At this stage there appears to be no relationship between the severity of depression as estimated by the Hamilton score and the parameters for [^{3}H]imipramine binding (Table 1); however, more patients should be studied before conclusions about this relationship are drawn.

Recent studies have demonstrated [^{3}H]imipramine binding sites in postmortem and neurosurgical samples of human brain (*9*). In the regions studied in detail—A_{34} (hippocampus), A_{10} (cortex), hypothalamus, and caudate nucleus—the K_d and B_{max} values closely resemble those found in rat brain (*9*). Thus the decrease in [^{3}H]imipramine binding sites seen in the platelets of depressed subjects may be a reflection of similar changes in the brain. It has been recently demonstrated that changes in platelet monoamine oxidase activity are correlated with the levels of 5-hydroxyindoleacetic acid in cerebral spinal fluid in human control volunteers (*10*), suggesting that changes in platelets may indeed reflect similar changes in the central nervous system.

[^{3}H]Imipramine binding in platelets from depressed patients may be decreased in response to the biochemical or humoral changes that trigger or maintain the depressive state. It is tempting to speculate that the differences in the binding of [^{3}H]imipramine observed in platelets may reflect sensitivity changes resulting from altered levels of a circulating substance having a role in mood regulation. This unknown substance could act directly on the [^{3}H]imipramine binding site. Compatible with the possible existence of a mood-regulating endogenous ligand for the [^{3}H]imipramine binding site is the fact that similar endogenous ligands have been found for (i) the [^{3}H]morphine binding site (*11*), now accepted to be the opiate receptor, and, more recently, for (ii) the [^{3}H]diazepam binding site (*12*), thought to be involved in the anxiolytic effect.

Although the significance of [^{3}H]imipramine binding is not yet clear, several possibilities can be suggested. (i) The binding site may be a specific receptor for an endogenous ligand. (ii) A direct or indirect relationship with the site of neuronal serotonin uptake has been suggested (*5*) on the basis of the high affinity of certain serotonin-uptake blockers. (iii) Finally, the [^{3}H]imipramine binding site could be a modulatory site for one of the established neurotransmitter receptors in analogy to the interaction between the γ-aminobutyric acid receptor and the benzodiazepine binding site (*13*).

In summary, maximal [^{3}H]imipramine binding is significantly lower in the platelets of untreated depressed patients than in the control group, whereas the affinity constant is unchanged. The study of [^{3}H]imipramine binding in depressed patients (before treatment and after treatment with tricyclic or atypical drugs, electroconvulsive shock, lithium, and even social and psychological therapies) represents a new approach in biological psychiatry. At this stage there are still many questions to be answered concerning the pathogenesis and therapy of depression, but it would appear that [^{3}H]imipramine binding both in platelets and in postmortem brain samples will be a powerful tool in our attempts to answer them.

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References and Notes

1. J. J. Schildkraut and S. S. Kety, *Science* **156**, 21 (1967).
2. B. N. Rosloff and J. M. Davis, *Psychopharmacologia* **40**, 53 (1974).
3. R. S. G. Jones and M. H. T. Roberts, *Br. J. Pharmacol.* **65**, 501 (1978).
4. S. Z. Langer, M. S. Briley, R. Raisman, in *Enzymes and Neurotransmitters in Mental Disease*, E. Usdin, Ed. (Wiley, Chichester, United Kindom, in press).
5. R. Raisman, M. Briley, S. Z. Langer, *Eur. J. Pharmacol.* **54**, 307 (1979); *Nature (London)* **281**, 148 (1979); *Eur. J. Pharmacol.* **61**, 373 (1980).
6. M. S. Briley, R. Raisman, S. Z. Langer, *Eur. J. Pharmacol.* **58**, 347 (1979).
7. S. Z. Langer, M. Briley, R. Raisman, J. F. Henry, P. L. Morselli, *Naunyn-Schmiedebergs Arch. Pharmakol.*, in press.
8. R. Raisman, D. Sechter, M. S. Briley, E. Zarifian, S. Z. Langer, in preparation.
9. R. Raisman, Y. Agid, M. S. Briley, S. Z. Langer, in preparation.
10. O. Oreland *et al.*, cited in C. J. Fowler, B. Ekstedt, T. Egashira, H. Kinemuchi, L. Oreland, *Biochem. Pharmacol.* **28**, 3063 (1979).
11. J. Hughes, T. W. Smith, B. Morgan, L. Fothergill, *Life Sci.* **16**, 1753 (1975).
12. P. Skolnick, P. J. Marangos, F. K. Goodwin, M. Edwards, S. Paul, *ibid.* **23**, 1473 (1978); M. Nielson, O. Gredal, C. Braestrup, *ibid.* **25**, 679 (1979); P. J. Marangos, S. M. Paul, F. K. Goodwin, *ibid.*, p. 1093.
13. M. S. Briley and S. Z. Langer, *Eur. J. Pharmacol.* **52**, 129 (1978); A. Guidotti, G. Toffano, E. Costa, *Nature (London)* **275**, 553 (1978).
14. We thank the doctors and nursing staff of the Service du Professeur Deniker, Hôpital Sainte Anne, for their help in this study and C. Féret for preparing the manuscript.

