ning radars and conventional radars (12). These cooperative observations should lead to a better understanding of the physical and meteorological characteristics of lightning.

RICHARD E. ORVILLE
Department of Atmospheric Science,
State University of New York at Albany,
Albany 12222

G. G. LALA
Atmospheric Sciences Research Center,
State University of New York at Albany
VINCENT P. IDONE
Department of Atmospheric Science,
State University of New York at Albany

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β-Adrenergic Receptors in Aged Rat Brain: Reduced Number and Capacity of Pineal Gland to Develop Supersensitivity

Abstract. *The density but not the affinity of β-adrenergic receptors declined significantly with age in rat pineal gland, corpus striatum, and cerebellum, as determined by the binding of tritiated dihydroalprenolol. Exposing rats to light for 12 hours increased the binding of this radioligand in 3-month-old but not in 24-month-old rats. The reduced responsiveness to catecholamines seen in aging may be due to a decrease in the number of β-adrenergic receptors which, in turn, may be caused by an impaired capacity of receptors in aged animals to adapt to changes in adrenergic neuronal input.*

The aging process is associated with reduced biochemical and physiological responses to various hormones (1). In the case of steroid hormones, decreased responsiveness is accompanied by a decline in the number but not in the affinity of steroid receptors in target tissues (2). Diminished β-adrenergic responsiveness to catecholamine hormones has also been reported in tissues from senescent rodents and humans (3, 4). Since many of the physiological effects that result from the interaction of catecholamines with β-adrenergic receptors are mediated through activation of adenylate cyclase (E.C. 4.6.1.1) (5), in aging there may be an alteration of this receptor-enzyme complex. In fact, decreased sensitivity of adenylate cyclase to norepinephrine and isoproterenol has been reported in brain (6), liver (7), vascular smooth muscle (3), erythrocytes (8), and adipocytes (9) isolated from aged rats. The reason for the age-related loss of β-adrenergic responsiveness has yet to be elucidated. An understanding of the mechanisms responsible for these changes may provide greater insight into the aging process and may ultimately suggest a new rationale for treating geriatric patients.

The recent development of a potent β-adrenergic receptor antagonist with high affinity and specificity for β-adrenergic receptors. [³H]dihydroalprenolol ([³H]-DHA) (10), permitted us to characterize these receptors in the brain of rats of various ages. In the work reported here we found an age-related decrease in the density but not in the affinity of β-adrenergic receptors in several areas of the rat brain. We also found that aged rats have a decreased ability to produce adaptive changes in these receptors.

Male rats (Fischer 344, Charles River), ranging in age from 1 to 24 months, were used in these experiments. These rats have a maximum life-span of 33 months, with 50 percent mortality at approximately 28 months of age. They were acclimatized for 2 weeks on a 12-hour light-dark cycle and were decapitated 5 to 7 hours after initiation of the light cycle, unless otherwise specified. Direct labeling of β-adrenergic receptor sites in homogenates of pineal gland, corpus striatum, and cerebellum was carried out according to procedures described previously (11). The assay was conducted in 150 μl of 50 mM tris-HCl buffer containing 3 mM Mg^{2+}, 0.6 to 40 nM of [³H]DHA (New England Nuclear; specific activity, 32 to 48 Ci/mmole), and tissue homogenate (0.4 to 2 mg of tissue). To determine nonspecific binding, incubation mixtures also contained 20 μM of (±)-propranolol, which selectively displaces [³H]DHA from β-adrenergic sites. Specific binding of [³H]DHA is defined as the total [³H]DHA binding minus the nonspecific binding determined in the presence of excess propranolol. Protein concentration was determined by the method of Lowry *et al.* (12).

Specific [³H]DHA binding was saturable at approximately 20 nM [³H]DHA in pineal gland and striatum and 10 nM in cerebellum. At these concentrations specific binding represented more than 80 percent of total binding in pineal gland, but only about 40 to 60 percent of total binding in cerebellum and striatum, respectively. In confirmation of previous reports (11, 13), specific [³H]DHA binding in these brain areas was stereospecific and of high affinity and was displaced by unlabeled β-adrenergic agonists and antagonists. For example, (−)-alprenolol was nearly two orders of magnitude more potent than (+)-alprenolol in displacing specific [³H]DHA binding, and (−)-isoproterenol was five to ten times as potent as (−)-norepinephrine.

