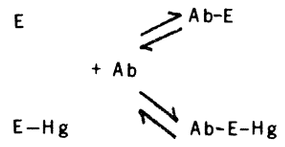
ed with estriol labeled with electroactive mercuric acetate. A small amount of estriol antibody (Ab) is added to a solution containing estriol (E) and a known amount of labeled estriol (E-Hg). The competitive binding reaction between Ab, E, and E-Hg can be expressed generally as



The amount of E-Hg in equilibrium is determined by reduction of E-Hg at a dropping mercury electrode by differential pulse polarography. The magnitude of the polarographic wave for E-Hg reduction is proportional to the concentration of E in a manner analogous to RIA.

4-Mercuric acetate estriol (E-Hg) was synthesized from estriol (Sigma Chemical Co.) by a reported procedure (*10*). A differential pulse polarogram of 4-mercuric acetate estriol in a water-ethanol mixture (90:10) with a 0.15*M* phosphate buffer (*p*H 8.0) as the supporting electrolyte gives a distinct reduction peak at −300 mV versus a standard calomel electrode (SCE). This labeled estriol is distinguishable from unlabeled estriol, which is nonelectroactive over the potential range −200 to −900 mV.

The feasibility of electrochemical immunoassay has been demonstrated by measuring the polarographic current for E-Hg in the presence of increasing amounts of specific Ab. Figure 1 shows the labeled antigen-antibody binding curve obtained when the peak current (i_p) for the reduction wave of 4-mercuric acetate estriol at −300 mV versus SCE is plotted as a function of increasing Ab concentration. The Ab concentration was varied by adding successive 100-μl portions of estriol antiserum (NEA-059A, New England Nuclear). As shown in Fig. 1, the peak current decreases as E-Hg binds to the increasing amount of Ab present in solution. The curve shape is analogous to that obtained by RIA (*11*). In a separate experiment, the addition of unlabeled estriol to a solution of Ab-E-Hg caused an increase in peak current due to competitive displacement of E-Hg by E from the antibody-antigen complex. Thus, binding of E-Hg with Ab is reversible. In addition, no change in peak current was seen when portions of nonspecific Ab were added sequentially in the absence of specific Ab.

Separation of free estriol from estriol bound to the antibody was unnecessary (*12*). The reduction wave for Ab-E-Hg occurred at a more negative potential than the wave for E-Hg. Consequently, polarograms could be performed directly on the mixture. This is advantageous, since most labels require separation of free-labeled antigen from bound-labeled antigen.

The low detection limits of modern electroanalytical techniques (*13*) such as differential pulse polarography ($\sim 10^{-8}M$) and differential pulse anodic stripping voltammetry ($\sim 10^{-10}M$) make electroactive labels potentially useful for immunoassay. Relatively inexpensive and reliable instrumentation for such techniques is commercially available.

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β_1- and β_2-Adrenergic Receptors in Rat Cerebral Cortex Are Independently Regulated

Abstract. *Repeated administration of the tricyclic antidepressant desmethylimipramine to adult rats for 10 days caused a 40 percent decrease in the density of β_1-adrenergic receptors in the cerebral cortex but had no effect on the density of β_2-adrenergic receptors. Conversely, destruction of noradrenergic neurons by administration of 6-hydroxydopamine to neonatal rats caused a 64 percent increase in the density of β_1-adrenergic receptors in adult cerebral cortex with no change in the density of β_2-adrenergic receptors. These results suggest that the β-adrenergic receptors in rat cortex involved in neuronal function are primarily of the β_1 subtype.*

The density of β-adrenergic receptors in rat cerebral cortex is controlled by the noradrenergic input to these receptors (*1*). A reduction in this input by destruction of adrenergic neurons with 6-hydroxydopamine (6-OHDA) or by receptor blockade during treatment with the β-adrenergic antagonist propranolol leads to an increase in the density of receptors (*1*). Conversely, pharmacologically induced increases in the amount of norepinephrine having access to the receptor by repeated treatment with the inhibitor of norepinephrine uptake desmethylimipramine (DMI) or the monoamine oxidase inhibitor pargyline decreases the density of β-adrenergic receptors in this brain area (*1*). These changes in receptor

Table 1. Affinity of IHYP and zinterol for β_1- and β_2-adrenergic receptors after repeated drug treatment. The K_d values for IHYP were determined by Scatchard analysis of IHYP binding. The K_d values for zinterol were calculated by a computerized analysis of biphasic Hofstee plots, as previously described (*3*). Animals were treated as described in Fig. 1. Each value is the mean ± standard error for ten animals (neonatal 6-OHDA) or 15 animals (DMI).

