tion signal (*21*). The field trap design illustrates a means of determining the behavioral activity of repellent or inhibitory pheromones under conditions similar to those in nature. *trans*-Verbenol, alone or in combination with other inhibitors, such as verbenone (*10, 22*), ipsdienol (*14*), or *cis*-verbenol (*3*), may be useful in protecting trees from attack by *D. brevicomis*.

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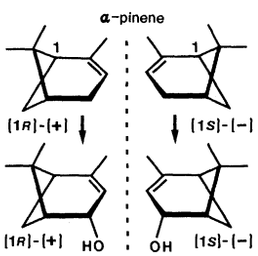
References and Notes

1. M. C. Birch and D. L. Wood, *J. Chem. Ecol.* **1**, 101 (1975).
2. J. A. Byers and D. L. Wood, *ibid.* **6**, 149 (1980).
3. ———, *ibid.* **7**, 9 (1981).
4. J. A. Rudinsky, *Science* **166**, 884 (1969); ——— and R. R. Michael, *ibid.* **175**, 1386 (1972); G. B. Pitman and J. P. Vité, *Environ. Entomol.* **3**, 886 (1974); J. A. Byers, *J. Chem. Ecol.* **9**, 129 (1983).
5. R. M. Silverstein, R. G. Brownlee, T. E. Bellas, D. L. Wood, L. E. Browne, *Science* **159**, 889 (1968); G. W. Kinzer, A. F. Fentiman, Jr., T. F. Page, Jr., R. L. Foltz, J. P. Vité, G. B. Pitman, *Nature (London)* **22**, 475 (1969); D. L. Wood *et al.*, *Science* **192**, 896 (1976).
6. Both (+) and (−) enantiomers of α-pinene occur in ponderosa pine resin, but the (−) enantiomer predominates in California [E. Zavarin and F. W. Cobb, Jr., *Phytochemistry* **9**, 2509 (1970)].
7. P. R. Hughes, *Z. Angew. Entomol.* **73**, 294 (1973).
8. J. A. A. Renwick, *Contrib. Boyce Thompson Inst.* **23**, 355 (1967).
9. ———, *ibid.* **24**, 337 (1970).
10. ——— and J. P. Vité, *ibid.*, p. 283.
11. J. A. A. Renwick, P. R. Hughes, I. S. Krull, *Science* **191**, 199 (1976) [in figure 2 (+)-*trans*-verbenol and (+)-myrtenol should have been represented by the (1*R*, 4*S*, 5*R*) configuration instead of the (1*S*, 4*R*, 5*S*) model shown].
12. Hydroxylation of (1*R*)-(+)- and (1*S*)-(−)-α-pinene enantiomers (non-superimposable mirror images) to (1*R*)-(+)- and (1*S*)-(−)-*trans*-verbenol enantiomers in *D. brevicomis*:



