MFO system cleaves [^{14}C-*phenyl*]-(±)-profenofos (CIBA-Geigy) at both the P–O–phenyl and P–S–propyl groups, in each case requiring NADPH, as indicated by thin-layer chromatography with authentic standards.
11. The oxime HS-6 [1-(2-hydroxyiminomethylpyridinium)-1-(3-carboxamidopyridinium)-dimethyl ether] is an antidote for organophosphate poisoning acting on the cholinergic system in part by reactivating AChE [J. G. Clement, *Fundam. Appl. Toxicol.* **1**, 193 (1981)].
12. A. M. A. Khasawinah, R. B. March, T. R. Fukuto, *Pestic. Biochem. Physiol.* **9**, 211 (1978); C. P. Robinson and D. Beiergrohslein, *ibid.* **13**, 267 (1980); C. M. Thompson and T. R. Fukuto, *J. Agric. Food Chem.* **30**, 282 (1982).
13. G. L. Ellman *et al.*, *Biochem. Pharmacol.* **7**, 88 (1961).
14. Supported in part by grant P01 ES00049 from the National Institute of Environmental Health Sciences. L. Cole provided valuable advice and assistance.

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Guanosine Triphosphate Activation of Brain Adenylate Cyclase: Enhancement by Long-Term Antidepressant Treatment

Abstract. *Activation of adenylate cyclase by a stable guanosine 5′-triphosphate analog was augmented in brain membrane preparations from rats treated on a long-term basis with tricyclic antidepressants or electroconvulsive shock. These treatments may facilitate cyclase activation by promoting the interaction of the regulatory and catalytic subunits of the enzyme. This finding suggests a possible mechanism for the changes in sensitivity to various neurotransmitters seen after antidepressant administration.*

A clinical response to antidepressant therapy typically does not develop until one or more weeks after the initiation of treatment (*1*). Accordingly, investigators of antidepressant mechanisms have recently focused on biochemical and physiological changes induced by long-term treatment that may be correlated with the delayed therapeutic effect (*2, 3*). Specifically, altered sensitivity of neurotransmitter receptors has been detected in a variety of systems and has been proposed as a common mechanism underlying the clinical response to various antidepressant therapies in humans (*2*).

Much of the current interest in antidepressant-induced receptor changes stems from early observations that both tricyclic antidepressants and electroconvulsive treatment (ECT) can reduce the norepinephrine-stimulated production of adenosine 3′,5′-monophosphate (cyclic AMP) in rat brain slices (*4, 5*). Cyclic AMP has been proposed to function in synaptic transmission as a "second messenger," perhaps mediating the action of certain neurohumors at a postreceptor level (*6*). The activation of adenylate cyclase, the plasma membrane–associated enzyme that catalyzes the formation of cyclic AMP from adenosine triphosphate, is regulated by a guanosine 5′-triphosphate (GTP)–binding protein (G unit) that couples the catalytic moiety with neurotransmitter (or other hormone) receptors (*7, 8*). Decreases in β-receptor density after long-term antidepressant treatment have been correlated with decreases in isoproterenol-induced cyclic AMP accumulation (*9*). In several cases (*2*), however, altered receptor binding following antidepressant treatment cannot fully account for changes in hormone-stimulated accumulation of cyclic AMP. Accordingly, it is of interest to examine the effects of antidepressant treatment on the regulation of adenylate cyclase through the G unit. We report that long-term, but not short-term, treatment of rats with tricyclic antidepressants or ECT produces an increase in G unit–dependent activity of adenylate cyclase in the brain. An enhanced physical association between the adenylate cyclase G unit and the catalytic moiety may be responsible for this effect.

Adult male albino rats (Charles River) weighing 225 to 300 g were treated for 15 to 20 days with daily intraperitoneal injections of 10 mg of amitriptyline (Merck Sharpe & Dohme), desipramine (USV Pharamaceuticals), imipramine (Ciba-Geigy), or iprindole (Wyeth) per kilogram. Controls received daily injections of 0.9 percent saline in the same volume (1 ml/kg). Animals receiving long-term treatment were killed 22 to 28 hours after the last injection. Animals receiving single injections were killed 60 minutes later.