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Long-Lasting Depletion of Striatal Dopamine by a Single Injection of Amphetamine in Iprindole-Treated Rats

Abstract. *A single injection of amphetamine given to rats treated concurrently with iprindole so that they could not metabolize the amphetamine by* para*-hydroxylation resulted in a decrease in the concentration of striatal dopamine 1 week later. The decrease was antagonized by amfonelic acid, an inhibitor of uptake into dopamine neurons. The long-lasting depletion of cerebral dopamine by amphetamine may be analogous to the depletion of cerebral serotonin by halogenated derivatives of amphetamine.*

Certain analogs of amphetamine are neurotoxic. For example, when a single dose of *p*-chloroamphetamine is injected into rats, the concentration of cerebral serotonin is decreased and remains decreased for months (*1*). Along with this reduction, there are long-lasting reductions in 5-hydroxyindoleacetic acid concentration, tryptophan hydroxylase activity, and high-affinity serotonin uptake (*1*), all of which imply the destruction of serotonin-containing neurons. Supporting this idea are histological findings that cell bodies degenerate in the serotonin-rich raphe region of rat brain (*2*).

Two characteristics of *p*-chloroamphetamine seem necessary for this neurotoxic effect on serotonin neurons: persistence of *p*-chloroamphetamine for many hours because of its slow rate of metabolic removal, and continued active accumulation of *p*-chloroamphetamine in serotonin neurons during this time because of its high affinity for the membrane uptake system on the neurons (*3*, *4*). Amphetamine itself has a high affinity for the membrane uptake system on dopamine neurons (*4*, *5*). Amphetamine is metabolized more rapidly than *p*-chloroamphetamine in the rat because amphetamine is hydroxylated in the *para* position of the phenyl ring, the position occupied by the chlorine substituent in *p*-chloroamphetamine (*6*). This hydroxylation can be inhibited by drugs like iprindole; in iprindole-treated rats the persistence of amphetamine in tissues resembles that of *p*-chloroamphetamine (*7*).

We conducted the present experiments to ascertain whether a single dose of amphetamine causes long-lasting effects on dopamine neurons in iprindole-treated rats (*8*). It has been shown that, although depletion of serotonin by halogenated amphetamines is initially re-

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versible by inhibiting uptake of the drug, the depletion becomes irreversible within 24 to 36 hours (*9*). A similar time course would probably be followed if amphetamine caused long-lasting depletion of dopamine. Therefore iprindole should prevent metabolism of amphetamine for at least 24 hours. A preliminary experiment showed that iprindole (10 mg/kg, intraperitoneally) inhibited amphetamine metabolism for up to 48 hours, as determined by the ability of iprindole pretreatment to increase brain levels of amphetamine measured 2 hours after amphetamine injection (*10*). Thus iprindole can inhibit amphetamine metabolism throughout the time that would be required for long-lasting effects of amphetamine to be produced.

