ phentolamine or 0.4 $\mu M$ (±)-propranolol was used to define specific ligand binding to $\alpha_2$-receptors and $\beta$-receptors, respectively. Protein concentrations were determined by the method of Lowry (*8*). The $^3$H-labeled WB-4101 (New England Nuclear, 25 Ci/mmole) binding to cortex $\alpha_1$-receptors was determined (*9*).

Incubation of cortical slices with 100 $\mu M$ (−)-isoproterenol for 120 minutes reduced the number of [$^3$H]dihydroalprenolol $\beta$-receptor binding sites by 40 to 60 percent ($P < .01$) (Table 1) (*4*, *10*). In contrast, in these same tissues, the number of *p*-[$^3$H]aminoclonidine $\alpha_2$-receptor sites was significantly increased by 50 to 60 percent ($P < .01$), with no change in the receptor affinity ($K_d$) for this ligand (Table 1).

To ensure pharmacological specificity of the $\beta$-receptor decrease, slices were coincubated for 120 minutes with 100 $\mu M$ (−)-isoproterenol and 20 $\mu M$ sotalol, a specific $\beta$-receptor blocking agent (*4*). Sotalol coincubation completely prevented the increase in the maximum density of *p*-[$^3$H]aminoclonidine binding ($B_{max}$) in addition to preventing the decrease in the $B_{max}$ of [$^3$H]dihydroalprenolol binding (Table 1). The lowered binding affinity of [$^3$H]dihydroalprenolol in sotalol-treated slices was presumably due to the continued presence of residual sotalol in the tissue (*11*). This blockade of the $\alpha_2$-response to isoproterenol by sotalol suggests that the isoproterenol-induced increase in $\alpha_2$-receptor binding depends on the activation of $\beta$-receptors and is not due to a weak direct $\alpha_2$-receptor agonist action of isoproterenol (*9*).

Previous studies have shown that the decrease in $\beta$-receptor binding in brain tissue in vitro has a rapid onset (*4*). To determine if the onset of the $\alpha_2$-receptor binding increase was equally rapid, we examined its time course. A maximum reduction in [$^3$H]dihydroalprenolol binding sites, apparent 30 to 60 minutes after incubation, was sustained for 120 minutes, in accord with previous data (Fig. 1) (*10*). The increase in the number of $\alpha_2$-receptor sites appeared to follow a similar, or possibly more rapid, time course, with maximum elevation occurring after 30 minutes of incubation; the increase was sustained at least another 90 minutes (Fig. 1). Over the 120-minute incubation period, no significant alteration was observed in $\alpha_2$- or $\beta$-receptor number in slices exposed to isoproterenol-free medium. In addition, no alteration was observed in the binding of [$^3$H]WB-4101 to cortex $\alpha_1$-receptors in the same isoproterenol-incubated tissues over this time period (Fig. 1). Furthermore, preliminary experiments indicated that, like the $\beta$-receptor decrease (*4*, *10*), the $\alpha_2$-receptor increase is reversible, returning to control values after 30 minutes of reincubation in drug-free medium (data not shown).

The determination of the precise mechanism of this inverse modulation of central nervous system $\alpha_2$- and $\beta$-adrenergic receptors depends on a better understanding of the synaptic location of the relevant $\alpha_2$-receptor population. Since the majority of brain $\alpha$-receptors do not appear to be located on NE terminals (*2*, *3*), it is conceivable that the $\alpha_2$-receptor population susceptible to isoproterenol-induced modulation is located on the same postsynaptic membranes as the affected $\beta$-receptors, possibly in close proximity. In any event, the inverse reciprocal modulation of adrenergic receptors at brain NE synapses may be another mechanism for the homeostatic control of central noradrenergic activity.