trans-Verbenol

13. J. A. Byers, D. L. Wood, L. E. Browne, R. H. Fish, B. Piatek, L. B. Hendry, *J. Insect Physiol.* **25**, 477 (1979).
14. J. A. Byers, *J. Chem. Ecol.* **8**, 363 (1982).
15. The same proportions of (+) and (−) enantiomers found in the samples of (+) and (−)-α-pinene were found in the beetle-produced (+)- and (−)-*trans*-verbenol, as judged by the highest reported rotations for (−)-*trans*-verbenol ($[\alpha]_D^{22} = -129.6°$) and (+)-α-pinene ($[\alpha]_D^{22} = +52.4°$) (*11*).
16. The *trans*-verbenol had a molecular weight of 152 and mass spectra (mass-to-charge ratio) in decreasing magnitude from a base peak of 109: 94 (59 percent of base peak), 91, 81, 119, 95, 69, 83, 137, 92, 84, 41, 67, 107, 79, and 134 which were in agreement with spectra of other samples of *trans*-verbenol obtained by G. Odham, University of Lund, and G. Bergström, University of Göteborg, Sweden. However, the spectra were not in agreement with those obtained by J. A. A. Renwick (*8*), who reported a base peak of 119 and major fragments of 91 and 134 for *trans*-verbenol obtained from *D. brevicomis*.
17. J. A. Byers, *Insect Biochem.* **11**, 563 (1981).
18. J. M. Brand, J. W. Bracke, A. J. Markovetz, D. L. Wood, L. E. Browne, *Nature (London)* **254**, 136 (1975).
19. J. A. Byers and D. L. Wood, *Science* **213**, 763 (1981).
20. ———, J. Craig, L. B. Hendry, in preparation.
21. J. A. A. Renwick and J. P. Vité, *Nature (London)* **224**, 1222 (1969); G. B. Pitman and J. P. Vité, *Can. Entomol.* **101**, 143 (1969); J. A. Rudinsky and R. R. Michael, in (*2*).
22. W. D. Bedard, P. E. Tilden, K. Q. Lindahl, Jr., D. L. Wood, P. A. Rauch, *J. Chem. Ecol.* **6**, 997 (1980).
23. I thank D. L. Wood, G. O. Poinar, Jr., C. Löfstedt, J. Löfqvist, and F. Schlyter for reviews; W. F. Haddon and E. Zavarin for assistance in the chemical analyses; and W. D. Bedard and P. E. Tilden for use of their research facilities. Supported in part by grants from the Rockefeller Foundation and the U.S. Forest Service, and by Regional Research Project grant W-110, SEA/USDA (to D. L. Wood).
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Recognition Sites for Norepinephrine Uptake: Regulation by Neurotransmitter

Abstract. *Recognition sites for the uptake of norepinephrine on adrenergic neurons in the brain and periphery were labeled with [^{3}H]desipramine. The number of these uptake sites varied with the concentration of transmitter; depletion of norepinephrine with reserpine reduced the number of uptake sites, whereas increasing the concentration of norepinephrine induced by treatment with monoamine oxidase inhibitors raised the number of binding sites. These dynamic alterations in norepinephrine uptake recognition sites may regulate synaptic function homeostatically, providing less inactivation of reuptake when the synaptic concentration of the transmitter is low and increased inactivation when it is high.*

The ability to label neurotransmitter receptors by their binding of radioactive drugs has enhanced our understanding of synaptic processes (*1*). The number of neurotransmitter receptor binding sites varies with the concentration of synaptically active transmitter, usually increasing in parallel with the functional supersensitivity that follows a reduction in the synaptic transmitter and decreasing with increased transmitter (*2*).

Biogenic amine and amino acid neurotransmitters are inactivated after they are released from synapses by specific uptake systems at nerve terminals (*3*). Recognition sites for the uptake of γ-aminobutyric acid (GABA) and glutamate can be labeled by [^{3}H]GABA and [^{3}H]glutamate, respectively, in the presence of sodium (*4*). Tricyclic antidepressant drugs potently inhibit the uptake of norepinephrine, dopamine, and serotonin (*5*). The uptake sites for serotonin and norepinephrine can be labeled with [^{3}H]imipramine (*6*) and [^{3}H]desipramine (*7–11*), respectively, thereby permitting

Table 1. Effects of reserpine on central and peripheral adrenergic parameters. Reserpine (5 mg/kg) was given subcutaneously once a day for 5 days. On day 6, rats were decapitated and binding of [^{3}H]desipramine (2 n*M*), [^{3}H]dihydroalprenolol (1 n*M*), [^{3}H]WB-4101 (1 n*M*), and *p*-[^{3}H]aminoclonidine (1 n*M*) to cerebral cortex membranes and salivary gland (submaxillary and sublingual) membranes and the uptake of [^{3}H]norepinephrine in crude synaptosomal preparations were determined (*7, 8*). Binding data are expressed as picomoles per gram of tissue, uptake data as picomoles per gram of tissue in 5 minutes, and norepinephrine level as nanomoles per gram of tissue. The numbers in parentheses show the percentage of control values. Results are the mean ± standard error of four separate determinations performed in triplicate. N.D., not determined.

