interesting that predictions of this thickness (*14*) agree well with the observed heights. Comparative examination of dust devils on Earth and Mars may help clarify many of these issues by demonstrating dependence on different conditions.

Ryan and colleagues (*17*) have interpreted several events in Viking Lander meteorological records as the probable passage of dust devils. The distinguishing property of these events is a rotating wind vector. Our observations support their interpretation, but simultaneous imaging and meteorological data do not exist for any of the detections. High-resolution imaging coverage is so sparse that occurrence statistics must remain uncertain until better data are obtained.

References and Notes

1. G. A. Briggs, W. A. Baum, J. Barnes, *J. Geophys. Res.* **84**, 2795 (1979).
2. R. Greeley and J. D. Iversen, *Wind as a Geological Process* (Cambridge Univ. Press, Cambridge, 1985).
3. J. A. Ryan, *J. Geophys. Res.* **69**, 3759 (1964); F. M. Neubauer, *ibid.* **71**, 2419 (1966); P. J. Gierasch and R. M. Goody, *J. Atmos. Sci.* **30**, 169 (1973).
4. A. S. Monin and A. M. Yaglom, *Statistical Fluid Mechanics: Mechanics of Turbulence* (Massachusetts Institute of Technology Press, Cambridge, 1965).
5. J. D. Iversen *et al.*, *Icarus* **29**, 381 (1976); R. Greeley *et al.*, *Geophys. Res. Lett.* **7**, 121 (1980); J. B. Pollack *et al.*, *Icarus* **29**, 395 (1976).
6. J. A. Ryan and R. D. Sharman, *J. Geophys. Res.* **86**, 3247 (1981); R. E. Arvidson, E. A. Guinness, H. J. Moore, J. Tillman, S. D. Wall, *Science* **232**, 463 (1983). A dust storm–related wind of 30 m sec^{-1} had the highest of the recorded speeds at the Viking Lander 1 site.
7. J. A. Ryan and J. J. Carroll, *J. Geophys. Res.* **75**, 531 (1970).
8. H. H. Kieffer *et al.*, *ibid.* **82**, 4249 (1977).
9. D. H. Scott, K. L. Tanaka, G. Schaber, *U.S. Geological Survey Map I-1276* (1981).
10. J. B. Pollack *et al.*, *J. Geophys. Res.* **84**, 2929 (1979).
11. D. H. Scott and M. H. Carr, *U.S. Geological Survey Map I-1083* (1978).
12. J. B. Pollack *et al.*, *J. Atmos. Sci.* **38**, 3 (1981).
13. S. L. Hess, *J. Geophys. Res.* **82**, 4559 (1977).
14. J. L. Sutton, C. B. Leovy, J. E. Tillman, *J. Atmos. Sci.* **35**, 2346 (1978); P. J. Gierasch and R. M. Goody, *Planet. Space Sci.* **16**, 615 (1968).
15. T. Maxworthy, *ibid.* **30**, 1717 (1973).
16. R. M. Goody and P. J. Gierasch, *ibid.* **31**, 1021 (1974).
17. J. A. Ryan and R. D. Lucich, *J. Geophys. Res.* **88**, 11005 (1983).

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A Potent Nonpeptide Cholecystokinin Antagonist Selective for Peripheral Tissues Isolated from *Aspergillus alliaceus*

Abstract. *A new, competitive, nonpeptide cholecystokinin (CCK) antagonist, asperlicin, was isolated from the fungus* Aspergillus alliaceus. *The compound has 300 to 400 times the affinity for pancreatic, ileal, and gallbladder CCK receptors than proglumide, a standard agent of this class. Moreover, asperlicin is highly selective for peripheral CCK receptors relative to brain CCK and gastrin receptors. Since asperlicin also exhibits long-lasting CCK antagonist activity in vivo, it should provide a valuable tool for investigating the physiological and pharmacological actions of CCK.*

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Research has been accelerating in recent years on the discovery, mechanism of action, and function of neuropeptides (*1*). Nonpeptide agonists and antagonists of neuropeptides are being sought to aid studies of their physiological functions and ultimately to provide leads for potential therapeutic developments. We report now the discovery from a microbial source of a potent, competitive, nonpeptide antagonist of the classic gastrointestinal transmitter cholecystokinin (CCK).