Electroconvulsive treatment was administered with sinusoidal current (80 mA, 60 Hz, 0.1 second) through saline-moistened corneal electrodes. This treatment reliably produced convulsions with tonic extension, followed by clonus persisting for 10 to 25 seconds (*10*). One group received ECT on alternate days for a total of five to seven treatments; a second group was given a single treatment, and a third received sham ECT (electrodes applied without current). All

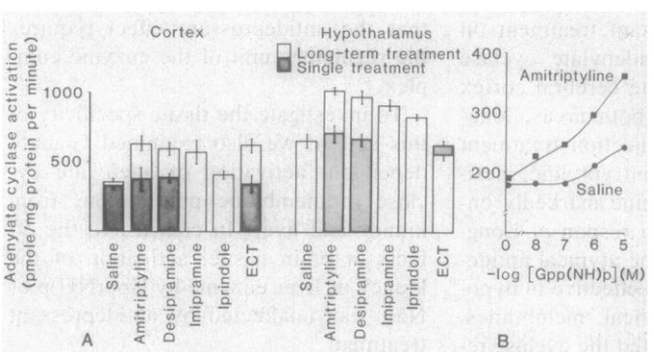

Fig. 1. (A) Effects of long- and short-term antidepressant treatment on Gpp(NH)p activation of adenylate cyclase in membrane particles from the cerebral cortex or hypothalamus. Hypothalamic membranes were prepared as described by Wheeler *et al.* (*29*). Hypothalami were homogenized 1:20 (weight to volume) in ice-cold 2 m*M* tris maleate with 0.8 m*M* EGTA (*p*H 7.4) by a Teflon-on-glass homogenizer at 1000 rev/min. Homogenates were centrifuged for 5 minutes at 600*g* and the pellet was discarded. Supernatants were centrifuged for 10 minutes at 7500*g* and the resulting pellets were resuspended in 0.32*M* sucrose with 50 m*M* tris maleate (*p*H 7.8), 5 m*M* $MgSO_4$, 1 m*M* EDTA, and 1 m*M* dithiothreitol. Following two cycles of centrifugation (7500*g* for 10 minutes) and washing, the pellets were resuspended in the same media to a protein concentration of 1 to 2 mg/ml and stored under liquid nitrogen until assay. Adenylate cyclase assays (*13*) were performed in 50 μl of a reaction mixture containing 80 m*M* tris maleate (*p*H 7.4), 1 m*M* isobutylmethylxanthine, 2 m*M* $MgSO_4$, 0.5 m*M* adenosine triphosphate (ATP) with 60 m*M* phosphenol pyruvate and pyruvate kinase (0.5 mg/ml), 0.5 m*M* dithiothreitol, and 0.4 m*M* EGTA. Hypothalamic membranes (1 to 2 mg of protein per milliliter) were incubated with 5 μ*M* Gpp(NH)p for 10 minutes before the reaction mixture was added. Reactions were allowed to proceed for 10 minutes and were then stopped by boiling for 4 minutes. Cortical membrane particles were prepared in a similar manner (*13*), and the adenylate cyclase assay was identical except for the concentration of Gpp(NH)p, which was 2 μ*M*. Reaction rates in both cases were linear for 20 minutes. In the presence of the ATP-regenerating system, ATP concentrations were unchanged for up to 20 minutes (*13*). Accumulated cyclic AMP was assayed by a modification of the method of Brown *et al.* (*30*) involving a binding protein from rabbit skeletal muscle. Protein content was determined in accordance with the method of Bradford (*31*). Values are means ± standard deviations for one of at least four experiments performed in duplicate (cortex) or triplicate (hypothalamus). (B) Effect of varying the concentration of Gpp(NH)p on the activation of adenylate cyclase in hypothalamic membranes from animals given repeated saline or amitriptyline treatments. Tissue was prepared and assays were carried out as described in (A), except that Gpp(NH)p and the reaction mixture were added to the membranes simultaneously. Standard deviations are less than 10 percent of the means graphed.