Parameter	Cerebral cortex		Salivary gland	
	Control	Reserpine	Control	Reserpine
[^{3}H]Desipramine binding (norepinephrine uptake site)	2.69 ± 0.19	1.58 ± 0.21† (59)	5.61 ± 0.26	2.79 ± 0.04† (50)
[^{3}H]Norepinephrine accumulation	108.4 ± 2.4	27.5 ± 4.78‡ (25)	N.D.	N.D.
Norepinephrine level	2.35 ± 0.14	<0.01 (0.4)	N.D.	N.D.
[^{3}H]Dihydroalprenolol binding (β-receptor)	2.64 ± 0.17	3.70 ± 0.12† (140)	2.43 ± 0.21	4.19 ± 0.27† (172)
[^{3}H]WB-4101 binding (α_1-receptor)	4.57 ± 0.17	5.62 ± 0.20† (123)	2.60 ± 0.22	2.54 ± 0.17 (89)
p-[^{3}H]Aminoclonidine binding (α_2-receptor)	2.43 ± 0.11	2.81 ± 0.13 (115)	0.72 ± 0.08	1.12 ± 0.12 (150)

*$P < .05$. †$P < .02$. ‡$P < .005$.

a molecular analysis of uptake sites. Evidence that [^{3}H]desipramine binding lables the recognition sites for norepinephrine uptake includes (i) a parallel between drug potencies at the binding sites and the influence on norepinephrine uptake, (ii) similar regional distributions of [^{3}H]desipramine binding and norepinephrine uptake, and (iii) parallel reductions of [^{3}H]desipramine binding and norepinephrine uptake after destruction of norepinephrine neurons by neurotoxins or by surgical lesions (*7–10*). In initial studies (*7, 9–11*), norepinephrine was less than 1 percent as potent at [^{3}H]desipramine binding sites as it was in inhibiting uptake accumulation at synaptosomes. However, subsequent studies with potassium-treated membranes and varying sodium concentrations (*8*) reveal the same affinities of norepinephrine for both sites.

The high-affinity uptake system for neuronal choline is influenced by synaptic release of acetylcholine, with increases and decreases in choline transport capacity linked, respectively, to increases or decreases in cholinergic activity (*12*). In the present study, we demonstrated that the binding of [^{3}H]desipramine associated with norepinephrine uptake sites is regulated by changes in transmitter concentration.

Depletion of central biogenic amines with reserpine (5 mg/kg) reduced [^{3}H]desipramine binding in rat cerebral cortex (Fig. 1 and Table 1). After 9 days of treatment, [^{3}H]desipramine binding was reduced by 54 percent. A highly significant reduction (25 percent) was observed 1 day after reserpine treatment, but at 2 hours, binding was not reduced. Although reserpine depletes the brain of serotonin as well as norepinephrine, [^{3}H]imipramine binding associated with serotonin uptake sites was much less affected by reserpine treatment (20 percent decrease after 9 days). Treatment with reserpine (0.5 mg/kg) also decreased the binding of [^{3}H]desipramine but not of [^{3}H]imipramine; the decrease in [^{3}H]desipramine binding was greater with the larger dose of reserpine.

Although reserpine depletes dopamine and serotonin as well as norepinephrine from the brain, the effects of reserpine in the salivary gland suggest that the norepinephrine depletion is responsible for