Using saturating concentrations of [³H]DHA, we found that specific binding in the three brain areas varied significantly with age (Fig. 1). Specific binding in pineal gland (Fig. 1A) was maximal at approximately 1 month of age. By 3 months the specific binding declined significantly and then remained at the reduced level through 24 months of age. Specific binding in cerebellum and striatum, on the other hand, increased significantly between 1 and 6 months of age and then decreased significantly through 24 months of age (Fig. 1, B and C). In striatum and cerebellum the decline in specific binding sites with age was accompanied by an increase in nonspecific binding sites (of approximately 24 fmole of [³H]DHA bound per milligram of protein), suggesting that other types of binding sites may be developing with age. Such sites may be associated with glial cells, since the glia/neuron ratio has been shown to increase with age (14).

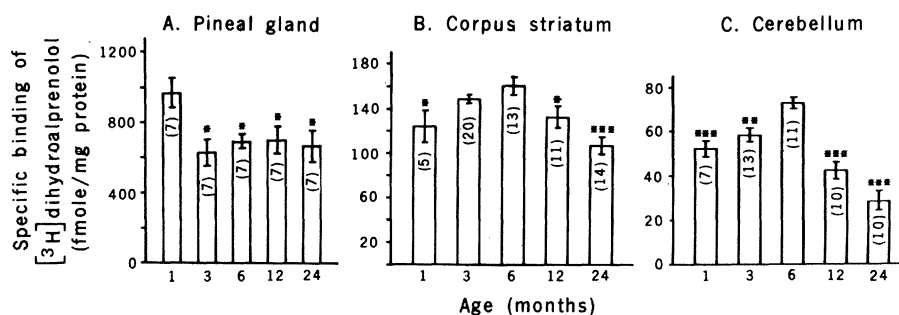To determine whether the decline in specific [³H]DHA binding was due to a

Fig. 1. Effect of age on the specific binding of [³H]DHA in rat pineal gland (A), corpus striatum (B), and cerebellum (C). The brain areas were rapidly dissected from rats varying in age from 1 to 24 months and were homogenized in a buffer containing 50 mM tris-HCl and 3 mM Mg²⁺, pH 8.0, using a Brinkmann Polytron homogenizer. Specific [³H]DHA binding was determined by using the assay conditions described in the text. Concentrations of [³H]DHA sufficient to saturate the β-adrenergic receptors in each tissue were utilized (10 nM, cerebellum; 20 nM, pineal gland and striatum). Incubations were conducted at 37°C for 10 minutes and terminated by rapid dilution of the samples with 2 ml of tris-Mg²⁺ buffer, followed by rapid vacuum filtration of the diluted samples through Whatman GF/C glass fiber filters. The filters were rapidly washed with 15 ml of cold buffer, added to scintillation vials containing 5 ml of Scintiverse fluid (Fisher), and counted for radioactivity. Each experiment always included rats from all five age groups. Each bar represents the mean ± standard error of the mean (S.E.M.) of the number of experiments shown in parentheses. Asterisks indicate the statistical significance of the differences between the specific [³H]DHA binding at each age and the binding at the age at which the greatest degree of binding occurred (1 month, pineal gland; 6 months, striatum and cerebellum): (*) $P < .05$, (**) $P < .01$, (***) $P < .001$; Student's t-test).

decrease in the density or affinity of β-adrenergic receptors for β-adrenergic antagonists, we measured the specific [³H]DHA binding in the three brain areas at various concentrations of [³H]DHA (0.6 to 40 nM). We performed four to six separate experiments with each brain area, comparing rats of different ages, and analyzed the data by the method of Scatchard (15) to determine the densities of specific binding sites (B_{max}) and the apparent dissociation constants (K_D). There was a significant age-related decrease in the density of specific [³H]DHA binding sites in all three brain regions. In pineal gland B_{max} values decreased from 1130 ± 140 fmole of [³H]DHA bound per milligram of protein at 2 months of age to 640 ± 100 fmole/mg ($P < .05$) at 24 months of age. In striatum, B_{max} values declined from 150 ± 9 to 120 ± 3 fmole/mg ($P < .05$) between 6 and 24 months of age, and in cerebellum, from 97 ± 8 to 53 ± 2 fmole/mg ($P < .01$) between 6 and 24 months. By contrast, the K_D values for [³H]DHA binding did not change significantly with age in any of the areas (approximately 5 nM in all three areas). There was also no apparent age-related change in the concentration of (±)-propranolol required to inhibit specific [³H]DHA binding by 50 percent (IC$_{50}$) in cerebellar or striatal tissue, a further indication that the properties of the receptors did not change with age.