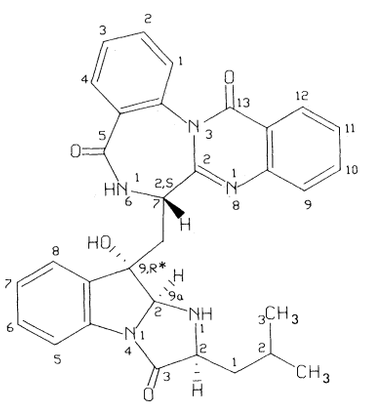

Fig. 1. Structure of asperlicin.

CCK is a hormonal regulator of pancreatic and gastric secretion, contraction of the gallbladder, and gut motility (*2*). CCK also exists in the brain and may have an equally important role as a central nervous system transmitter (*3*). However, in spite of the potential therapeutic utilities for CCK antagonists, previously reported nonpeptide antagonists such as proglumide, dibutyryl guanosine 3′,5′-cyclic monophosphate (dibutyryl cyclic GMP), and benzotript exhibit only low in vitro potencies, which limit their in vivo usefulness (*4*). Some peptide fragments of CCK have exhibited greater in vitro potency as CCK antagonists (*5*), but are subject in vivo to rapid degradation in physiological fluids (*6*).

Our new CCK antagonist, asperlicin, was isolated from an *Aspergillus alliaceus* strain which has been deposited at the American Type Culture Collection with accession number ATCC 20655 (*7*). The structure of asperlicin (Fig. 1) was determined by mass spectrometry, nuclear magnetic resonance, and x-ray crystallography (*8*). Its chemical designation is [2S-[2α,9β,9(*R**),9*a*β]]-6,7-dihydro-7-[[2,3,9,9*a*-tetrahydro-9-hydroxy-2-(2-methylpropyl)-3-oxo-1*H*-imidazo [1,2-*a*]indol-9-yl]methyl]-quinazolino [3,2-*a*][1,4]benzodiazepine-5,13-dione. To our knowledge asperlicin is the first nonpeptide antagonist of a neuropeptide to have been isolated from microbial sources.

Asperlicin was compared with known CCK antagonists for its ability to displace the specific binding of ^{125}I-labeled CCK-33 to CCK receptors in rat pancreatic tissue (*9*). All agents inhibited the specific binding of CCK in a concentration-related manner. The concentration of asperlicin causing half-maximal inhibition of binding (IC_{50} = 1.4 μ*M*) was considerably less than those of the nonpeptide CCK antagonists proglumide, benzotript, and dibutyryl cyclic GMP and similar to the potent peptide antagonist *N*-carbobenzoxy-CCK-(27-32) (Table 1). The specific binding of ^{125}I-labeled CCK to guinea pig brain tissues (*9*) and of ^{125}I-labeled gastrin to guinea pig gastric glands (*10*) was not affected by asperlicin at concentrations 70 times its IC_{50} for inhibiting pancreatic ^{125}I-labeled CCK binding; this result indicates a high degree of selectivity for peripheral CCK receptors.

To examine the mechanism by which asperlicin inhibits CCK binding in pancreatic tissue, ^{125}I-labeled CCK binding

Table 1. Effect of CCK antagonists on specific binding of ^{125}I-labeled CCK-33 in rat pancreas and brain and ^{125}I-labeled gastrin binding in guinea pig gastric glands, determined as described (*9, 10*). Drug displacement studies were conducted with five to six concentrations of unlabeled drug, and IC_{50} values were determined by log probit analysis. Values are the mean ± standard error of at least three determinations performed in triplicate.