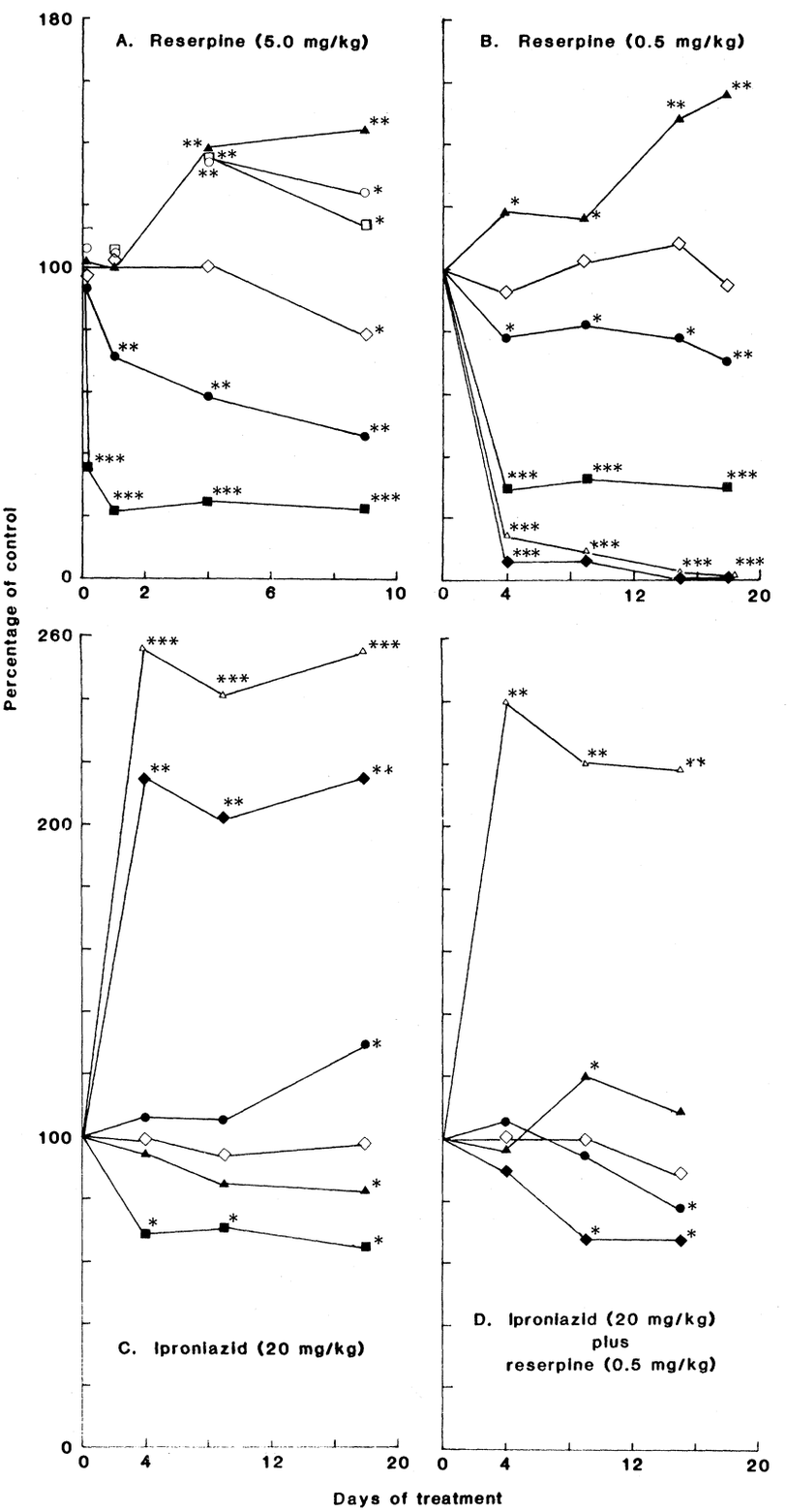

Fig. 1. Time course for effects of (A) reserpine (5 mg/kg, subcutaneously), (B) reserpine (0.5 mg/kg, intraperitoneally), (C) iproniazid (20 mg/kg, intraperitoneally), and (D) reserpine (0.5 mg/kg, intraperitoneally) plus iproniazid (20 mg/kg, intraperitoneally) on various adrenergic and serotonergic parameters. Direct binding of (□) 1 n*M* [^{3}H]WB-4101, (○) 1 n*M* *p*-[^{3}H]aminoclonidine, (▲) 1 n*M* [^{3}H]dihydroalprenolol, (●) 2 n*M* [^{3}H]desipramine, and (◇) 2 n*M* [^{3}H]imipramine to α_1-, α_2-, and β-adrenergic receptors, norepinephrine uptake recognition sites, and serotonin uptake recognition sites, respectively, in rat cerebral cortex membranes were measured by filtration assays (*7, 8*). The uptake of (■) 50 n*M* (±)-[^{3}H]norepinephrine by cerebral cortex homogenates rich in synaptosomes was determined by a filtration assay (*7, 8*). Levels of (◆) norepinephrine and (△) serotonin in 0.1*N* perchloric acid extracts were determined by high-performance liquid chromatography with electrochemical detection (*18*). The results are the means of four to eight separate determinations performed in triplicate and are expressed as the percentage of control values; the controls were treated with saline during the same time course. $*P < .05$, $**P < .02$, and $***P < .005$ compared to control.