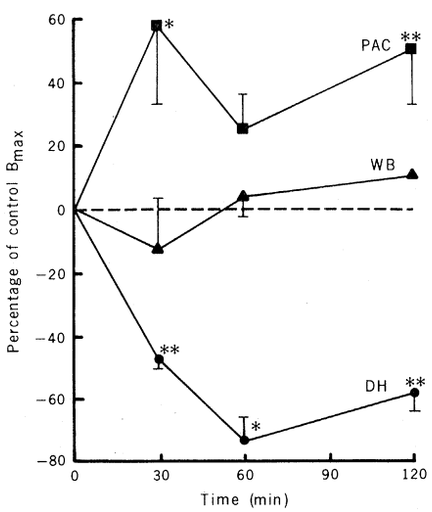


Fig. 1. Time course of isoproterenol-induced alterations of receptor binding sites in membranes from rat cerebral cortex. Receptors were labeled with [$^3$H]dihydroalprenolol (*DH*) ($\beta$-receptors), $^3$H-labeled WB-4101 (*WB*) ($\alpha_1$-receptors), and *p*-[$^3$H]aminoclonidine (*PAC*) ($\alpha_2$-receptors). Slices were incubated at 37°C with 100 $\mu M$ (−)-isoproterenol for various times and assayed with four to five concentrations of $^3$H-labeled ligand as in Table 1. Control $B_{max}$ values for all three $^3$H-labeled ligands did not vary with the time of incubation in isoproterenol-free medium, and the control values ± S.E. over the entire incubation period were *p*-[$^3$H]aminoclonidine: $B_{max}$, 1.2 ± 0.15 pmole per milligram of protein; $K_d$, 1.6 ± 0.2 n$M$; [$^3$H]dihydroalprenolol: $B_{max}$, 0.55 ± 0.05 pmole/mg; $K_d$, 2.4 ± 0.2 n$M$; WB-4101: $B_{max}$, 0.032 ± 0.003 pmole/mg; $K_d$, 0.90 ± 0.02 n$M$. Each point is the mean ± S.E. of three to ten separate experiments—for *p*-[$^3$H]aminoclonidine, 30 and 120 minutes and for [$^3$H]dihydroalprenolol, 120 minutes—or the mean ± the variation of two separate experiments. The WB-4101 point at 120 minutes represents a single experiment. Significant differences at each time point from control values were determined by *t*-tests. * $P < .05$, ** $P < .01$.


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**References and Notes**

1. W. J. Strittmatter, J. N. Davis, R. J. Lefkowitz, *J. Biol. Chem* **252**, 5478 (1977); R. J. Lefkowitz, D. Mullikin, C. L. Wood, T. B. Gore, C. Mukherjee, *ibid.*, p. 5295; M. R. Wessels, D. Mullikin, R. J. Lefkowitz, *ibid.* **253**, 3371 (1978); R. J. Lefkowitz, D. Mullikin, L. T. Williams, *Mol. Pharmacol.* **14**, 376 (1978); Y.-F. Su, L. X. Cubeddu, J. P. Perkins, *J. Cyclic Nucl. Res.* **2**, 257 (1976); J. R. Sporn, T. K. Harden, B. B. Wolfe, P. B. Molinoff, *Science* **194**, 624 (1976); D. C. U'Prichard and S. H. Snyder, *Eur. J. Pharmacol.* **51**, 145 (1978).
2. D. C. U'Prichard, W. D. Bechtel, B. R. Rouot, S. H. Snyder, *Mol. Pharmacol.* **16**, 47 (1979).
3. D. C. U'Prichard, T. D. Reisine, S. T. Mason, H. C. Fibiger, H. I. Yamamura, *Brain Res.*, in press.
4. N. Chagnon and W. Irons, Eds., *Evolutionary Acad. Sci. U.S.A.* **76**, 2057 (1979); M. D. Dibner and P. B. Molinoff, *J. Pharmacol. Exp. Ther.*, in press.
5. S. Z. Langer, *Biochem. Pharmacol.* **23**, 1973 (1974); H. D. Taube, K. Starke, E. Borowski, *N.S. Arch. Pharmacol.* **299**, 123 (1977).
6. S. Kakiuchi and T. W. Rall, *Mol. Pharmacol.* **4**, 367 (1968).
7. B. R. Rouot and S. H. Snyder, *Life Sci.* **25**, 769 (1979).
8. O. H. Lowry, N. J. Rosebrough, A. L. Farr, R. J. Randall, *J. Biol. Chem.* **193**, 265 (1951).
9. D. C. U'Prichard, D. A. Greenberg, S. H. Snyder, *Mol. Pharmacol.* **13**, 454 (1977).
10. D. C. U'Prichard and S. J. Enna, *Eur. J. Pharmacol.* **59**, 297 (1979).
11. In some experiments the β-blocker propranolol was used instead of sotalol. However, prevention of the β-receptor decrease by propranolol could not be demonstrated, because it was difficult to remove residual propranolol from the slices.


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# Hemoglobin Switching: A Cellular Model


Abstract. *Regulation of hemoglobin synthesis depends in part on the population of cells available for erythroid differentiation. Mouse erythroleukemia cells were cloned, and the clones were induced with dimethyl sulfoxide to test the relative induction of β minor and β major synthesis. Cells of line 745 produced approximately 35 percent β minor after induction, and 39 clones of line 745 produced from 23 to 61 percent β minor. Further subcloning of the clone that produced 61 percent β minor led to three subclones, all of which produced more than 90 percent β minor. Thus one kind of hemoglobin regulation occurs at the cellular level.*