These studies showed further that although the amount of nonspecific binding (100 to 200 fmole/mg) was similar in the three brain regions, the density of

specific [³H]DHA binding sites was 10 to 20 times greater in the pineal gland than in the corpus striatum and cerebellum.

Our results are in agreement with the recent demonstration of a decreased concentration of β-adrenergic receptors in aged human lymphocytes (16) and rat
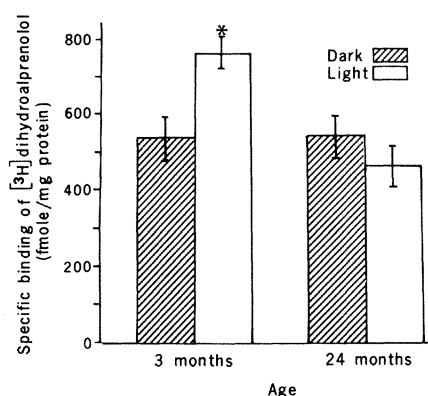

Fig. 2. Effect of age on the binding of [³H]DHA in pineal glands of rats following exposure to light or darkness. Rats 3 and 24 months old were kept in alternating periods of 12 hours of light and 12 hours of darkness. The rats were killed at the end of the light or dark cycle (a red light was used when animals were killed in the dark); the pineal glands were removed and were homogenized as described in the legend of Fig. 1. Specific [³H]DHA binding was determined by using 20 nM [³H]DHA, as described in the text and in the legend of Fig. 1. Each bar represents the mean of six experiments (± S.E.M.). Each experiment included dark- and light-exposed rats from both age groups. There was a statistically significant increase ($P < .01$; Student's t-test) in specific [³H]DHA binding in pineals from 3-month-old rats exposed to light compared with the binding in rats of the same age kept in darkness or in any of the 24-month-old rats.

erythrocytes (17) and suggest that the loss of these receptors with age may be a general phenomenon. Whether the decreases in receptor density result from a loss of receptors in individual adrenergic target cells or from a loss of target cells relative to other cell populations cannot be ascertained from our data. However, in human lymphocytes there was an age-related decrease in the receptor number per cell (16), suggesting that receptors may be lost from individual target cells.

Previous studies showed that reduction of adrenergic neuronal input to pineal gland and other brain structures (18–20) resulted in an increased responsiveness of the adenylate cyclase system to catecholamines. More recent evidence suggests that postsynaptic responsiveness to catecholamines may be mediated in part by changes in the density of β-adrenergic receptors (20, 21). The question arises, then, whether the decrease in β-adrenergic receptor density observed in aged brains may be due to an inability of these tissues to show adaptive changes in response to altered adrenergic neuronal input. To investigate this question, we examined pineal glands from young and old rats.

The pineal gland is innervated by sympathetic nerves (22) whose firing rate can be altered by changing the environmental lighting; rats kept in darkness have an increased adrenergic input to the pineal gland, whereas rats kept in constant light have a decreased input (23). Earlier studies showed that light exposure increases the sensitivity of pineal adenylate cyclase to norepinephrine (19) and increases the density of pineal β-adrenergic receptor bindings sites (24).

In our study, 3- and 24-month-old rats were acclimated to a 12-hour light-dark cycle and killed after 12 hours of exposure to dark or light. The pineal glands from 3-month-old rats exposed to light for 12 hours showed a significant increase in specific [³H]DHA binding compared with that found in rats exposed to 12 hours of darkness. By contrast, there was no significant light-induced increase in [³H]DHA binding in the pineals of the 24-month-old rats (Fig. 2). These results suggest that the pineal glands of aged rats are not able to develop the normal alterations in β-adrenergic receptor binding in response to diurnal changes in lighting conditions and therefore may not exhibit the supersensitive responses to norepinephrine that develop in young rats following light exposure.

In summary, the results of this study suggest that the reduced responsiveness to β-adrenergic agonists that occurs in aged animals and humans may be due, at

least in part, to a decrease in the density of β-adrenergic receptors. They suggest further that the decline of these receptors may be caused by an inability of aged animals to increase the density of adrenergic receptors in response to reduced activity of the sympathetic nervous system.

