labeling medium composed of methionine-free minimal essential medium, 10 percent heat-inactivated horse serum, 5 percent chicken embryo extract, nerve growth factor (10 ng/ml), 15 μ*M* unlabeled methionine, and [^{35}S]methionine (1 mCi/ml). The expanded side compartments contained 3.5 ml of the same medium, except that 4 m*M* unlabeled methionine replaced the radioactive methionine. Incubations were for 40 hours at 37°C in 10 percent CO_2. After labeling, 50 μl of medium from each compartment was aspirated and the protein precipitated by trichloroacetic acid. The free [^{35}S]methionine that remained in solution was counted in a beta counter at a counting efficiency of approximately 70 percent. This procedure provided an estimate of the leakage of radioactive label into the side compartment. After removing the rest of the supernatant media, the axons in the side compartment were washed twice with Dulbecco's phosphate-buffered saline (Gibco), and the cellular material was collected by dissolution in a solution of 2 percent SDS and 5 percent β-mercaptoethanol at a temperature of 90°C, pooled, and processed for two-dimensional polyacrylamide gel electrophoresis (*3*). The Ampholine solution of the isoelectric focusing step was composed of 1.6 percent Ampholine 5/7 (LKB) and 0.4 percent Ampholine 3/10. The second dimension was run in a 10 to 17.5 percent acrylamide gradient. The preparation of the gels for fluorography was done according to the principles worked out by W. M. Bonner and R. A. Laskey [*Eur. J. Biochem.* **46**, 83 (1974)] but with a commercially available acetic acid–based enhancer (EN^{3}HANCE, New England Nuclear). Exposure was for 4 to 5 weeks at −70°C.

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12. This minor contamination, which does not affect the outcome of the study, has to be considered as inherent to the design of this compartmental cell culture system. The plates used for all labeling studies were carefully tested for the absence of hydrostatic bulk flow between the compartments (*2*). Proteins synthesized by cells of the side compartments might be labeled by absorption of labeled amino acid or proteins secreted from DRG axons or by uptake and retrograde transport by VSC axons reaching into the center compartment. These possibilities were experimentally excluded (data not shown). Thus, we conclude that the minor buildup of radioactive amino acid in the side compartment (about 0.25 percent of the concentration of the center compartment after 40 hours) has to be the result of diffusion through the film of medium between the compartments.

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Strain Differences in Rat Brain Epinephrine Synthesis: Regulation of α-Adrenergic Receptor Number by Epinephrine

Abstract. *Inbred rat strains Fischer 344 (F344) and Buffalo (BUF) differ in several physiological and behavioral measures. It was found that the activity of adrenomedullary and regional brain phenylethanolamine* N*-methyltransferase is at least four times higher in F344 rats than in BUF rats; these strain-dependent differences corresponded directly with the epinephrine content of the medulla-pons and hypothalamus. Conversely, α-adrenergic receptor density in brain regions containing phenylethanolamine* N*-methyltransferase is two to three times lower in F344 rats than in BUF rats; α-receptors in frontal cortex (a brain region lacking phenylethanolamine* N*-methyltransferase activity and epinephrine) are similar in both strains. These findings suggest that strain-dependent differences in α-receptors are regulated by inherited differences in presynaptic adrenergic neuronal function in different brain regions.*

Phenylethanolamine *N*-methyltransferase (PNMT), the enzyme that converts norepinephrine (NE) to epinephrine (EPI), is present in adrenomedullary cells and in medulla-pons and hypothalamic neurons (*1, 2*). PNMT-containing neurons may play key roles in physiological processes such as regulation of blood pressure and responses to stress (*2*). Recent studies have demonstrated the importance of genetic factors influencing regulation of catecholamine biosynthetic enzymes and, by association, catecholamine-mediated physiological functions (*3*). In this regard, two inbred rat strains, Fischer 344 (F344) and Buffalo (BUF), demonstrate differences in midbrain and cortical tyrosine hydroxylase (TH) activity, spontaneous motor activity, apomorphine-induced stereotypy, NE- and amphetamine-induced behavioral responsiveness, and NE-sensitive adenosine 3′,5′-monophosphate (cyclic AMP) accumulation in midbrain slice preparations (*4, 5*). Differences in brain receptor sensitivity to the various catecholamines may explain the behavioral and physiological strain differences (*4*); thus, Helmeste *et al.* (*5*) have demonstrated differences in brain dopamine receptor number. It is likely, however, that other neurotransmitter systems, including adrenergic ones, are involved in the many differences seen between these inbred rat strains. We observed that adrenomedullary PNMT activity was five times higher in F344 rats than in BUF rats; this observation led us to explore differences in PNMT activity and adrenergic receptors in brain regions of F344 and BUF rats.