Treatment	K_d for IHYP ($\times 10^{-10}M$)	K_d for zinterol (μM)	
		β_1	β_2
Control	0.84 ± 0.054	2.2 ± 0.24	0.042 ± 0.0066
Desmethylimipramine	0.90 ± 0.056	2.06 ± 0.24	0.033 ± 0.0041
Control	0.72 ± 0.034	1.52 ± 0.09	0.023 ± 0.004
Neonatal 6-hydroxydopamine	0.86 ± 0.027	1.71 ± 0.16	0.040 ± 0.008

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density are paralleled by and temporally correlated with changes in catecholamine-stimulated adenosine 3′,5′-monophosphate (cyclic AMP) accumulation.

Physiological evidence suggests that two subtypes of β-adrenergic receptors exist (*2*). β_1-Adrenergic receptors have similar affinities for epinephrine and norepinephrine and are found mainly in heart and adipose tissue. β_2-Adrenergic receptors have a high affinity for epinephrine but a very low affinity for norepinephrine and are found mainly in muscle and liver. We recently developed a method for the simultaneous measurement of β_1- and β_2-adrenergic receptors in tissues that contain both subtypes (*3*). In this report evidence for the independent regulation of these two receptor subtypes in rat cerebral cortex is presented.

The radiolabeled high-affinity antagonist [^{125}I]iodohydroxybenzylpindolol (IHYP) has a similar affinity for β_1- and β_2-adrenergic receptors (*3*). Scatchard analysis of specific IHYP binding (*4*) is used to determine the total number of β-adrenergic receptors in homogenates of rat cerebral cortex. The percentage of each receptor subtype is calculated by analyzing displacement curves of specific IHYP binding by drugs that show selectivity for β_1 (practolol, metoprolol) or β_2 (zinterol, salmefamol) receptors in vitro (*3*). In tissues containing both receptor subtypes, inhibition of specific IHYP binding by these drugs results in biphasic Hofstee plots. Analysis of these curves by a computer-based procedure which iteratively corrects each component for the contribution of the other component provides a quantitative estimate of the percentage of each receptor subtype. These percentages are then multiplied by the total receptor density to yield the density of each subtype (*3*).

As previously reported (*1*), repeated administration of the tricyclic antidepressant DMI to adult rats for 10 days caused a 32 percent decrease in total β-adrenergic receptor density in the cerebral cortex (Fig. 1). Desmethylimipramine is a weak β-adrenergic receptor antagonist (*1*); however, the dissociation constant (K_d) for IHYP after DMI administration was the same as that in control animals (Table 1), indicating that there was no residual drug in the tissue. Inhibition of specific IHYP binding in the control and treated tissues by the β_2-selective drug zinterol is shown in Fig. 1. In agreement with previous results (*3*), 81.7 ± 2.46 percent of the receptors in control animals were of the β_1 subtype. In DMI-treated animals the density of β_1 receptors was decreased from 50.3 ± 2.7 to 30.0 ± 2.3 fmole of IHYP bound per milligram of protein. The density of β_2 receptors did not change (10.9 ± 1.4 and 11.5 ± 0.6 fmole/mg in control and DMI-treated animals, respectively). The affinity of zinterol calculated for each receptor subtype was identical in the two groups (Table 1).

Neonatal 6-OHDA treatment has been shown to cause an increase in the density of β-adrenergic receptors in adult rat cerebral cortex (*1*). This treatment caused a 55 percent increase in the total density of β-adrenergic receptors (Fig. 1). The K_d for IHYP was unchanged by 6-OHDA administration (Table 1). As observed in experiments performed after DMI treatment, the change was restricted to the β_1 subtype. In control animals 85.7 ± 1.0 percent of the receptors were of the β_1 subtype. Neonatal 6-OHDA treatment caused an increase in the density of β_1 receptors from 69.1 ± 3.0 to 113.4 ± 4.1 fmole/mg. The density of β_2 receptors was unchanged (11.7 ± 1.1 and 11.9 ± 2.2 fmole/mg in control and 6-OHDA-treated animals, respectively). The affinity of zinterol, calculated for each receptor subtype, was again identical in the two groups (Table 1).

In the current experiments rats were treated with neurally active drugs which affect the amount of norepinephrine having access to the receptor. Increasing the availability of norepinephrine by repeated treatment with DMI led to a decrease in the density of β_1-adrenergic receptors. Conversely, destruction of noradrenergic terminals with 6-OHDA led to an increase in the density of β_1-adrenergic receptors. In neither case was any change observed in the density of β_2-adrenergic receptors. The finding that drugs affecting neuronal activity induce changes in β_1 receptors suggests that these receptors receive a neuronal input.