The effect of a single injection of amphetamine on dopamine concentration in rat cerebral hemispheres after 1 week is given in Table 1. Amphetamine given alone produced no significant change in dopamine concentration, but when given to rats treated concurrently with iprindole, it caused a 31 percent decline in dopamine concentration. (Iprindole given alone had no effect on dopamine concentration.) In this experiment, dopamine concentration in the hypothalamus, brainstem, and midbrain was unchanged after amphetamine treatments with or without iprindole. In a second experiment, we analyzed a smaller dopamine-rich region in the cerebral hemispheres, the striatum (Table 1). All rats in this experiment were treated concurrently with iprindole. Again, amphetamine caused a 28 percent decrease in dopamine concentration after 1 week. The decreases in dopamine concentration induced by amphetamine after 1 week are similar to (perhaps slightly smaller than) the decreases in serotonin concentration usually seen 1 week after injection of *p*-chloroamphetamine at an equimolar dose.

In the case of *p*-chloroamphetamine, the decrease in serotonin concentration is antagonized by an inhibitor of uptake into serotonin neurons (*3*). Therefore we examined the effect of amfonelic acid, an inhibitor of uptake into dopamine neurons (*11*), on the decline in dopamine concentration after amphetamine injection. As shown in Table 2, the long-lasting, amphetamine-induced depletion of striatal dopamine was antagonized by inhibition of uptake into dopamine neurons. Amfonelic acid given alone caused no significant effect on dopamine concentration in the striatum or the hypothalamus. The depletion of dopamine by amphetamine in the striatum was, however, significantly antagonized by amfonelic acid. In this experiment, the norepinephrine concentration in the striatum and the norepinephrine and epinephrine concentrations in the hypothalamus were also measured. No significant change was induced by amphetamine or amfonelic acid.

Table 1. Amphetamine-induced depletion of dopamine concentration in rat cerebral hemispheres and striata. (+)Amphetamine sulfate (18.4 mg/kg) and iprindole hydrochloride (10 mg/kg) were injected intraperitoneally 1 week before the rats were killed. Data are mean values for five or six rats per treatment.

Treatment	Dopamine concentration (nmole/g)
Dopamine measured in cerebral hemispheres	
None	3.57 ± 0.05
Amphetamine	3.44 ± 0.17
Iprindole	3.62 ± 0.09
Amphetamine + iprindole	2.45 ± 0.10*
Dopamine measured in striata of iprindole-treated rats	
None	48.2 ± 1.9
Amphetamine	34.5 ± 1.4*

*Significant depletion compared to controls ($P < .005$, Student's t-test).

These results extend earlier findings of persistent dopamine depletion after long-term administration of amphetamine. Ellison *et al.* (*12*) reported chronic biochemical and histologic changes in dopaminergic innervation of rat caudate nucleus after removal of subcutaneous pellets containing amphetamine. Hotchkiss *et al.* (*13*) found persistent decreases in tyrosine hydroxylase, the rate-limiting enzyme in dopamine biosynthesis, in the neostriatum of rats treated with five successive doses of methamphetamine. Wagner *et al.* (*14*) reported long-lasting decreases in dopamine concentration in rat striatum after repeated administration of methamphetamine. Thus amphetamine and related drugs may cause long-lasting effects on dopamine neurons in much the same way as halogenated derivatives of amphetamine cause long-lasting effects on serotonin neurons. Single injections cause these changes, provided the drugs are not metabolized too rapidly, and the effects are dependent on neuronal membrane uptake mechanisms. The structural dependence, total duration, stereospecificity, and irreversibility of the effect of amphetamine on dopamine neurons remain to be studied.

Table 2. Antagonism of the amphetamine-induced decrease in striatal dopamine by amfonelic acid. (+)Amphetamine sulfate (18.4 mg/kg) and amfonelic acid (5 mg/kg) were injected intraperitoneally 1 week before the rats were killed. All rats received iprindole hydrochloride (10 mg/kg, intraperitoneally) at zero time. Data are mean values for five or six rats per treatment.