Antagonist	IC_{50} (μ*M*)		
	^{125}I-labeled CCK-33 in		^{125}I-labeled gastrin in
	Pancreas	Brain	Gastric glands
Asperlicin	1.4 ± 0.2	>100	>100
N-Carbobenzoxy-CCK-(27–32)	3.5 ± 0.4	6.8 ± 0.8	1.0 ± 0.2
Dibutyryl cyclic GMP	87 ± 11	1600 ± 300	1200 ± 400
Benzotript	102 ± 18	84 ± 13	59 ± 24
Proglumide	600 ± 58	875 ± 125	900 ± 200

in the presence and absence of asperlicin (1.0 μ*M*) was analyzed according to Scatchard (*11*) (Fig. 2). Asperlicin reduced the slope but not the *x*-intercept of the Scatchard plot, indicating a change in the dissociation constant (K_d) of CCK for the receptor without a change in the maximum number of receptors (B_{max}). The data suggest that asperlicin interacts competitively with CCK receptors in pancreatic tissues with an inhibition constant (K_i) of 0.6 ± 0.2 μ*M* (*12*).

The well-known actions of CCK-8 in contracting the isolated guinea pig ileum and gallbladder were used to further study the interaction of asperlicin with peripheral CCK receptors (*13*). Asperlicin antagonized the contractions produced by CCK-8 in both the ileum and gallbladder as a function of concentration. The antagonism was characterized by a parallel shift to the right of the CCK-8 concentration-response curves without significant reduction in the maximum contractile response. Schild plots (*14*) of the data gave slopes not significantly different from unity, indicating competitive antagonism. The pA_2 values for asperlicin as an antagonist of CCK-8 in the guinea pig ileum and gallbladder were 6.4 ± 0.4 and 6.0 ± 0.2, respectively. Similar studies with proglumide and benzotript in the guinea pig ileum gave pA_2 values of 3.9 ± 0.3 and less than 4.7, respectively, indicating that, as well as in the pancreas, asperlicin was considerably more potent than these agents as a CCK antagonist (*15*). The pA_2 values for asperlicin in the guinea pig ileum and gallbladder are in good agreement with its K_i value ($-\log K_i = 6.2$) in displacing ^{125}I-labeled CCK pancreatic binding, thus demonstrating a similar affinity of asperlicin for CCK receptors in each tissue.

Asperlicin also demonstrated specificity for antagonism of contractions in the guinea pig ileum produced by CCK-8 compared with other contractile agonists of this tissue. Concentrations of asperlicin (13 μ*M*), which were 33 times its pA_2 value for antagonism of CCK-8, did not significantly affect the concentration-response curves or maximum contractile responses to acetylcholine, histamine, substance P, or pentagastrin, nor did it affect contractions of this tissue produced by electrical stimulation (*16*).

Since, chemically, asperlicin contains a benzodiazepine structure common to many compounds exhibiting diverse pharmacological activities, its potential interaction with classical central (*17*) and peripheral (*18*) benzodiazepine receptors was also investigated. Asperlicin only weakly affected ^{3}H-labeled diazepam (IC_{50} = 50 μ*M*) or ^{3}H-labeled RO-5-4864 [7-chloro-5-(4′-chlorophenyl)-1,3-dihydro-1-methyl-2*H*-1,4-benzodiazepin-2-one] (IC_{50} >20 μ*M*) binding in rat brain or kidney, respectively, further demonstrating its selectivity for peripheral CCK receptors.

The ability of asperlicin to antagonize the action of CCK on peripheral tissues in vitro was also evident in vivo after intravenous or intraperitoneal administration. Asperlicin (12 mg/kg, intravenous) effectively antagonized contractions of the gall bladder produced by intravenous CCK-8 in anesthetized guinea pigs (*19*). CCK-8 dose-response curves were displaced to the right to the same degree when asperlicin was administered either 1 or 4 hours before CCK-8, indicating a long duration of action in vivo (Fig. 3). The dose of CCK-8 necessary for 50 percent maximal contraction (ED_{50}) was increased approximately fourfold at both time intervals. A similar antagonism of CCK-8 was observed when asperlicin (30 mg/kg) was administered intraperitoneally (*20*). In agreement with the low potency observed in vitro, proglumide (50 mg/kg, intravenous) did not significantly alter the dose-response curve to CCK-8 in this preparation under the same conditions.