Table 2. Effects of reserpine and iproniazid treatment on the binding constants for [^{3}H]dihydroalprenolol and [^{3}H]desipramine binding in cerebral cortex. Reserpine and iproniazid were given intraperitoneally once daily for 18 days. On day 19, the rats were decapitated and the binding of [^{3}H]desipramine and [^{3}H]dihydroalprenolol to cerebral cortical membranes were determined at seven different concentrations of the respective radioactive ligand in the range 0.25 to 8 n*M* (*7, 8*). The numbers in parentheses represent the percentage of control values. Results are the means ± standard error of three separate determinations performed in triplicate. N.D., not determined.

Treatment	[^{3}H]Desipramine		[^{3}H]Dihydroalprenolol	
	K_d (n*M*)	B_{max} (pmole/g)	K_d (n*M*)	B_{max} (pmole/g)
Control (saline)	2.5 ± 0.4	7.3 ± 0.2	0.91 ± 0.21	3.8 ± 0.4
Reserpine (0.5 mg/kg)	3.8 ± 0.2	5.5 ± 0.4* (75)	0.85 ± 0.06	5.7 ± 0.2* (150)
Iproniazid (20 mg/kg)	2.7 ± 0.3	9.5 ± 0.2* (130)	N.D.	N.D.

*$P < .05$.

the changes in [^{3}H]desipramine binding (Table 1). Norepinephrine is the transmitter for the sympathetic nerves in the salivary gland, where dopamine serves only as a precursor and no substantial levels of serotonin exist. However, treatment with reserpine reduced salivary gland [^{3}H]desipramine binding by 50 percent, which is similar to the reduction in the cerebral cortical binding observed in parallel experiments (Table 1).

The pharmacological efficacy of reserpine is apparent in its profound depletion of endogenous norepinephrine and serotonin and the marked reduction of [^{3}H]norepinephrine accumulation, both of which reflect the impairment of the granular storage of neuronal norepinephrine (Table 1 and Fig. 1).

As observed previously (*13*), transmitter depletion by reserpine is associated with a marked augmentation of β-adrenergic receptor binding with some increase in α_2-adrenergic receptor binding and an increase in α_1-receptor binding in the brain but not the salivary gland (Table 1).

To determine whether the apparent down regulation of [^{3}H]desipramine binding by reserpine was elicited by the reduction in transmitter level, we attempted to raise the norepinephrine concentration by treatment with the monoamine oxidase inhibitor iproniazid (Fig. 1). Iproniazid (20 mg/kg, intraperitoneally) for 18 days augmented [^{3}H]desipramine binding by 30 percent. Its effective inhibition of monoamine oxidase was apparent in the marked increase in endogenous norepinephrine and serotonin. As observed earlier for other monoamine oxidase inhibitors (*14*), β-adrenergic receptor binding was reduced significantly 18 days after treatment with iproniazid (Fig. 1). To determine whether the effect of iproniazid is related to its inhibition of monoamine oxidase and the associated augmentation of amine levels, or to some other effect, we evaluated the influence of pargyline (10 mg/kg), a chemically different monoamine oxidase inhibitor. At 10 and at 18 days after daily treatment with pargyline, a 15 percent increase in [^{3}H]desipramine binding was observed (data not shown). The reduction in [^{3}H]norepinephrine accumulation resulting from iproniazid and pargyline treatment may be related to the inhibitory effect of these drugs on the neuronal uptake of norepinephrine (*15*).

Combined treatment with reserpine and iproniazid elicited much less depletion of norepinephrine than treatment with reserpine alone, while endogenous serotonin was still twice that in controls (Fig. 1D). [^{3}H]Desipramine binding was not reduced at 4 or 9 days after the combined treatment, but a significant reduction (20 percent) was observed after 15 days.

The reduced [^{3}H]desipramine binding after reserpine treatment and the increased binding after iproniazid treatment reflect significant changes in the number of binding sites, with no significant change in affinity for desipramine, although a limited nonsignificant increase in the dissociation constant (K_d) was observed after reserpine treatment (Table 2). As observed by others (*13*), the increased β-adrenergic receptor binding after treatment with reserpine involves an increased number of binding sites with no change in affinity.