Breeding colonies of F344 and BUF rats were established from parental stock obtained from Microbiological Associates and maintained by brother-sister mating for at least two generations. Individual rat brain regions were processed to allow simultaneous assay of TH, PNMT, catecholamines, and receptor sites (*6, 7*). Adrenal and regional brain PNMT and brain TH were assayed with modifications of published methods (*8*), and brain catecholamine levels were measured by high-performance liquid chromatography with electrochemical detection (*9*). We followed standard methods in our radioligand binding assays for adrenergic and opiate receptors (*10*) and used [^{3}H]prazosin (PRAZ), an α_1-receptor–specific antagonist; *p*-[^{3}H]-aminoclonidine (PAC), an α_2 partial agonist with selectivity for the α_2(H) state (*11*); [^{3}H]rauwolscine (RAUW), an α_2 antagonist with some selectivity for the α_2(L) state (*12*); [^{125}I]iodocyanopindolol (ICYP), a β-receptor antagonist; and [^{3}H]naloxone (NAL), an opiate antagonist (*11*).

Adrenal PNMT activity in male F344 rats (N = 41) was 6.52 ± 0.07 U per adrenal pair (mean ± standard error; U = 1.0 nmole of product per hour at 37°C), compared to 1.34 ± 0.02 U per adrenal pair in BUF (N = 34) adrenals. The same fivefold difference in adrenal PNMT activity was seen between female rats of the two strains. Similar differences were also observed in two brain regions (Table 1). Medulla-pons PNMT activity was four times higher in F344 rats than in BUF rats. Differences between strains were even more striking for hypothalamic PNMT activity. As expected, little or no PNMT activity (or EPI) was detected in cortical tissue (*12*). Strain-dependent differences in TH activity were similar to those reported previously (*4*), with F344 rats showing 30 to 40 percent greater TH activity.

Regional catecholamine levels generally reflected differences in synthetic enzyme activity. Most notably, the prominent between-strain differences in medulla-pons and hypothalamic PNMT activity were accompanied by three- to fivefold higher EPI content in F344 than in BUF rats (Table 1). Cortical, hypothalamic, and medulla-pons NE levels reflected brain TH activity differences, with NE levels up to 40 percent higher in F344 than in BUF rats.

Agonist regulation of adrenergic re-

Table 1. Activity of PNMT, EPI concentration, and adrenergic receptor binding in brain regions of F344 and BUF rats. Female rats were used in the experiments, except for the determination of medulla-pons EPI levels and PRAZ binding to α_1-receptors, when tissue from male rats was used. No sex differences were observed in either strain for any variable in the three brain regions, and the same between-strain differences were observed in both male and female F344 and BUF rats for all measured variables. Radioligand concentrations in experiments with paired tissue samples were: PRAZ, 0.9 n*M*; PAC, 0.6 to 0.9 n*M*; RAUW, 0.9 to 1.5 n*M*; and ICYP, 25 to 35 p*M*. Values are means ± standard errors for the number of experiments shown in parentheses. N.D., not determined.

Substance	Medulla-pons		Hypothalamus		Frontal cortex	
	F344	BUF	F344	BUF	F344	BUF
PNMT (nmole/hour-g)	0.53 ± 0.05 (12)	0.14 ± 0.02 (13)	0.89 ± 0.04 (9)	0.09 ± 0.03 (10)	Trace*	Trace*
EPI (ng/g)	6.6 ± 0.3 (8)	2.8 ± 0.3 (8)†	73.7 ± 4.3 (9)	14.2 ± 1.6 (10)	N.D.	N.D.
PRAZ (fmole/mg protein)	16.0 ± 3.1 (7)	39.0 ± 5.9 (5)‡	12.3 ± 3.5 (3)	27.2 ± 4.4 (3)§	24.9 ± 4.9 (3)	28.6 ± 5.2 (3)
PAC (fmole/mg protein)	4.4 ± 0.9 (5)	20.0 ± 4.4 (5)§	14.6 ± 2.6 (3)	27.1 ± 3.4 (3)‡	22.5 ± 3.9 (5)	20.6 ± 3.2 (5)
RAUW (fmole/mg protein)	5.1 ± 0.6 (5)	18.6 ± 3.6 (5)‡	19.4 ± 1.7 (3)	33.3 ± 2.8 (3)‡	29.0 ± 2.8 (5)	26.4 ± 2.6 (5)
ICYP (fmole/mg protein)	13.6 ± 1.6 (5)	14.1 ± 2.0 (5)	19.5 (1)	27.8 (1)	32.4 (1)	41.0 (1)