LOUISE H. GREENBERG
BENJAMIN WEISS

Department of Pharmacology,
Medical College of Pennsylvania,
Philadelphia 19129

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Attraction by Repellents: An Error in Sensory Information Processing by Bacterial Mutants

Abstract. *Normal* Escherichia coli *bacteria are repelled by acetate, benzoate, and indole and attracted by α-aminoisobutyrate. We have isolated mutants that are attracted to acetate, benzoate, and indole and may be repelled by α-aminoisobutyrate. These reversed-taxis mutants are defective in a central processing component: a set of methylated proteins known as MCP 1. The mechanism of reversal of taxis is discussed.*

We set out to search for a mutant that is attracted to a chemical which normally repels *Escherichia coli*. From such a reversal of taxis we hoped, ultimately, to learn about the mechanism that a bacterium uses to determine whether it should treat a chemical as an attractant or repellent. We report the discovery and characteristics of such mutants.

The first strains known to exhibit reversed taxis were isolated by selecting for mutants that were attracted into a capillary containing acetate (*1*), which is normally a repellent for *E. coli* (*2*). The chemotactic response to acetate of such a mutant, AW630, and its parent is shown in Fig. 1. The attractant response

in the mutant has a threshold of approximately $10^{-3}M$ and a maximum at $1 \times 10^{-1}M$ to $3 \times 10^{-1}M$ acetate. Since repulsion by acetate in the parent shows a similar dependency on concentration (*2*), it appears that the attraction observed in the mutant is not a response to a chemical contaminating the acetate but represents a true reversal of the chemotactic response. Furthermore, since the receptor dissociation constant (*3*) and the specificity (*4*) are the same for acetate attraction in the mutant as for acetate repulsion in the parent, it appears that the same receptor is involved in both processes.

To ascertain whether this reversal of

chemotaxis was specific to the acetate receptor or, alternatively, affected the responses also to chemicals detected by other chemoreceptors, we tested various attractants and repellents in the mutant. The chemotactic response to some chemicals was drastically altered, while the response to others was relatively normal. These alterations show a remarkable similarity to the pattern of defects characteristically exhibited by previously isolated *tsr* mutants (*5–8*), although *tsr* strains were not known to be reversed for taxis. However, additional experiments now show that the *tsr* mutants indeed do exhibit such a reversal, and that they and AW630 belong to a single complementation group, the *tsr* cistron (*9*).

Table 1 presents a summary of the responses of a typical *tsr* mutant and its parent in temporal assays (*10, 11*) of chemotactic behavior. Certain chemicals (type 1) show either a reversal or a loss of the response when the mutant is compared to the parent. For example, acetate, propionate, and *n*-butyrate—all detected by the acetate receptor (*2, 11*)—and benzoate and indole—each detected by a separate receptor (*2*)—act as attractants in the mutant, in contrast to their action as repellents in the parent (*2*). This has been confirmed by the capillary assay for acetate (Fig. 1) and for benzoate and indole (*12*). L-Leucine, normally a repellent for *E. coli*, and detected by yet another receptor (*2, 11*), fails to either repel or attract the mutant, as judged by both temporal (Table 1) and capillary (*12*) assays. α-Aminoisobutyrate, a type 1 chemical that is an attractant for the parent, may repel *tsr* mutants; this is indicated by the chemical-in-plug assay (*13*); but results from temporal assays (Table 1) show weak repulsion or, in some experiments, no response. In contrast to type 1 chemicals, the response of *tsr* mutants to type 2 chemicals is relatively normal (Table 1). L-Aspartate, which acts as an attractant in the parental strain, also acts as an attractant for the mutant, and cobalt and nickel ions act as repellents for both parent and mutant.

Since the reversed taxis mutants are all in the *tsr* cistron, the loss of a functional *tsr* product must, in some way, cause the reversal of taxis. It was discovered recently that *tsr* codes for one of the two methyl-accepting chemotaxis proteins (MCP's) (*7, 8*). This protein (actually a set of bands on a sodium dodecyl sulfate gel) is called MCP 1, and it is defective in *tsr* mutants. The other protein (again actually a set), MCP 2, is coded for by the *tar* gene and is defective in *tar*

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