Treatment	Dopamine concentration (nmole/g)	
	Striatum	Hypothalamus
None	47.6 ± 1.4	1.17 ± 0.02
Amphetamine	35.3 ± 2.7*	1.13 ± 0.09
Amphetamine + amfonelic acid	42.7 ± 1.7*†	1.17 ± 0.06
Amfonelic acid	46.0 ± 2.8	1.15 ± 0.02

*Significant depletion compared to controls ($P < .05$). †Significant antagonism of amphetamine effect ($P < .05$).

Amphetamine is widely abused. The recognition that a single dose of amphetamine can cause long-lasting, possibly neurotoxic changes in certain brain neurons may be of value in exploring a neurochemical basis for persistent behavioral changes in chronic amphetamine abusers.

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References and Notes

1. E. Sanders-Bush, J. A. Bushing, F. Sulser, *Eur. J. Pharmacol.* **20**, 385 (1972); R. W. Fuller and H. D. Snoddy, *Neuropharmacology* **13**, 85 (1974); E. Sanders-Bush and L. R. Steranka, *Ann. N.Y. Acad. Sci.* **305**, 208 (1978).
2. J. A. Harvey, S. E. McMaster, L. M. Yunger, *Science* **187**, 841 (1975); J. A. Harvey, S. E. McMaster, R. W. Fuller, *J. Pharmacol. Exp. Ther.* **202**, 581 (1977); J. A. Harvey, *Ann. N.Y. Acad. Sci.* **305**, 289 (1978).
3. J. L. Meek, K. Fuxe, A. Carlsson, *Biochem. Pharmacol.* **20**, 707 (1971); R. W. Fuller, R. J. Schaffer, B. W. Roush, B. B. Molloy, *ibid.* **21**, 1413 (1972); R. W. Fuller and J. C. Baker, *J. Pharm. Pharmacol.* **26**, 912 (1974); R. W. Fuller, *Ann. N.Y. Acad. Sci.* **305**, 147 (1978).
4. D. T. Wong, J.-S. Horng, R. W. Fuller, *Biochem. Pharmacol.* **22**, 311 (1973).
5. B. K. Koe, *J. Pharmacol. Exp. Ther.* **199**, 649 (1976); S. H. Snyder, K. M. Taylor, J. T. Coyle, J. L. Meyerhoff, *Am. J. Psychiatry* **127**, 117 (1970).
6. L. G. Dring, R. L. Smith, R. T. Williams, *Biochem. J.* **116**, 425 (1970); R. W. Fuller and C. W. Hines, *J. Pharm. Sci.* **56**, 302 (1967).
7. J. J. Freeman and F. Sulser, *J. Pharmacol. Exp. Ther.* **183**, 307 (1972).
8. Male Wistar rats (Harlan Industries) weighing 130 to 150 g were housed separately in hanging wire cages and given free access to food and water. (+)Amphetamine sulfate (Chemical Procurements) was injected at a dose of 18.4 mg/kg (0.1 mmole/kg, a dose previously used to demonstrate long-lasting depletion of serotonin by *p*-chloroamphetamine). This dose, although high, was not lethal even to rats also treated with iprindole. Iprindole hydrochloride (Wyeth) and amfonelic acid (Sterling Winthrop) were injected at doses of 10 and 5 mg/kg, respectively. All injections were intraperitoneal. One week later, the rats were decapitated. Each brain was rapidly excised and dissected into cerebral hemispheres (with cerebellum, brainstem, hypothalamus, and midbrain removed) or striatum. Tissue samples (mean wet weight, 1170 mg for cerebral hemispheres and 54 mg for striatum) were frozen on dry ice. Concentrations of dopamine, norepinephrine, and epinephrine were determined by high-performance liquid chromatography with electrochemical detection [R. W. Fuller and K. W. Perry, *Biochem. Pharmacol.* **26**, 2087 (1977)]. Amphetamine in whole brain was extracted and identified by reaction with fluorescamine [R. W. Fuller, K. W. Perry, J. C. Baker,