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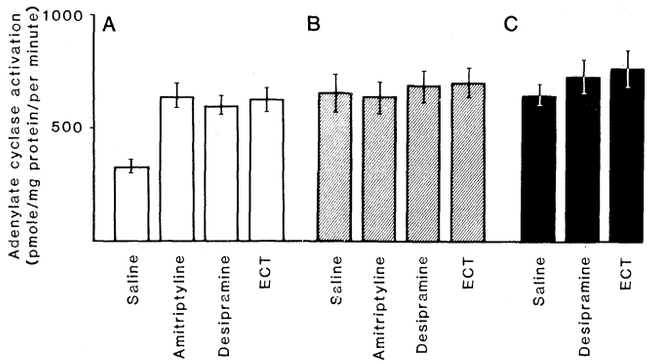

Fig. 2. Effect of *cis*-vaccenic acid and colchicine on Gpp(NH)p-activated adenylate cyclase in cortical membranes from control and antidepressant-treated animals. (A) Activation of adenylate cyclase by 2 μM Gpp(NH)p in the various long-term treatment groups. The same experiment was repeated in the presence of 100 μM *cis*-vaccenic acid (B) and 2.0 μM colchicine (C). The detection assay was performed as described in the legend to Fig. 1A. Values are means ± standard deviations.

the rats were killed 24 hours after the last convulsion.

After treatment with drugs or ECT, the rats were decapitated and their brains were rapidly dissected on ice. Cerebral cortical tissue rostral to a coronal section through the optic chiasm was separated from the striatum and immediately transferred to ice-cold buffer. The hypothalamus was also excised (*11*).

G unit–dependent activation of adenylate cyclase was measured with the nonhydrolyzable GTP analog guanylylimidodiphosphate (Gpp(NH)p) (*8*). Figure 1A shows the effect of long- and short-term antidepressant treatment on Gpp(NH)p-activated adenylate cyclase in membranes from the cerebral cortex or hypothalamus. In both areas, long-term but not single-injection treatment with the tricyclics amitryptyline, desipramine, and imipramine markedly enhanced the Gpp(NH)p response. Long-term treatment with the atypical antidepressant iprindole was effective in hypothalamic, but not cortical, membranes. Long-term ECT elevated the cyclase response to Gpp(NH)p in the cortex but was without effect in the hypothalamus. The effect of ECT, like that of the tricyclics, appears to depend on long-term treatment, since a single shock had no significant effect (Fig. 1A). Adenylate cyclase activation in rats given sham ECT resembled that in controls given saline.

The drug-enhanced G unit activation of adenylate cyclase in hypothalamic membranes was studied over a range of Gpp(NH)p concentrations. Long-term amitriptyline treatment elevated the cyclase response to Gpp(NH)p present at concentrations from 10^{-8} to $10^{-5} M$ (Fig. 1B). A similar response was found at $10^{-4} M$ Gpp(NH)p in cortical membranes from imipramine-treated rats (*12*). These findings are consistent with the possibility that long-term treatment with antidepressants increases the ability of the nucleotide-bound G unit to activate the catalytic moiety, although an enhanced binding of Gpp(NH)p to the G unit or an increase in the number of G units cannot be ruled out.

G unit–dependent adenylate cyclase activity can also be assessed with fluoride ions (*13, 14*). Activation of adenylate cyclase by NaF was enhanced in cortical membranes from rats given long-term treatment with antidepressants. By contrast, direct activation of the adenylate cyclase catalytic moiety by Mn^{2+} (*13–15*) was not altered in cortical membranes from treated animals, suggesting that the antidepressant effect requires the regulatory unit of the enzyme complex.

To investigate the tissue specificity of this action, we also examined G unit–dependent activation of adenylate cyclase in membrane preparations from kidney and liver. In contrast to the effects in brain tissue, activation of the kidney or liver enzyme by Gpp(NH)p or NaF was unaffected by antidepressant treatment.

Free fatty acids, such as *cis*-vaccenic acid, may facilitate G unit activation of adenylate cyclase, presumably by fluidizing the membrane and allowing greater interaction between the G unit and the catalytic moiety (*15, 16*). Microtubule-disrupting agents, such as colchicine or vinblastine, may enhance G unit activation of adenylate cyclase by releasing cytoskeletal constraints on the G unit–catalytic moiety interaction without altering membrane fluidity (*17*). To evaluate possible mechanisms whereby antidepressant treatment might facilitate G unit activation of adenylate cyclase, we investigated the effect of membrane fluidizers and microtubule disrupters on enzyme preparations from control and treated animals.