The reserpine-induced reduction in [^{3}H]desipramine binding and the increase in β-adrenergic receptor binding are reversible. In rats treated with daily injections of reserpine (0.5 mg/kg) for 18 days, we examined [^{3}H]desipramine and [^{3}H]dihydroalprenolol binding 18 days after the last injection when endogenous norepinephrine levels had returned to control values. At this time no reduction in [^{3}H]desipramine or increase in [^{3}H]dihydroalprenolol binding could be detected. The changes in [^{3}H]desipramine binding elicited by reserpine or iproniazid are not direct effects of the drugs themselves, since addition of 10 μ*M* of either drug in vitro did not effect [^{3}H]-desipramine binding (data not shown).

To determine whether antidepressant treatment itself altered [^{3}H]desipramine binding, we treated groups of four rats each with daily intraperitoneal injections of desipramine (10 mg/kg), imipramine (10 mg/kg), or iprindole (10 mg/kg) for 18 days and killed the rats on day 19. In brain membranes washed to remove residual drug, no alteration in [^{3}H]desipramine, [^{3}H]imipramine, *p*-[^{3}H]aminoclonidine, or [^{3}H]WB-4101 binding to cerebral cortex membranes was apparent. However, as reported by others (*16*) this chronic treatment with desipramine and imipramine did produce a significant reduction (20 percent) in [^{3}H]dihydroalprenolol binding to β-receptors (data not shown).

Our finding that norepinephrine uptake recognition sites are regulated by transmitter concentration may have functional consequences. The norepinephrine uptake system inactivates synaptically released transmitter, and the efficacy of this process is dependent on the number of available uptake sites (*3*). After norepinephrine is depleted by reserpine, synaptic levels of the transmitter are markedly reduced. Conceivably, fewer uptake sites would facilitate the synaptic actions of these lower norepinephrine concentrations. Alternatively, an increased number of uptake sites would remove more rapidly the higher synaptic levels of norepinephrine produced by inhibition of monoamine oxidase.

We could not readily determine whether the change in the number of desipramine binding sites reflects functional alterations in norepinephrine uptake. We measured [^{3}H]norepinephrine accumulation by homogenates rich in synaptosomes, but this process involves uptake by the neuronal membrane and also vesicular uptake and granular storage, a process that is impaired by reserpine (*3*). The profound reduction in [^{3}H]norepinephrine uptake 2 hours after reserpine treatment (Fig. 1) indicates that the process of vesicular uptake and granular storage is the principal determinant of the total [^{3}H]norepinephrine accumulation since the neuronal membrane transport of norepinephrine is unaffected by reserpine (*15*). The only way to monitor norepinephrine accumulation by the neuronal membrane transport

process separately from granular storage is to use synaptosomes from reserpinized preparations, an approach not altogether feasible in the present experimental model.

The changes in the binding of [^{3}H]desipramine to presynaptic norepinephrine uptake sites are in a direction opposite to changes in binding to postsynaptic adrenergic receptors. However, both alterations appear to reflect homeostatic attempts of these synaptic systems to normalize norepinephrine synaptic transmission after perturbation by drugs. Thus, depletion of norepinephrine by reserpine gives rise to an increased number of postsynaptic adrenergic receptors, providing a supersensitivity to the smaller amount of synaptic transmitter.

In contrast to the substantial changes in desipramine binding sites, treatment with reserpine and iproniazid has little effect on [^{3}H]imipramine binding to serotonin uptake sites. The only change detected was a 20 percent reduction 9 days after a high dose of reserpine (5 mg/kg). Conceivably, this finding reflects a less stringent regulation of synaptic serotonin than of norepinephrine. Thus, injections of tryptophan produce considerable increases in the brain levels and possibly the synaptic concentrations of serotonin, whereas treatment with tyrosine produces much smaller changes in norepinephrine (*17*).

