Moreover, the cells of one ommatidium appear to be electrically coupled [P. G. Lillywhite, *J. Comp. Physiol.* **125**, 13 (1978)]. For a description of ommatidial structure, see M. Wilson, P. Garrard, S. McGinness, *Cell Tissue Res.* **195**, 205 (1978).

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15. Lights-on ("dawn") was at 0600, lights-off ("dusk") at 1800. Illuminance from daylight-fluorescent plus incandescent lighting was 1000 lux in the center of each white-sided cage (36 by 36 by 46 cm). Temperature outside the cage was 20° to 35°C. Animals were fed bran and wheat. At least 24 hours before fixation (including the period when eyes were masked), animals were kept at 25° ± 2°C and 48 ± 2 percent humidity, under the same cyclic lighting as that used during rearing.
16. Before primary fixation, dissection was limited to one or two slices through the surface of the eye around the central region, where the inter-ommatidial angle is constant. Whole heads were then quickly immersed in primary fixative: 2.5 percent glutaraldehyde plus 2 percent paraformaldehyde buffered in 0.08*M* NaH_2PO_4-NaOH plus 0.06*M* D-glucose. After 3 to 12 hours, the eyes were trimmed, fixed in OsO_4, dehydrated, and embedded in Araldite. Sections for electron microscopy were stained with uranyl acetate and lead citrate. They were cut so that the ommatidia at the boundary between masked and unmasked areas were in transverse section. Measurements of rhabdom cross-sectional areas were made with an image analyzer (Kontron MOP-AMO3), from electron micrographs magnified 3300 times.
17. Shed membrane is aggregated as vesicles in multivesicular bodies, which degrade through multilamellar bodies to residual bodies (*5*). This pathway is common to most arthropods [E. Eguchi and T. H. Waterman, *Cell Tissue Res.* **169**, 419 (1976); A. D. Blest, L. Kao, K. Powell, *ibid.* **195**, 425 (1978); A. D. Blest, S. Stowe, D. G. Price, *ibid.* **205**, 229 (1980); G. S. Hafner, G. Hamond-Soltis, T. Tokarski, *ibid.* **206**, 319 (1980); (*4*)].
18. G. A. Horridge and P. B. T. Barnard, *Q. J. Microsc. Sci.* **106**, 131 (1965).
19. Usually at the boundary between masked and unmasked ommatidia, a row of ommatidia had rhabdoms of an intermediate size. These ommatidia were probably incompletely masked, so that their rhabdoms received an intermediate light intensity. Rhabdom size has been shown to be modulated according to "intermediate" light intensities in a mosquito [R. H. White and E. Lord, *J. Gen. Physiol.* **65**, 583 (1975)]. In the shedding experiment, the intermediate-sized rhabdoms are a result of a smaller amount of shedding, since no rhabdomeral disarray was found to indicate that full shedding followed by some assembly of new PRM had occurred. The intermediate size in the assembly experiment, however, could have resulted from either assembly of less PRM, or full assembly followed by some shedding.
20. Early experiments, in which polarized or monochromatic light was used to selectively stimulate some of the photoreceptors in a crab, *Libinia* [E. Eguchi and T. H. Waterman, *Z. Zellforsch. Mikrosk. Anat.* **84**, 87 (1968)], and in a honey bee [F. G. Gribakin, *Nature (London)* **233**, 639 (1969)], respectively, provided evidence that breakdown of microvillar PRM can be restricted within an ommatidium to the individual photoreceptors. In the bee, the breakdown did not appear to constitute normal shedding, since it was associated with swelling of the microvilli rather than the common form of internalization by pinocytosis [E. Eguchi and T. H. Waterman, *Z. Zellforsch. Mikrosk. Anat.* **79**, 209 (1967); R. H. White, *J. Exp. Zool.* **169**, 261 (1968)]. In *Libinia*, the extent of shedding that did not coincide with the daily rhythm was determined (by relative numbers of secondary lysosomes; no size change was manifest), rather than the initiation of a cell's normal shedding response as in the present report.
21. Efferent neurons project to the lamina in insect and crustacean compound eyes [N. J. Strausfeld and D. R. Nässel, in *Handbook of Sensory Physiology*, H. Autrum, Ed. (Springer-Verlag, New York, 1981), vol. 7/6B, p. 1].
22. Local control has been shown in photoreceptor cell pigment (and other photomechanical) movements in *Limulus* lateral eye [M. E. Behrens, *J. Comp. Physiol.* **89**, 45 (1974)]; pigment in fly [D. G. M. Beersma, thesis, Rijksuniversiteit Groningen (1979)]; and pigment-palisade interplay in locust [this report (Fig. 1)]. Retinomotor movements during dark and light adaptation in fish retinas are also controlled locally [S. S. Easter and A. Macy, *Vision Res.* **18**, 937 (1978)].
23. This was established for photoreceptor sensitivity in the *Limulus* lateral eye [J. E. Lisman and J. E. Brown, *J. Gen. Physiol.* **59**, 701 (1972)]; the honey bee drone [C. R. Bader, F. Bauman, D. Betraud, *J. Gen. Physiol.* **67**, 475 (1976)]; and in the fly [H. Muijser, *J. Comp. Physiol.* **132**, 87 (1979)]; and for photoreceptor cell pigment movements in the fly [K. Kirschfeld and K. Vogt, *Naturwissenschaften* **67**, 515 (1980)].