*Activity was below 0.1 U/g and variable (*13*). †Four of the eight BUF samples had EPI values at the limits of sensitivity for the amount of tissue processed and were assigned values of 2 ng/g for statistical evaluation. ‡$P < 0.01$. §$P < 0.05$.

ceptor number and function has been demonstrated directly and indirectly in several neural systems (*13, 14*), and we examined the possibility of between-strain differences in brain receptors that might be associated with differences in EPI content. Initial experiments showed significant between-strain differences in α-adrenergic receptor radioligand binding (Table 1). In contrast to the higher PNMT and EPI contents observed in F344 rats, BUF rats had two- to fivefold greater binding of all α_1- and α_2-receptor ligands in the medulla-pons and hypothalamus. There were, however, no between-strain differences in α-receptor binding in frontal cortex or in ICYP binding to β-adrenergic receptors in any brain region. Likewise, F344 and BUF rats exhibited similar NAL binding to opiate receptors in medulla-pons (1.6 n*M* NAL; 43.6 ± 1.1 versus 56.6 ± 5.9 fmole per milligram of protein; $N = 5$).

Binding of α-receptor ligands in F344 and BUF medulla-pons was examined further to evaluate the nature of the between-strain differences. Representative Scatchard plots of α-receptor binding isotherms are shown in Fig. 1. Analyses from three similar experiments in tissue from female rats (*15, 16*) indicated no between-strain differences in the binding affinity (K_d) for PRAZ (means ± standard errors for F344 and BUF rats, respectively: 0.29 ± 0.20 and 0.27 ± 0.06 n*M*), PAC (0.88 ± 0.08 and 0.69 ± 0.12 n*M*), or RAUW (1.7 ± 0.29 and 1.6 ± 0.28 n*M*). There were, however, prominent differences between F344 and BUF rats in the maximum number of binding sites (B_{max}; fmole per milligram of protein) for PRAZ (34.2 ± 8.2 and 97.6 ± 10.5; $P < 0.05$), PAC (35.8 ± 3.2 and 82.9 ± 24.1; $P < 0.05$), and RAUW (22.5 ± 5.4 and 67.6 ± 12.5; $P < 0.05$). Similar K_d and B_{max} values for medulla-pons α-receptor binding were observed in male F344 and BUF rats. These data support the conclusion that the inbred BUF strain, with the lower PNMT activity and epinephrine content in the medulla-pons, has a two- to threefold higher density of α_1- and α_2-receptors than the F344 strain.

The present results demonstrate a parallel genetic regulation of both adrenal medulla and brain PNMT activity in inbred F344 and BUF rat strains. Furthermore, in the two strains there is an inverse relation between brain α-receptor sites and PNMT activity. The receptor differences may be functional concomitants of the differences in PNMT activity and are due to the ability of receptor number to be regulated by the amount of physiological agonist present (*14*). Thus differential genetic regulation of PNMT may initiate a cascade of events (differences in catecholamine levels resulting in differential regulation of adrenergic receptors) that finally leads to strain differences in physiological and behavioral responsiveness to adrenergic agents. The greater density of α_1-receptors in BUF medulla-pons is in agreement with the greater accumulation of cyclic AMP induced by NE in midbrain slices from these rats (*4*).

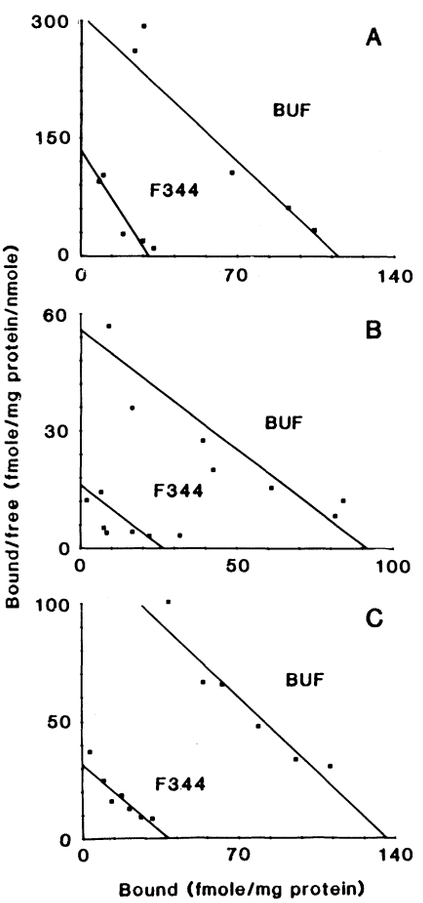

Fig. 1. Alpha-adrenergic receptor binding in medulla-pons from female F344 and BUF rats. The same batch of membranes (0.5 mg of protein pooled from two to three animals) was incubated with increasing concentrations of radioligand. Scatchard plots from one of three similar experiments for female rats are shown. (A) Binding of PRAZ to α_1-receptors, (B) RAUW binding to α_2-receptors, and (C) PAC binding to α_2-receptors.