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References and Notes

1. H. I. Yamamura, S. J. Enna, M. J. Kuhar, Eds., *Neurotransmitter Receptor Binding* (Raven, New York, 1978).
2. R. J. Lefkowitz, *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **37**, 123 (1978); B. B. Wolfe, T. K. Harden, P. B. Molinoff, *Annu. Rev. Pharmacol. Toxicol.* **17**, 575 (1977); T. Reisine, *Neuroscience* **6**, 1471 (1981); I. Creese and D. Sibley, *Annu. Rev. Pharmacol. Toxicol.* **21**, 357 (1981); S. H. Snyder, *N. Engl. J. Med.* **300**, 465 (1979).
3. L. L. Iversen, in *Handbook of Psychopharmacology*, L. L. Iversen, S. D. Iversen, S. H. Snyder, Eds. (Plenum, New York, 1975), vol. 3, p. 381.
4. S. J. Enna and S. H. Snyder, *Brain Res.* **100**, 81 (1975); S. R. Vincent and E. McGeer, *ibid.* **184**, 99 (1980); M. Baudry and G. Lynch, *J. Neurochem.* **36**, 811 (1981).
5. A. S. Horn, J. T. Coyle, S. H. Snyder, *Mol. Pharmacol.* **7**, 66 (1971); S. B. Ross and A. L. Renyi, *Acta Pharmacol. Toxicol.* **36**, 382 (1975); R. M. Maxwell, R. M. Ferris, J. E. Burcsu, in *The Mechanism of Neuronal and Extraneuronal Transport of Catecholamines*, D. M. Paton, Ed. (Raven, New York, 1976), p. 95.
6. J. Talvenheimo, P. J. Nelson, G. Rudnick, *J. Biol. Chem.* **254**, 4631 (1979); S. Z. Langer, E. Zarifan, M. Briley, R. Raisman, D. Shechter, *Life Sci.* **29**, 211 (1981); S. M. Paul, M. Rehavi, K. Rice, Y. Ittah, P. Skolnick, *ibid.* **28**, 2253 (1981).
7. C.-M. Lee and S. H. Snyder, *Proc. Natl. Acad. Sci. U.S.A.* **78**, 5250 (1981).
8. C.-M. Lee, J. A. Javitch, S. H. Snyder, *J. Neurosci.* **2**, 1515 (1982); J. A. Javitch, C.-M. Lee, S. H. Snyder, in preparation.
9. M. Rehavi, P. Skolnick, B. Hulihan, S. M. Paul, *Eur. J. Pharmacol.* **70**, 597 (1981); M. Rehavi *et al.*, *J. Neurochem.* **38**, 889 (1982).
10. S. Z. Langer, R. Raisman, M. Briley, *Eur. J. Pharmacol.* **72**, 423 (1981); R. Raisman, M. Sette, C. Pimoule, M. Briley, S. Z. Langer, *ibid.* **78**, 345 (1982).
11. P. O. Hrdina, K. Elson-Hartman, D. C. S. Roberts, B. A. Pappas, *Eur. J. Pharmacol.* **73**, 375 (1981); P. O. Hrdina, *Prog. Neuro-Psychopharmacol.* **5**, 553 (1981).
12. D. J. Jenden, R. S. Jope, M. H. Weiler, *Science* **194**, 635 (1976); J. R. Simon and M. J. Kuhar, *J. Neurochem.* **27**, 93 (1976); L. A. Barker, *Life Sci.* **18**, 725 (1976); L. C. Murrin and M. J. Kuhar, *Mol. Pharmacol.* **12**, 1082 (1976); G. M. Samaras and J. F. Contrera, *J. Neurochem.* **28**, 1373 (1977).
13. D. C. U'Prichard and S. H. Snyder, *Eur. J. Pharmacol.* **51**, 145 (1978); D. B. Bylund and J. R. Martinez, *Nature (London)* **285**, 229 (1980); S. Treiser and K. J. Keller, *Eur. J. Pharmacol.* **58**, 85 (1979); K. Dismukes and J. W. Daly, *Mol. Pharmacol.* **10**, 933 (1974); G. C. Palmer, H. R. Wagner, R. W. Putman, *Neuropharmacology* **15**, 695 (1976).
14. I. C. Campbell, D. L. Murphy, D. W. Gallager, in *Monoamine Oxidase: Structure, Function and Altered Functions*, T. P. Singer, R. W. Von Korff, D. L. Murphy, Eds. (Academic Press, New York, 1979), p. 517; M. M. Sellinger-Barnette, J. Mendels, A. Frazer, *Neuropharmacology* **19**, 447 (1980).
15. L. L. Iversen, *The Uptake and Storage of Noradrenaline in Sympathetic Nerves* (Cambridge Univ. Press, New York, 1967); E. D. Hendley and S. H. Snyder, *Nature (London)* **220**, 1330 (1968).
16. B. B. Wolfe, T. K. Harden, J. R. Sporn, P. B. Molinoff, *J. Pharmacol. Exp. Ther.* **207**, 446 (1978); K. Sarai, *Biochem. Pharmacol.* **27**, 2179 (1978); A. Maggi, D. C. U'Prichard, S. J. Enna, *Eur. J. Pharmacol.* **61**, 91 (1980).
17. R. J. Wurtman and J. D. Fernstrom, *Am. J. Clin. Nutr.* **28**, 638 (1975).
18. R. Zaczek and J. T. Coyle, *J. Neural Transm.* **53**, 1 (1982).
19. We thank M. Bowman for technical assistance and D. C. Hanks for secretarial assistance. Supported by USPHS grants MH-18501, DA-00266, and NS-16375; research scientist award DA-00074 (to S.H.S.); a National Huntington's Disease Association fellowship (to C.-M.L.); and MSTP training grant GM07309 (to J.A.J.).
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12 October 1982