The lack of effect of drug treatments on β_2 receptors may represent an example of neuronal receptors that are not regulated by their endogenous input. However, in most cases that have been

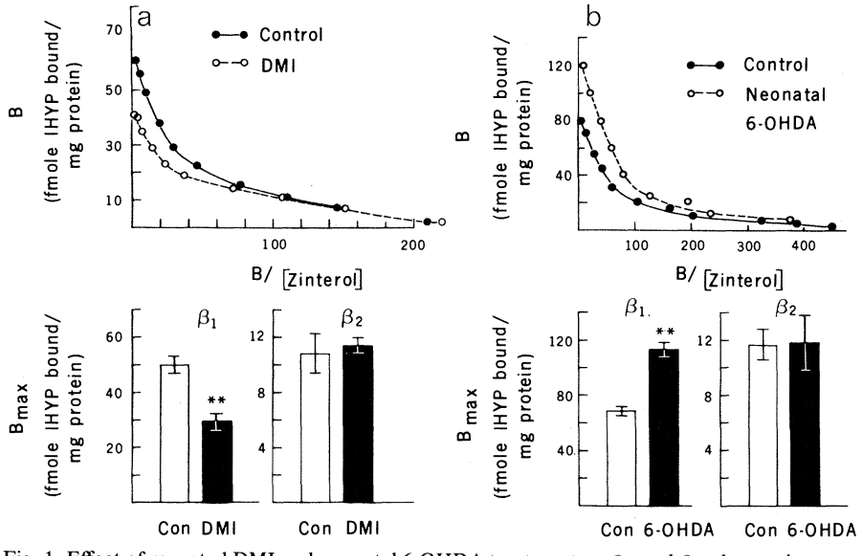

Fig. 1. Effect of repeated DMI and neonatal 6-OHDA treatment on β_1- and β_2-adrenergic receptor densities in rat cerebral cortex. (a) Adult rats received DMI (10 mg/kg, intraperitoneally) or vehicle twice daily for 10 days. Animals were killed 16 hours after the last injection of DMI and the entire cerebral cortex, dissected free from white matter and midbrain structures, was homogenized in isotonic saline. The density of β-adrenergic receptors was determined by Scatchard analysis of specific IHYP binding (*3*). Nonspecific binding was defined as binding inhibited by 10 μ*M* *l*-isoproterenol. Assay mixtures also contained 0.1 m*M* phentolamine and 100 μ*M* guanosine 5′-triphosphate (*1*, *3*). Inhibition of specific IHYP binding (50 p*M*) by the β_2-selective drug zinterol resulted in a biphasic Hofstee plot (top). Dissection of this curve into its two components by a computer-based iterative procedure (*3*) provided a measure of the densities of β_1 (low-affinity) and β_2 (high-affinity) receptors in each group. In the Hofstee plots, the abscissa is the mean of the amount bound (*B*) and the ordinate is the mean of the amount bound divided by the concentration of zinterol (micromoles per liter). The histograms at the bottom show the mean ± standard error (S.E.) of the density of β_1- and β_2-adrenergic receptors for each group (N = 15). (b) Rats received 6-OHDA (100 mg/kg, subcutaneously) or vehicle on each of the first 4 days of life. After 6 weeks, the animals were killed and total β-adrenergic receptors and β_1 and β_2 receptors were determined as described above. (Top) Combined Hofstee plots for the inhibition of specific IHYP binding by the β_2-selective drug zinterol. (Bottom) Histograms representing the mean ± S.E. of the density of β_1 and β_2 receptors for the two groups (N = 10). Norepinephrine was assayed by the method of Henry *et al.* (*11*). In the control group the cerebral cortex contained 2.7 ± 0.3 ng of norepinephrine per milligram of protein. Administration of 6-OHDA caused a 91 percent depletion of norepinephrine. Treated cortices contained 0.2 ± 0.04 ng of norepinephrine per milligram of protein. (**) Significantly different from control, $P < .001$ by Student's t-test.

examined, increases in the density of receptors do occur as a consequence of denervation. This has been well characterized with nicotinic cholinergic receptors on skeletal muscle (*5*) and has also been observed with α-adrenergic (*6*) and dopaminergic (*7*) receptors as well as with β-adrenergic receptors. On the other hand, denervation causes other changes in addition to altering receptor density (*8*). For example, in smooth muscle changes in membrane potential appear to account for a relatively nonselective increase in neuronal sensitivity (*9*).