C. J. Parli, N. Lee, W. A. Day, B. B. Molloy, *ibid.* **23**, 3267 (1974)].
9. R. W. Fuller, K. W. Perry, B. B. Molloy, *Eur. J. Pharmacol.* **33**, 119 (1975); R. W. Fuller and K. W. Perry, *IRCS J. Med. Sci.* **6**, 117 (1978); R. W. Fuller *et al.*, *J. Pharmacol. Exp. Ther.* **212**, 115 (1980).
10. Iprindole hydrochloride (10 mg/kg) was injected intraperitoneally at various times before the injection of (+)amphetamine sulfate (18.4 mg/kg, intraperitoneally). Brain levels of amphetamine measured 2 hours after injection of amphetamine were 55 ± 6 ng/g in rats not receiving iprindole. When amphetamine was injected 1, 4, 16, 24, or 48 hours after administration of iprindole, amphetamine concentrations in brain tissue were 157 ± 8, 148 ± 10, 167 ± 5, 160 ± 10, and 80 ± 4 ng/g, respectively. All of these values differed significantly from those in the group not first treated with iprindole ($P < .05$). Blank values for untreated rats or rats treated with iprindole alone were not significantly different from those at zero time.
11. R. W. Fuller, K. W. Perry, F. P. Bymaster, D. T. Wong, *J. Pharm. Pharmacol.* **30**, 197 (1978); S. B. Ross, *Life Sci.* **24**, 159 (1979).
12. G. Ellison, M. S. Eison, H. S. Huberman, F. Daniel, *Science* **201**, 276 (1978).
13. A. J. Hotchkiss, M. E. Morgan, J. W. Gibb, *Life Sci.* **25**, 1373 (1979).
14. G. C. Wagner *et al.*, *Brain Res.* **181**, 151 (1980).

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β-Lipotropin: A New Aldosterone-Stimulating Factor

Abstract. *β-Lipotropin stimulated the production of aldosterone in collagenase-dispersed rat adrenal capsular cells. The maximum response obtained with β-lipotropin was the same as the response obtained with corticotropin and was greater than that obtained with angiotensin II. These data suggest that β-lipotropin may play a role in aldosterone regulation.*

Although it is well known that angiotensin II (AII), potassium ion, and adrenocorticotropic hormone (ACTH) stimulate aldosterone secretion, it has become evident that all aspects of the regulation of aldosterone secretion cannot be fully explained by the known stimulators. Previous reports (*1*, *2*) suggest that non-ACTH pituitary factors may play a role in regulation of aldosterone secretion. In 1965, β-lipotropin (β-LPH) was isolated from the ovine pituitary gland (*3*). Both ACTH and β-LPH are contained in a common precursor molecule present in the pituitary gland (*4*). Recently β-LPH became the focus of interest as a prohormone of opioid peptides, but there is no information available regarding the effect of β-LPH on the adrenal gland. In this report, we discuss the effect of sheep and human β-LPH on adrenal steroidogenesis in rat adrenal collagenase-dispersed capsular and decapsular cells. Results indicate that β-LPH has a potent effect on aldosterone production.