Treatment of control cortical membranes with *cis*-vaccenic acid or colchicine increased the Gpp(NH)p activation of adenylate cyclase (*15*) approximately to the level seen in antidepressant-treated animals (Fig. 2). By contrast, *cis*-vaccenic acid or colchicine treatment of membranes from animals given long-term treatment with amitriptyline, desipramine, or ECT produced only a minimal further enhancement of Gpp(NH)p activation of adenylate cyclase. Thus, in the presence of *cis*-vaccenic acid or colchicine, the difference in G unit activation of adenylate cyclase between control and antidepressant-treated animals was abolished (Fig. 2). Similar results were obtained with other fluidizing or microtubule-disrupting agents, such as linoleic acid or vinblastine.

These results suggest that long-term treatment of rats with antidepressants or ECT may enhance G unit–catalytic moiety interaction in a manner similar to that produced by addition of colchicine or *cis*-vaccenic acid in vitro. To further examine this possibility, we made direct measurements of membrane fluidity in membranes from control and treated animals with the technique of fluorescence depolarization (*18*). The technique, in which 1,6-diphenylhexatriene is used as a fluorescent probe, failed to show an effect of antidepressant treatment on cortical membrane fluidity. Thus, the effect of antidepressant administration is analogous to that of colchicine, rather than that of *cis*-vaccenic acid, in that both enhance G unit–catalytic moiety interaction without producing a gross alteration in membrane fluidity.

We have shown that antidepressant treatment enhances G unit activation of adenylate cyclase in membrane particles from the cerebral cortex and hypothalamus (*19*). The effect requires long-term antidepressant treatment (Fig. 1) and, in the case of the tricyclic antidepressants, is probably not a result of drug accumulation, since concentrations of these compounds in the brain are higher 1 hour after a single injection than 24 hours after the final injection in a sequence (*5*). Moreover, addition of tricyclic antidepressants in vitro, even at high concentrations, fails to alter Gpp(NH)p activation of adenylate cyclase (*20*). Short-term treatment with amitriptyline markedly elevates cortical levels of cyclic AMP in vivo (*21*); whether altered G unit–catalytic moiety interaction is involved in this phenomenon remains to be established.

Some evidence suggests that an enhanced physical association of the G unit with the catalytic moiety may underlie the augmented activation of adenylate cyclase by Gpp(NH)p and NaF observed

after antidepressant treatment. The ability of *cis*-vaccenic acid or colchicine to increase Gpp(NH)p activation of cyclase in membranes from control but not treated animals is consistent with the idea that G unit–catalytic moiety interaction is already enhanced in the latter.

The possibility that such an enhanced interaction is associated with antidepressant action is suggested by the finding that the effect is induced by typical and atypical tricyclic antidepressants as well as by ECT. Moreover, long-term treatment is required for the effect to become manifest.

Since a facilitated G unit–catalytic moiety interaction is generally associated with enhanced hormone responsiveness (*16*), the present results appear to be inconsistent with the observation that ECT or tricyclic antidepressants decrease norepinephrine-stimulated accumulation of cyclic AMP in brain slices (*4, 5*). However, the α_2-adrenergic receptor system is regulated by guanyl nucleotides (*22*) and may inhibit adenylate cyclase in a GTP-dependent manner (*23*). Thus, enhanced G unit–catalytic moiety interaction might augment α_2-adrenergic inhibition of cyclase by norepinephrine and thereby reduce the net stimulation of cyclic AMP accumulation in brain slices by norepinephrine (*24*).

Repeated ECT markedly enhances the stimulation of cyclic AMP levels in cortical slices by norepinephrine in the presence of adenosine (*25*). Similarly, recent electrophysiological and behavioral studies have shown enhanced α-adrenergic (*26*), serotonergic (*27*), and dopaminergic (*28*) responsiveness in the brain after long-term antidepressant treatment. It would be of particular interest to examine the role of G unit alterations in these effects as a means of integrating data on receptor binding with data on the physiological and behavioral effects of antidepressant treatment.