Between-strain differences were limited to α-adrenergic receptors in brain areas containing PNMT and EPI, suggesting that α-receptors in these areas are primarily regulated by inherited differences in adrenergic neuronal function. Autoradiographic studies have shown that α_2-receptors especially are concentrated in regions of adrenergic innervation (*17*). The selective influence of EPI is further suggested by the finding of consistent between-strain differences in TH activity and NE content across all brain regions despite prominent regional variation in α-receptor between-strain differences. β-Receptors do not appear to be subject to the same influence.

The difference in α-receptor number is substantially greater than that reported in studies comparing spontaneously hypertensive (SH) and Wistar-Kyoto (WKY) normotensive inbred strains (*18*). Apart from these studies, the only other comparison of brain α- and β-receptors in different rat strains published to our knowledge showed no differences between F344 and Sprague-Dawley rats

(*19*). Between-strain differences in striatal dopamine receptor number in F344 and BUF rats (*5*) and 11 inbred mouse strains (*20*) are also smaller than the present α-receptor differences. The parallelism in between-strain differences for tritiated agonist and antagonist binding to α_2-receptors indicates a true difference in the density of this receptor in the hypothalamus and medulla-pons; a similar parallelism was observed for dopamine receptor interstrain differences (*20*).

The different PNMT activity in F344 and BUF rat brains may reflect differences in the number of brainstem adrenergic neurons, as previously suggested for SH and WKY rats (*21*). In analyzing genetic differences in brain dopamine systems in two inbred mouse strains, Baker *et al.* (*22*) found quantitatively similar differences in midbrain TH activity and dopamine neuron number.

The present results, in addition to allowing a further genetic analysis of the interrelation between PNMT and α-receptors, suggest a model for examining the regulation of α-receptor number and function in pathological and therapeutic conditions in which both inherited factors and α-adrenergic receptor function are thought to be important (*18, 23*).

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Acetylcholine Mediates a Slow Synaptic Potential in Hippocampal Pyramidal Cells

Abstract. *The hippocampal slice preparation was used to study the role of acetylcholine as a synaptic transmitter. Bath-applied acetylcholine had three actions on pyramidal cells: (i) depolarization associated with increased input resistance, (ii) blockade of calcium-activated potassium responses, and (iii) blockade of accommodation of cell discharge. All these actions were reversed by the muscarinic antagonist atropine. Stimulation of sites in the slice known to contain cholinergic fibers mimicked all the actions. Furthermore, these evoked synaptic responses were enhanced by the cholinesterase inhibitor eserine and were blocked by atropine. These findings provide electrophysiological support for the role of acetylcholine as a synaptic transmitter in the brain and demonstrate that nonclassical synaptic responses involving the blockade of membrane conductances exist in the brain.*

The first identification of a synaptic transmitter in the vertebrate central nervous system (CNS) was in 1954 when Eccles *et al.* (*1*) demonstrated that the collaterals of spinal motoneurons use the transmitter acetylcholine (ACh) to excite Renshaw cells. Although a variety of studies since then have suggested that ACh might be a transmitter at various sites in the mammalian CNS (*2–6*), no convincing correlation at the intracellular level has been made between the actions of ACh and synaptic responses (*7–9*). We examined this question in the hippocampal slice preparation, in which pyramidal cells receive a dense cholinergic input from the medial septum (*7, 10*). Using intracellular recording in this preparation, we found that the muscarinic actions of ACh, which are slow and involve nonclassical ionic mechanisms (*3–6*), can be mimicked exactly by synaptic inputs. These inputs are blocked by the muscarinic antagonist atropine and are enhanced by the cholinesterase inhibitor eserine.

We have described the details of the rat hippocampal slice preparation elsewhere (*11*). Intracellular recordings were obtained from over 100 CA1 pyramidal cells using 2*M* potassium methyl sulfate–filled microelectrodes. Drugs were applied by superfusion. Stimulating electrodes were positioned in the CA2/CA3 region of stratum oriens to activate the cholinergic fibers that are concentrated there (*10*). A 20- to 40-Hz train of stimuli for 0.5 seconds was optimal. Stimulus