For each experiment, 18 to 20 female Sprague-Dawley rats (180 to 220 g), fed a regular sodium diet for at least 2 weeks, were used (*5*). After the animals were decapitated, the adrenal glands were removed and separated into capsular (mainly glomerulosa cells) and decapsular (fasciculata, reticularis, and medullary cells) portions. Each portion was minced with scissors, washed with medium 199 (*6*), and incubated with collagenase (2 mg per milliliter of medium 199) for 20 minutes at 37°C under 95 percent O_2 plus 5 percent CO_2. To disperse capsular and decapsular cells, medium 199 containing bovine serum albumin (BSA; 2 mg/ml) was added to the collagenase-treated tissues, which were then agitated with a Pasteur pipette. The cell suspensions were filtered through gauze into a 50-ml polyethylene centrifugation tube. The dispersed cells were pooled and centrifuged at 100*g* for 15 minutes, and the sedimented cells were resuspended in fresh medium 199 containing BSA (2 mg/ml). After the capsular and decapsular cells were harvested, these cell suspensions (average cell count, 100,000 per tube) were incubated in 1 ml of medium 199 containing BSA (2 mg/ml), with various amounts (10^{-11} to $10^{-5}M$) of sheep and human β-LPH, for 2 hours at 37°C under 95 percent O_2 and 5 percent CO_2. The β-LPH was isolated from sheep or human pituitary glands as described (*7*). Amino acid sequences of sheep and human β-LPH have been elucidated (*3*, *8*). The purity of the β-LPH preparations was verified; a single band was noted when disc electrophoresis on 7 percent polyacrylamide gel at *p*H 4.5 was used, and glutamic acid was the only amino terminal residue noted by the dansyl method (*9*). The β-LPH concentration was determined by ultraviolet absorption. In suspensions of capsular cells, the production of aldosterone in response to synthetic [Asp^1-Ile^5]AII ($10^{-4}M$) (Asp, aspartic acid; Ile, isoleucine) and porcine ACTH (86 I.U./mg; $4 \times 10^{-6}M$) were compared in each experiment. The maximum steroidogenic response to synthetic human ACTH was the same as it was to porcine ACTH. These concentrations are well above the maximum stimulating doses (*5*). In suspensions of decapsular cells, response to the same dose of porcine ACTH was compared in each experiment. Aldosterone and corticosterone were measured directly by radioimmunoassay (*5*, *10*).

At 10^{-8} to $10^{-7}M$, sheep β-LPH stimulated production of aldosterone by the capsular cells, but not that of corticosterone by the decapsular cells (Fig. 1). Human β-LPH produced a similar effect. In a single experiment with synthetic sheep β-LPH (*11*), aldosterone stimulation was similar to that caused by the natural product. A significant increase in aldosterone production was obtained with $10^{-9}M$ β-LPH [from the control value of 32.9 ± 1.9 (standard error) ng per 100,000 capsular cells to 38.6 ± 2.0 ng per 100,000 capsular cells; $P < .02$]; the half-maximum increase in aldosterone production was obtained at a β-LPH concentration between $3 \times 10^{-8}M$ and $10^{-7}M$ (Fig. 2). The maximum response

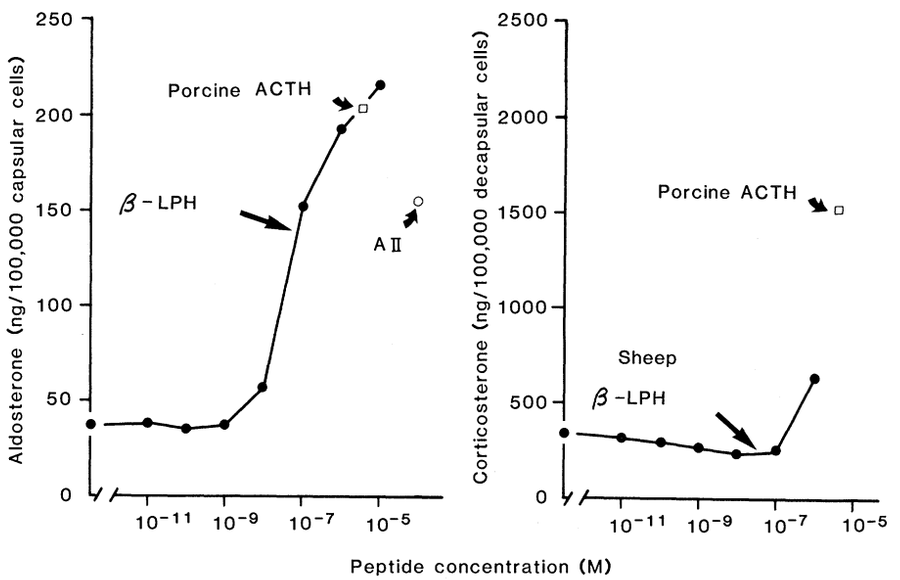

Fig. 1. Aldosterone and corticosterone production by sheep β-LPH in capsular and decapsular cells in a representative experiment. Each point represents the mean of duplicate incubations. Peptide concentrations are expressed as moles per liter of incubation medium.

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