24. Helpful comments on the manuscript were provided by S. Laughlin, S. Stowe, and D. Blest.

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Role of Serotonergic Input in the Regulation of the β-Adrenergic Receptor–Coupled Adenylate Cyclase System

Abstract. *The action of desipramine on the norepinephrine-sensitive adenylate cyclase system and the density of β-adrenergic receptors in rat cortex was studied after selective lesioning of serotonergic neurons with 5,7-dihydroxytryptamine. In animals with lesions desipramine failed to reduce the density of β-adrenoceptors but decreased the response of adenosine 3′,5′-monophosphate to isoproterenol and norepinephrine to the same degree as in animals without lesions. The results demonstrate a functional linkage between serotonergic and noradrenergic systems in the rat cortex, with β-adrenergic receptors and neurohormonal sensitivity of the adenosine 3′,5′-monophosphate–generating system being under separate regulatory control.*

Various prototypes of clinically effective antidepressant drugs have been shown to down-regulate the noradrenergic adenosine 3′,5′-monophosphate (cyclic AMP)–generating system and its β-adrenergic receptor population in the brain (*1–3*). The ability of antidepressants to reduce the sensitivity of the system and the density of β-adrenergic receptors depends on an intact noradrenergic neuronal input (*4–6*). However, an increased availability of norepinephrine (NE) appears to be only one prerequisite for the regulation of the receptor system. Thus, iprindole, which does not increase the availability of NE, nevertheless reduces both the sensitivity to NE and the density of β-adrenergic receptors (*4, 7, 8*). Conversely, cocaine, which increases the availability of NE, does not change the density of β-adrenergic receptors (*3, 9*). Since the terminal fields of serotonergic projections in the cortex overlap those of noradrenergic projections, we studied the consequences of selective lesioning of serotonergic neurons on the regulation by desipramine of the NE receptor–coupled adenylate cyclase system in the rat cortex. Our results demonstrate that desipramine fails to decrease the density of β-adrenergic receptors in the absence of serotonergic input while still reducing the sensitivity of the cyclic AMP–generating system to NE and the β-adrenergic agonist isoproterenol.

As subjects we used male Sprague-Dawley rats (250 to 300 g) kept under

Table 1. Effect of DHT lesions of the central serotonergic system on the recognition and action functions of the NE receptor–coupled adenylate cyclase system. The lesions were made 10 to 12 days before treatment with desipramine (15 mg/kg, intraperitoneally) daily for 7 days. Twenty-four hours after the last desipramine injection, the animals were decapitated and the cyclic AMP responses to NE and the density of β-adrenergic receptors were determined. Each response equals the stimulated concentration of cyclic AMP minus the basal level. For the determination of specific [^{3}H]DHA binding, no fewer than five different concentrations of ligand (0.3 to 3.5 n*M*) were used. Numbers in parentheses indicate the number of animals, each sample being analyzed in duplicate (cyclic AMP) or in triplicate (DHA binding). Values are means ± standard errors.

Treatment	Cyclic AMP (pmole/mg protein)		[^{3}H]DHA binding	
	Basal concentration	Response to 100 μ*M* NE	Maximum number of sites (fmole/mg protein)	Affinity (n*M*)
No lesion; saline	18.0 ± 2.5 (10)	65.2 ± 6.3 (17)	100 ± 10 (8)	1.31 ± 0.13
No lesion; desipramine	17.4 ± 2.0 (12)	27.0 ± 3.6* (17)	68 ± 4† (8)	1.32 ± 0.07
Lesion; saline	18.1 ± 1.3 (13)	50.7 ± 4.6 (23)	131 ± 4† (7)	1.56 ± 0.08
Lesion; desipramine	17.7 ± 1.7 (13)	23.9 ± 3.1‡ (20)	133 ± 25 (7)	1.71 ± 0.21

*Significantly different from corresponding value for nonlesioned animals given saline ($P < .001$). †$P < .025$. ‡Significantly different from corresponding value for lesioned animals given saline ($P < .001$).