The high potency and specificity of asperlicin as a CCK antagonist provides a valuable tool for investigating the physiological or pharmacological actions of CCK. The in vitro data showing a large separation in the ability of asperlicin to displace ^{125}I-labeled CCK binding in pancreatic tissue compared with that in brain or ^{125}I-labeled gastrin binding in gastric glands supports proposals for distinct CCK receptors in these tissues (*21–23*). The in vivo activity of asperlicin presents the opportunity to further explore functional correlates of the radioli-

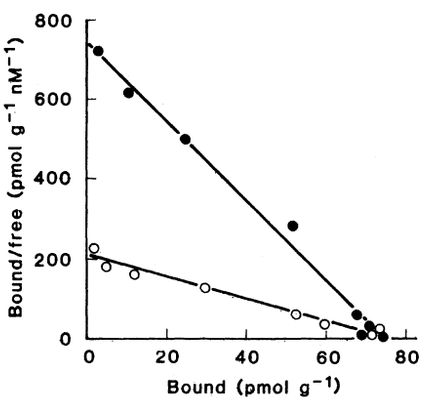

Fig. 2. Scatchard analysis of specific ^{125}I-labeled CCK-8 binding in pancreatic membranes in the absence (●) and presence (○) of 1.0 μ*M* asperlicin. Each point represents the mean of triplicate determinations replicated three times. The lines were determined by regression analysis. The K_d values in control and treated membranes were 0.11 ± 0.01 and 0.32 ± 0.04 n*M*, respectively. The B_{max} values in the presence of asperlicin were 91 ± 7 percent of control.

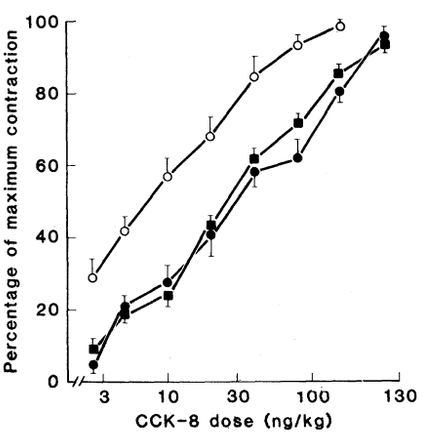

Fig. 3. Antagonism by intravenously administered asperlicin (12 mg/kg) of gallbladder contractions induced by CCK-8 in anesthetized guinea pigs. Control ED_{50} value for CCK-8 (○) was 6.45 (5.2 to 8.1) ng/kg (intravenous). ED_{50} values 1 hour (●) and 4 hours (■) after asperlicin were 29.5 (24.8 to 35.2) and 24.9 (21.4 to 29.1) ng/kg, respectively. Each value is the mean ± standard error of six animals.

gand binding data indicating differences among CCK receptors in various tissues, as well as to delineate the peripheral and central nervous system actions of CCK. The therapeutic utilities of potent antagonists, such as asperlicin, in CCK-related disorders of the gastrointestinal system remain to be determined.