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References and Notes

1. I. Oswald, V. Brezinova, D. L. F. Dunleavy, *Br. J. Psychiatry* **120**, 673 (1972); D. V. Klein, R. Gittelman, F. Quitkin, A. Rifkin, *Diagnosis and Drug Treatment of Psychiatric Disorders: Adults and Children* (Williams & Wilkins, Baltimore, ed. 2, 1980).
2. F. Sulser, J. Vetulani, P. L. Mobley, *Biochem. Pharmacol.* **27**, 257 (1978); D. S. Charney, D. B. Menkes, G. R. Heninger, *Arch. Gen. Psychiatry* **38**, 1160 (1981).
3. M. F. Sugrue, *Pharmacol. Ther.* **13**, 219 (1981).
4. A. Frazer, G. Pandey, J. Mendels, S. Neeleg, M. Kane, M. E. Hess, *Neuropharmacology* **13**, 1131 (1974).
5. J. Vetulani, R. J. Stawarz, J. V. Dingell, F. Sulser, *Naunyn-Schmiedeberg's Arch. Pharmacol.* **293**, 109 (1976).
6. F. E. Bloom, *Rev. Physiol. Biochem. Pharmacol.* **74**, 1 (1975); P. Greengard, *Nature (London)* **260**, 101 (1976).
7. Adenylate cyclase is composed of at least three plasma membrane–associated proteins: the hormone or neurotransmitter receptor, the guanyl nucleotide–binding regulatory subunit (G unit), and the catalytic moiety. The enzyme is activated when hormone, bound to the receptor, causes the G unit to bind GTP and subsequently activates the catalytic moiety (*8*).
8. M. Rodbell, *Nature (London)* **284**, 117 (1980).
9. S. P. Banerjee, L. S. Kung, S. J. Riggi, S. K. Chanda, *ibid.* **268**, 455 (1977); B. S. Wolfe, T. K. Harden, J. R. Sporn, P. B. Molinoff, *J. Pharmacol. Exp. Ther.* **207**, 446 (1978); R. Mishra, A. Janowsky, F. Sulser, *Neuropharmacology* **19**, 983 (1980).
10. E. A. Swinyard, in *Experimental Models of Epilepsy*, D. Purpura *et al.*, Eds. (Raven, New York, 1972), pp. 434–458.
11. J. Glowinski and L. L. Iversen, *J. Neurochem.* **13**, 655 (1966).
12. M. Turck, W. F. Schocker, A. M. Fathy, J. E. Schultz, *Arch. Pharm. (Weinheim, Ger.)* **313**, 768 (1980).
13. M. M. Rasenick and M. W. Bitensky, *Proc. Natl. Acad. Sci. U.S.A.* **77**, 4628 (1980).
14. E. Ross and A. G. Gilman, *Annu. Rev. Biochem.* **49**, 533 (1980).
15. M. M. Rasenick, P. J. Stein, M. W. Bitensky, *Nature (London)* **294**, 560 (1981).
16. J. Orly and M. Schramm, *Proc. Natl. Acad. Sci. U.S.A.* **72**, 3433 (1975); G. Rimon, E. Hanski, S. Braun, A. Levitzki, *Nature (London)* **276**, 295 (1978).
17. Although colchicine, vinblastine, and *cis*-isomers of unsaturated fatty acids are capable of enhancing Gpp(NH)p or NaF activation of adenylate cyclase in cortical synaptic membranes, there is a mechanistic distinction between the microtubule-disrupting agents and the fatty acids. The fatty acids increase synaptic membrane fluidity and may consequently enhance G unit–catalytic moiety interaction. Colchicine and vinblastine, however, do not appear to increase overall membrane fluidity but rather may liberate a constraint on the G unit, increasing its interaction with the catalytic moiety (*15*).
18. M. Shinitzky and Y. Barenholz, *J. Biol. Chem.* **249**, 2652 (1974).
19. Only the cortex shows increased Gpp(NH)p-activated adenylate cyclase activity after long-term ECT. Conversely, only the hypothalamus displays increased adenylate cyclase activity after long-term iprindole treatment. A possible explanation for this disparity is the heterogeneity of various brain regions. Hypothalamic and cortical adenylate cyclase varies in the "coupled" response to agonist as well as to fluoride [(*15, 29*); J. W. Daly, *Cyclic Nucleotides in the Nervous System* (Plenum, New York, 1977)].
20. M. A. Wheeler and M. M. Rasenick, unpublished observations.
21. G. C. Palmer, D. J. Jones, M. A. Medina, W. B. Stavinoha, *Neuropharmacology* **16**, 435 (1977).
22. D. C. U'Prichard and S. H. Snyder, *J. Biol. Chem.* **253**, 3444 (1978); D. J. Kahn, J. C. Mitrius, D. C. U'Prichard. *Mol. Pharmacol.* **21**, 17 (1982).
23. W. Saur and G. Scholtz, *FEBS Lett.* **85**, 167 (1978).
24. P. L. Mobley and F. Sulser, *Eur. J. Pharmacol.* **60**, 221 (1979).
25. A Sattin, in *Chemisms of the Brain*, S. Stahl and R. Rodnight Eds. (Churchill Livingstone, Edinburgh, Scotland, 1981).
26. D. B. Menkes, G. K. Aghajanian, R. B. McCall, *Life Sci.* **27**, 45 (1980); D. B. Menkes and G. K. Aghajanian, *Eur. J. Pharmacol.* **74**, 47 (1981); J. Maj, E. Mogilnicka, V. Klimek, *J. Neural Transm.* **44**, 221 (1979); J. Maj, E. Mogilnicka, A. Kordecka-Magiera, *Pharmacol. Biochem. Behav.* **13**, 153 (1981).
27. C. de Montigny and G. K. Aghajanian, *Science* **202**, 1303 (1978); R. Y. Wang and G. K. Aghajanian, *Commun. Psychopharmacol.* **4**, 83 (1980); E. Friedman and A. Dallob, *ibid.* **3**, 89 (1979); D. W. Costain *et al.*, *Psychopharmacology* **61**, 167 (1979).
28. A. R. Green, D. J. Heal, D. G. Grahame-Smith, *Psychopharmacology* **52**, 195 (1977); C. Spyraki and H. C. Fibiger, *Eur. J. Pharmacol.* **74**, 195 (1981).
29. M. Wheeler, M. Tishler, M. W. Bitensky, *Brain Res.* **231**, 387 (1982).
30. B. Brown, R. Elkins, J. Albano, *Adv. Cyclic Nucleotide Res.* **2**, 25 (1972).
31. M. Bradford, *Anal. Biochem.* **72**, 248 (1976).
32. We thank G. Aghajanian and D. U'Prichard for helpful discussions. Supported by NIH grants AM 20179, AM 06287, and GM 07205.
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Parathyroid Hormone: Effects of the 3–34 Fragment in vivo and in vitro