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standard laboratory conditions (12:12 hour light-dark cycle; free access to water and standard laboratory feed). Specific lesions of serotonergic neurons were produced by injecting 5,7-dihydroxytryptamine (DHT) (150 μg in 20 μl of saline with 0.1 percent ascorbate, intraventricularly) after prior treatment (45 to 60 minutes) with desipramine (25 mg/kg, intraperitoneally) to prevent destruction of noradrenergic neurons (*10*). Control animals received desipramine followed by an intraventricular injection of a corresponding volume of the vehicle. The animals were allowed to recover for 10 to 12 days, and then were injected with desipramine (15 mg/kg, intraperitoneally) or saline daily for 7 days. At 24 hours after the last injection, the animals were decapitated and their brains were rapidly removed. One-half of the frontal cortex was immediately used for studies of the cyclic AMP response to NE or isoproterenol, and the other half was frozen and used for studies of β-adrenergic receptor binding and for analysis of serotonin and NE. The sensitivity of the cyclic AMP–generating system to NE and isoproterenol was determined in cortical slices (*11, 12*). Cyclic AMP was isolated by ion-exchange chromatography and assayed by the protein binding method of Gilman (*13*). The status of β-adrenergic receptors was determined in accordance with the method of Bylund and Snyder (*14*), with [^{3}H]dihydroalprenolol (DHA) used as a ligand. The data on specific DHA binding were subjected to Scatchard analysis (*15*). Proteins were assayed by the method of Lowry *et al.* (*16*), and the concentrations of serotonin and NE were determined chromatographically (*17*).

Administration of DHT significantly reduced the concentration of serotonin in the cortex, from 326 ± 8 (N = 36) to 44 ± 3 ng/g (N = 38) ($P < .001$, Student's t-test). Norepinephrine concentrations were unaffected, indicating the specificity of the lesion. Although the maximum number of sites for [^{3}H]DHA binding was slightly increased in animals lesioned with DHT, the cyclic AMP responses to NE (Table 1) and isoproterenol (Table 2) and were not altered in these animals.

Long-term administration of desipramine to animals without lesions caused the expected decrease in the cyclic AMP responses to NE and isoproterenol. This was linked to a decrease in the density of β-adrenergic receptor sites without alteration in ligand binding affinity (Tables 1 and 2). In the absence of serotonergic neuronal input, desipramine failed to reduce the density of β-adrenergic receptors, confirming the results of Brunello *et al.* (*18*). However, the responsiveness of the cyclic AMP–generating system to both NE and isoproterenol was reduced in lesioned animals to approximately the same extent as in control animals (Tables 1 and 2). No significant changes in binding affinities were seen after the administration of DHT or desipramine or both.

Table 2. Effect of DHT lesions of the central serotonergic system on the cyclic AMP response to isoproterenol. The lesions were made 10 to 12 days before treatment with desipramine (15 mg/kg, intraperitoneally) daily for 7 days. Twenty-four hours after the last desipramine injection, the animals were decapitated and the cyclic AMP responses to isoproterenol were determined. The phosphodiesterase inhibitor 4-[3-butoxy-4-methoxy]-2-imidazolidone (RO 20-1724, 100 μM) was added 15 minutes before the addition of isoproterenol. Each response equals the isoproterenol-stimulated level of cyclic AMP minus the basal level in the presence of RO 20-1724. Numbers in parentheses indicate the number of animals, each sample being analyzed in duplicate. Values are means ± standard errors.

Treatment	Cyclic AMP (pmole/mg protein)	
	Basal concentration	Response to 10 μM isoproterenol
No lesion; saline	50.5 ± 4.0 (11)	66.9 ± 10.6 (12)
No lesion; desipramine	43.4 ± 4.3 (10)	39.7 ± 6.2* (10)
Lesion; saline	50.0 ± 2.5 (11)	62.7 ± 8.9 (12)
Lesion; desipramine	50.1 ± 5.0 (11)	30.0 ± 5.1† (10)

*Significantly different from corresponding value for nonlesioned animals given saline ($P < .001$). †Significantly different from corresponding value for lesioned animals given DHT ($P < .005$).

While an intact noradrenergic neuronal input is necessary for down-regulation of the "NE receptor–coupled" adenylate cyclase system (subsensitivity to agonists and reduced density of β-adrenergic receptors) by tricyclic antidepressants (*4–6*), these and previous results (*4–6*) demonstrate that both serotonergic neuronal input and NE are required for the regulation of the β-adrenergic receptor moiety of the system but not for the induction by desipramine of subsensitivity to NE and isoproterenol. These results provide direct evidence for a functional linkage between serotonergic and noradrenergic systems at the molecular level and indicate that β-adrenergic receptor density and neurohormonal sensitivity of the cyclic AMP–generating system are under separate regulatory control and do not necessarily change in the same direction. The temporal relation of this dissociation between the number of β-adrenergic receptors and sensitivity of the NE-sensitive adenylate cyclase remains to be elucidated.

The functional linkage between serotonergic and noradrenergic neuronal systems in the cortex might provide a basis for unifying the two main hypotheses of affective disorders, the serotonin and NE hypotheses. Moreover, elucidating the molecular mechanisms by which the serotonergic system regulates recognition and action function of central noradrenergic receptor systems should stimulate new studies of antidepressant drug action.

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