We feel that a more likely explanation for the lack of effect on β_2 receptors in the cerebral cortex is that the input to these receptors is not neuronal. In this case, alterations in neuronal firing would have no effect on these receptors. We previously showed that although the density of β_1-adrenergic receptors in discrete areas of rat brain varies almost 20-fold, the density of β_2 receptors varies only two- to threefold, suggesting that β_2 receptors are located on a more homogeneously distributed tissue constituent than are β_1 receptors (*10*). It should be noted that β_2 receptors have a low affinity for norepinephrine (the major adrenergic catecholamine in the central nervous system). One reasonable hypothesis is that β_2 receptors in the cerebral cortex are associated with blood vessels and that their endogenous input is epinephrine released from the adrenal.

In summary, the observation that the densities of β_1- and β_2-adrenergic receptors in the brain can be independently regulated provides strong evidence that they are indeed separate receptors, that they probably exist on distinct tissue components, and that they receive different endogenous inputs.

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Induction of Differentiation in Human Promyelocytic Leukemia Cells by Tumor Promoters

Abstract. *Phorbol diester tumor promoters and the promoter mezerein convert human promyelocytic leukemia cells in culture into adherent, nonproliferating cells with many of the characteristics of macrophages. Other types of promoters such as anthralin, phenobarbital, and saccharin do not have this effect. Various compounds that can inhibit some of the biological and biochemical effects of tumor promoters do not interfere with the induction of cell adherence and differentiation by the effective promoters.*

Tumor promoters are compounds that enhance tumor formation when administered after a low dose of a carcinogen (*1*). Phorbol diesters and some related plant diterpene esters that promote tumors in mouse skin in vivo have been shown to exert many biochemical and biological effects on cells in culture (*2*, *3*). Recently, it has been reported (*4*-*7*) that these compounds can reversibly inhibit terminal differentiation of various normal and malignant cells in culture. For example, they inhibit spontaneous and induced differentiation of Friend erythroleukemia cells (*4*, *6*). A possible relationship between this effect of promoters in vitro and the mechanism of tumor promotion in vivo has been discussed by Weinstein and Wigler (*8*) and Diamond *et al.* (*3*).

During an investigation of the generality of promoter-mediated inhibition of differentiation, we observed that these compounds can have more than one effect on differentiation, the effect depending on the target cell. While this work was in progress, Miao *et al.* (*9*) reported that in two Rauscher virus-transformed murine erythroid cell lines phorbol diester tumor promoters stimulated, rather than inhibited, differentiation along the normal erythroid pathway. We now report that in a human promyelocytic leukemia cell line, HL-60 (*10*), some tumor promoters can induce differentiation along a pathway apparently different from that normally followed. Thus, tumor promoters can have at least three different effects on terminal cell differentiation: inhibition of differentiation, stimulation of the normal pathway, and, as reported here, induction of an alternate pathway.

The HL-60 cell line was derived from the peripheral blood leukocytes of an adult human female with acute promyelocytic leukemia (*10*). Most of the cells are promyelocytes, but approximately 20 percent of the population consists of more mature myeloid cells. As with Friend murine erythroleukemia cells (*11*), HL-60 cells can be induced to differentiate by treatment with dimethyl sulfoxide, butyric acid, or dimethylformamide (*12*). Differentiation of HL-60 cells then occurs along the myeloid series and metamyelocytes, banded granulocytes, and mature granulocytes are formed.

When HL-60 cells were treated with the potent tumor promoter 12-*O*-tetradecanoylphorbol-13-acetate (TPA), they became adherent to the plastic substrate and gradually underwent morphological and biochemical changes indicative of differentiation along the monocytic rather than the normal myeloid series (Fig. 1). The cell shape changed dramatically, the azurophilic granulation typical of promyelocytes disappeared, and, after 48 hours, the nuclear : cytoplasmic ratio was shifted from greater than 1 to less than 1 (see Fig. 1). At an initial cell density of 2×10^5 per milliliter and a TPA concentration of 1.6×10^{-8} to $1.6 \times 10^{-7} M$, 20 to 40 percent of the cells were attached to the plastic surface after 18 hours and 80 to 90 percent were attached after 48 hours. Some cells also adhered to each other, forming small clumps that attached to the surface. The small fraction of cells that did not become adherent nevertheless lost all proliferative potential; no proliferating cells were ever recovered after exposure of HL-60 cells to TPA concentrations of $10^{-8} M$ or greater. Autoradiography (*13*) revealed that the number of cells incorporating [^{3}H]thymidine over a 24-hour period decreased from 90 percent in the untreated suspension culture to 12 percent of the adherent cells at 24 hours after TPA treatment ($1.6 \times 10^{-7} M$) and 0 percent at 48 hours.

The TPA also induced many biochemi-

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