References and Notes

1. D. T. Krieger, *Science* **222**, 975 (1983).
2. J. A. Williams, *Biomed. Res.* **3**, 107 (1982). Various molecular forms of CCK have been identified in gut and brain. The predominate and most biologically active form is the octapeptide, CCK-8, which may be derived from the larger molecule, CCK-33.
3. J. E. Morley, *Life Sci.* **30**, 479 (1982).
4. J. D. Gardner and R. T. Jensen, *Am. J. Physiol.* **246**, G471 (1984).
5. M. Spanarkel *et al.*, *J. Biol. Chem.* **258**, 6746 (1983).
6. D. Koulischer, L. Moroder, M. Deschodt-Lanckman, *Regul. Peptides* **4**, 127 (1982).
7. M. A. Goetz *et al.*, in preparation.
8. J. M. Liesch *et al.*, in preparation.
9. R. S. L. Chang, V. J. Lotti, T. B. Chen, *Biochem. Pharmacol.* **33**, 2334 (1984); R. S. L. Chang *et al.*, *Life Sci.* **32**, 871 (1983). Specific binding of ^{125}I-labeled CCK-33 (60 to 80 p*M*) is the difference between total and nonspecific binding defined in the presence of 1 μM CCK-8. In all binding studies, asperlicin and benzotript were dissolved in 100 percent methanol; *N*-carbobenzoxy-CCK-(27-32) and proglumide were dissolved in equivalent molar amounts of NH_4OH and NaOH, respectively. Control studies demonstrated that the volumes of each vehicle used (≤1 percent) did not affect specific ligand binding.
10. M. Praissman, M. E. Walden, C. Pellechia, *J. Receptor Res.* **3**, 647 (1983). Filtration through Whatman glass fiber (grade GF/B) under reduced pressure was used to separate bound and free ^{125}I-labeled gastrin (New England Nuclear). Specific binding of ^{125}I-labeled gastrin (60 p*M*) is the difference between total and nonspecific binding defined in the presence of 1 μM gastrin.
11. G. Scatchard, *Ann. N.Y. Acad. Sci.* **51**, 660 (1949).
12. The dissociation constant of the inhibitor (K_i) was calculated from the formula $K_i = (K_d/K_{d'} - K_d) \times I$. K_d and $K_{d'}$ are the dissociation constants of the ligand alone and in the presence of inhibitor concentration I, respectively.
13. R. S. L.Chang, V. J. Lotti, T. B. Chen, *Neuroscience Lett.* **46**, 71 (1984). Cumulative concentration-response curves to CCK-8 were determined alone and in the same tissues that had been treated for 20 minutes with three concentrations of asperlicin (1.3, 3.9, and 13.0 μM). At least four tissues were used at each concentration. Asperlicin, proglumide, and benzotript were dissolved in 100 percent methanol or dimethyl sulfoxide (DMSO). Control studies demonstrated that the volumes of vehicle used (<0.1 percent) did not affect repetitive concentration-response curves to CCK-8 or other agonists.
14. O. Arunlakshana and H. O. Schild, *Br. J. Pharmacol. Chemother.* **14**, 48 (1959).
15. The concentrations of proglumide (0.3, 0.6, 1.8, and 3.0 m*M*) gave a Schild plot slope not significantly different than unity (1.13 ± 0.30). Benzotript (22 μM) did not shift the CCK-8 concentration response curve indicating a pA_2 of <4.7. Higher concentrations of benzotript (67 and 222 μM) shifted the CCK-8 concentration-response curve disproportionally to the antagonist concentration. The Schild plot slope (1.87 ± 0.23) differed significantly from unity, indicating noncompetitive antagonism.
16. The methods used for studying these contractile agonists were identical to those described in (*13*). Electrical stimulation was in the form of square-wave pulses at 0.1 Hz presented for 0.5 msec at supramaximal voltage (30 to 60 V).
17. C. Braestrup and R. F. Squires, *Proc. Natl. Acad. Sci. U.S.A.* **74**, 3805 (1977).
18. G. Lefur *et al.*, *Life Sci.* **32**, 1839 (1983).
19. The animals were prepared for recording of gallbladder contractions in vivo as described by J. W. Fara and S. M. Erde [*Eur. J. Pharmacol.* **47**, 359 (1978)]. When administered intravenously, asperlicin was dissolved in 100 percent DMSO and administered in a volume of 0.1 ml per 100 g of body weight. Control studies demonstrated the reproducibility of cumulative dose-response curves to CCK-8 determined in the same animals before, 1, 3, and 4 hours after injection of vehicle.
20. For intraperitoneal administration, asperlicin was suspended in 0.5 percent methyl cellulose. In these studies the control CCK-8 ED_{50} was 5.5 (4.6 to 6.6) ng/kg injected intravenously (n = 5). The CCK-8 ED_{50} in the same animals 1 and 3 hours after asperlicin (30 mg/kg injected intraperitoneally) were 31.5 (23.3 to 42.6) and 33.5 (26.6 to 42.1) ng/kg (injected intravenously), respectively.
21. R. B. Innis and S. H. Snyder, *Proc. Natl. Acad. Sci. U.S.A.* **77**, 6917 (1980).
22. A. Saito, J. D. Goldfine, J. A. Williams, *J. Neurochem.* **37**, 483 (1981).
23. S. J. Hersey, D. May, D. Schyberg, *Am. J. Physiol.* **244**, G392 (1983).