Abstract. *The biologically active fragment of parathyroid hormone, consisting of residues 1–34, and its in vitro antagonist, fragment 3–34, were administered separately or in combination to chronically thyroparathyroidectomized dogs. These fragments were also studied in vitro with dog renal cortical membranes. Fragment 3–34 inhibited the stimulation of adenylate cyclase by fragment 1–34 in vitro, but had no agonist or antagonistic effects on renal phosphate transport in vivo.*

The native bovine parathyroid hormone (bPTH) molecule is a single-chain polypeptide of 84 amino acids. Many of its biological effects, however, can be reproduced by the first 34 residues at the amino terminus, bPTH-(1–34) (*1, 2*). The 1–34 fragment can thus increase urinary phosphate excretion (*1*), increase the excretion of adenosine 3′,5′-monophosphate (cyclic AMP) (*1*), mobilize calcium from bone (*1, 2*), and stimulate the adenylate cyclase system in vitro in both rat renal cortex and calvaria (*1, 2*). Furthermore, its potency is equal to that of the native molecule (on a molar basis) in most bioassays of parathyroid hormone action. Removal of the first two amino acid residues, to produce bPTH-(3–34), not only results in the loss of these agonist properties of the molecule, but produces a fragment that is antagonistic in vitro to both the 1–34 and 1–84 hormone molecule. This inhibitory effect has been demonstrated in the rat renal cortex adenylate cyclase system of the rat renal cortex (*3, 4*). Modification of the 3–34 fragment, [Nle-8, Nle-18, Tyr-34]bPTH-(3–34)-amide [sub-bPTH-(3–34)], produces a more potent and more stable antagonist (*4*).

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