Narcolepsy: Biogenic Amine Deficits in an Animal Model

Abstract. *Concentrations of biogenic amine metabolites in discrete brain areas differed significantly between dogs with genetically transmitted narcolepsy and age- and breed-matched controls. Dopamine and 3,4-dihydroxyphenylacetic acid were consistently elevated in the brains of narcoleptic animals, while homovanillic acid was not. Narcoleptic animals consistently exhibited lower utilization of dopamine and higher intraneuronal degradation of dopamine but no uniform decrease in serotonin utilization. Hence neuropathology appears to be associated with genetically transmitted canine narcolepsy. The data indicate a nonglobal depression of dopamine utilization or turnover or both.*

Narcolepsy, an incurable human sleep disorder, has been recognized for nearly a century, but its etiology is still undefined (*1*). The disease is characterized in its complete form by a severe and permanent daytime somnolence and by attacks of flaccid paralysis called cataplexy. These attacks can strike spontaneously or be elicited by laughter, excitement, anger, or fear. Two other frequent symptoms are sleep paralysis and hypnagogic hallucinations. Finally, polysomnographic testing of narcoleptic patients consistently shows pathognomonic periods of rapid eye movement (REM) at the onset of sleep. Thus, human narcolepsy is well defined pathophysiologically and can be accurately diagnosed. Many studies have indicated that human narcolepsy has genetic determinants (*2*).

In the past decade narcolepsy has been described in the dog and the horse (*3*). At Stanford University two breeds of dogs, Labrador retrievers and Doberman pinschers, have been isolated that transmit narcolepsy to their offspring through an autosomal recessive mechanism (*4*). A breeding colony now supplies a homogeneous population of affected animals.

Our early neurochemical studies were limited to sampling cerebrospinal fluid in narcoleptic and normal miniature poodles, a breed that apparently does not transmit the illness. The most consistent finding was a decreased turnover of serotonin in narcoleptic poodles and a trend toward decreased turnover of catecholamines (*5*). We attempted to replicate this result in normal and genetically narcoleptic Doberman pinschers. No significant differences in monoamine metabolites were found between control and affected animals (*6*). This suggested either that biogenic amines play no role in narcolepsy in this breed or that any deficits in the central nervous system were too subtle or localized to be reflected in cisternal cerebrospinal fluid.

We now report the results of a direct neurochemical analysis of brain tissues from genetically narcoleptic Doberman pinschers and age- and breed-matched controls. We measured the concentrations of norepinephrine, epinephrine, dopamine, serotonin, 5-hydroxyindoleacetic acid (5-HIAA), homovanillic acid (HVA), and 3,4-dihydroxyphenylacetic acid (DOPAC) in specific brain areas selected for study because of their suspected role in regulation of sleep and waking states.

Brains from five narcoleptic and seven