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Cloning of Shiga-Like Toxin Structural Genes from a Toxin Converting Phage of *Escherichia coli*

Abstract. *The genes controlling high-level production of Shiga-like toxin (SLT) in* Escherichia coli *were cloned from the SLT converting phage 933J. This phage was isolated from a strain of* E. coli *that caused a foodborne outbreak of hemorrhagic colitis. The genes that convert normal* E. coli *to organisms producing high levels of toxin were cloned into the plasmid pBR328 and expressed in* E. coli *HB101. DNA restriction mapping, subcloning, examination of the cloned gene products by minicell analysis, neutralization, and immunoprecipitation with antibodies to SLT were used to localize the toxin converting genes and identify them as structural genes for SLT. Southern hybridization studies established that the DNA fragment carrying the cloned toxin structural genes had homology with the DNA of* Shigella.

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Two Shiga-like toxin (SLT) converting phages, 933J and H19A/J, were isolated from *Escherichia coli* strains responsible for hemorrhagic colitis and infantile diarrhea, respectively (*1*). These two phages are members of a family of SLT converting phages that exist in *E. coli* strains in nature (*1–3*). The well-studied laboratory strain *E. coli* K12 makes small amounts of SLT (≤ 10^2 doses that have 50 percent cytotoxicity per milligram of protein in cell lysates) (*4, 5*) and is susceptible to infection by these phages. Lysogenization of *E. coli* K12 with phage 933J or phage H19A/J causes a 10,000-fold increase in the amount of SLT produced (*1*). These findings suggest that the toxin converting phages may either contain structural genes for SLT or regulatory elements that enhance the expression of structural genes for SLT in *E. coli* K12. We cloned the SLT converting genes from phage 933J, a phage isolated from *E. coli* 0157:H7 strain 933, examined the products of the cloned genes by minicell analysis, and demonstrated by neutralization and immunoprecipitation with antibodies that the products of the cloned genes expressed antigenic determinants of SLT. DNA-DNA hybridization studies showed that the cloned sequences encoding SLT were homologous to DNA sequences in Shiga toxin–producing strains of shigellae.

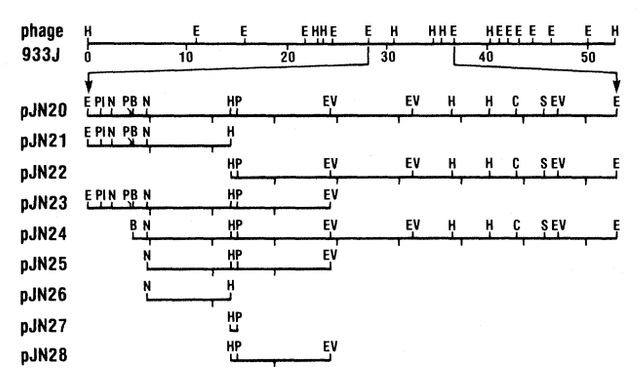

Fig. 1. Restriction endonuclease cleavage maps of phage 933J DNA and of DNA fragments subcloned into vector pBR328 to form plasmids pJN20 to pJN28. The circular map of phage 933J is displayed in a linear fashion and is arbitrarily broken between the two largest Hind III fragments. The molecular size of phage 933J is indicated in kilobases (kb) and increments of 1 kb are indicated below the maps for the cloned inserts. The toxinogenicity of *E. coli* strains lysogenic for phage 933J or containing each of the plasmids is indicated on the right. Toxin production by *E. coli* HB101 (pJN25) was seven times that of *E. coli* strain 933. Restriction enzymes are designated E, Eco RI; PI, Pst I; N, Nco I; P, Pvu II; B, Bam HI; H, Hind III; EV, Eco RV; C, Cla I; and S, Sma I.