Regulation of hemoglobin synthesis may occur at the cellular or the subcellular level. At the cellular level, modulation of globin expression results from growth and differentiation of cell populations with specific programs for globin synthesis. At the subcellular level, modulation depends on alterations in transcription, processing, or translation of globin genes. The relative contribution of cellular versus subcellular regulation in the types of hemoglobins produced is not established. We now present a murine model of erythropoiesis designed to determine the potential contribution of cellular selection in hemoglobin regulation. Mice of strain DBA/2 produce a major ($\alpha_2\beta_2$-major) and a minor ($\alpha_2\beta_2$-minor) hemoglobin. In utero the proportion of minor may exceed major, but in the adult the ratio of major to minor is approximately 80 to 20 (*1*). In mice, the shift from minor to major qualitatively resembles the switch from fetal to adult hemoglobin in humans. Mouse erythroleukemia cells, developed from DBA/2 mice infected with Friend leukemia viruses, provide a model for the switch. The cells grow in suspension as proerythroblasts, undergo erythroid differentiation when cultured with inducing agents, and produce mouse hemoglobin (*2*). The relative proportions of the major and minor hemoglobins depend on the inducing agent, as well as on the cell line. For example, induction of line 745 with dimethyl sulfoxide leads to synthesis of approximately 30 percent β minor while induction of the same line with hemin leads to 100 percent β minor.

Cytoplasmic messenger RNA (mRNA) which was obtained from induced erythroleukemia cells directed, in a wheat germ cell-free system, the synthesis of globin chains in a pattern similar to that seen in the induced intact cells (*3*). Thus inducing agents might alter globin gene transcription in erythroleukemia cells. However, these agents might also induce selective growth and differentiation of cells within the mass culture preprogrammed to produce a specific pattern of globin chains. To test this possibility, we cloned line 745 cells without inducing agents, and tested the pattern of β chain synthesis after induction of the clones with dimethyl sulfoxide. Cellular heterogeneity found in the induction patterns suggests that one form of hemoglobin regulation occurs at the cellular level.

Erythroleukemia cell line 745 was grown in Dulbecco's modified Eagle's medium in 10 percent fetal calf serum in an atmosphere of 10 percent $CO_2$ and passaged twice weekly (*4*). The line was in continuous culture for 2 years. For cloning, cells in the log phase of growth were suspended in medium at one cell per 2 ml, and 0.2 ml was placed in each well of Falcon Microtest II dishes. After 8 to 10 days, 10 percent of the wells had a visible clone in one area of the bottom of the well. Each clone was replated in 2 ml of medium in 35-mm Falcon dishes. When the cell density reached $1 \times 10^6$ to $3 \times 10^6$ cell/ml, the clones were replated at $2.5 \times 10^4$ cell/ml in 10 ml of medium (in 40-ml Falcon flasks) and passaged twice at that density to maintain logarithmic growth.

The cloned cells were then plated at $5 \times 10^4$ cell/ml in 10 ml of the medium described above, to which 2 percent (280 m*M*) dimethyl sulfoxide was added. After 4 days, the cells were centrifuged, and resuspended in 2 ml of medium lacking leucine, to which 25 μCi of $^3$H-labeled leucine ([3,4,5-$^3$H]leucine, 100 Ci/mmole; New England Nuclear) was added. After incubation at 37°C for 4 hours, the cells were harvested by centrifugation and washed three times with Hanks balanced salts with 1 percent bovine serum albumin. The cell pellets were lysed by the addition of 50 or 100 μl of 1 m*M* $Na_2HPO_4$ (*p*H 7.4) and incubation at 37°C for 15 minutes. Stroma and nuclei were removed by the addition of 25 μl of a mixture of carbon tetrachloride and toluene (1 : 1), vigorous vortexing, and centrifugation for 4 minutes at 10,000*g* (Brinkmann Microfuge 3200). Lysates were stored in liquid nitrogen.

Globin chains were analyzed by polyacrylamide gel electrophoresis (containing acid, urea, and 2 percent Triton X-100) (*5*) (Fig. 1). The β major, β minor, and α chains of mouse globin separate from each other and from nonglobin proteins. With this assay, globin synthesis was not detected in uninduced cells of line 745, but was seen in the electrophoresis of newly synthesized radioactive proteins from line 745 that had undergone induction with 2 percent dimethyl sulfoxide for 4 days (Fig. 1B). The gels were processed by fluorography (*6*), and the relative rates of globin chain synthesis were determined by integration of the areas under the peaks detected by densitometry (Fig. 2A). The β minor represented 32 percent of the total β chain synthesis. Line 745 was induced and globin synthesis was analyzed in 12 separate experiments (Fig. 3). The mean percentage of β minor was 35, with a range from 32 to 41, and 1 standard deviation of 3 percent.

In the absence of inducer, 39 clones of line 745 were obtained and then induced with 2 percent dimethyl sulfoxide for 4 days; globin synthesis was then evaluated (Fig. 3). The mean β minor was 32 percent, similar to the mean in the 12 studies of line 745. However, the range



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