avian parent virus was recovered from rectal swabs of infected squirrel monkeys.

These findings have implications for the production of an influenza A vaccine virus that is attenuated for man. Since the genetic determinants of attenuation of the avian virus for primates reside on one or more of the genes that do not code for the surface antigens, it should be possible to produce an avian-human reassortant virus that has the surface antigens of a new epidemic human virus and the attenuating genes derived from an avian influenza virus. If such a reassortant virus behaves in man as it does in monkeys, it should be sufficiently restricted in replication to be attenuated. To be useful for immunoprophylaxis, reassortant virus would have to replicate well enough in man to induce resistance to illness caused by the epidemic wild-type virus.

These findings also have implications for the production of live virus vaccines for other animal or human viral pathogens.

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References and Notes

1. H. M. Meyer, Jr., H. E. Hopps, P. D. Parkman, F. A. Ennis, *Am. J. Clin. Pathol.* **70**, 146 (1978).
2. T. W. Hoskins, J. R. Davies, A. J. Smith, C. L. Miller, A. Allchin, *Lancet* **1979-I**, 33 (1979).
3. R. M. Chanock and B. R. Murphy, *Rev. Infect. Dis.* **2**, 421 (1980).
4. B. R. Murphy, J. A. Kasel, R. M. Chanock, *N. Engl. J. Med.* **286**, 1329 (1972).
5. J.-L. Virelizier, *J. Immunol.* **115**, 434 (1975).
6. P. Palese, J. L. Sculman, M. B. Ritchey, *Perspect. Virol.* **10**, 57 (1978).
7. R. A. Lamb, P. R. Etkind, P. W. Choppin, *Virology* **91**, 60 (1978).
8. R. A. Lamb and P. W. Choppin, *ibid.* **112**, 729 (1981).
9. M. D. Tolpin *et al.*, *Infect. Immun.*, in press.
10. M. D. Tolpin, J. G. Massicot, M. G. Mullinix, H. W. Kim, R. H. Parrott, R. M. Chanock, B. R. Murphy, *Virology* **112**, 505 (1981).
11. M. Baez, J. J. Zazra, R. M. Elliott, J. F. Young, P. Palese, *ibid.* **113**, 397 (1981).
12. W. Rohde, E. Harms, C. Scholtissek, *ibid.* **79**, 393 (1977).
13. C. Scholtissek, E. Harms, W. Rohde, M. Orlich, R. Rott, *ibid.* **74**, 332 (1976).
14. C. Scholtissek, W. Rohde, V. von Hoyningen, R. Rott, *ibid.* **87**, 13 (1978).
15. C. Scholtissek and V. von Hoyningen-Huene, *ibid.* **102**, 13 (1980).
16. B. R. Murphy, D. L. Sly, N. T. Hosier, W. T. London, R. M. Chanock, *Infect. Immun.* **28**, 688 (1980).
17. B. R. Murphy *et al.*, *ibid.*, in press.
18. H. F. Maassab, *Nature (London)* **219**, 645 (1968).
19. P. Palese and J. L. Schulman, *J. Virol.* **17**, 876 (1976).
20. L. Bal-lan, R. G. Webster, V. S. Hinshaw, *Infect. Immun.* in press.
21. D. McCahon and G. C. Schild. *J. Gen. Virol.* **15**, 73 (1972).
22. R. G. Webster, M. Yakhno, V. S. Hinshaw, W. J. Bean, K. G. Murti, *Virology* **84**, 268 (1978).
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Benzodiazepine Receptor–Mediated Experimental "Anxiety" in Primates

Abstract. *The ethyl ester of β-carboline-3-carboxylic acid has a high affinity for benzodiazepine receptors in the brain. In the rhesus monkey this substance produces an acute behavioral syndrome characterized by dramatic elevations in heart rate, blood pressure, plasma cortisol, and catecholamines. The effects are blocked by benzodiazepines and the specific benzodiazepine receptor antagonist Ro 15-1788. The benzodiazepine receptor may consist of several subsites or functional domains that independently recognize agonists, antagonists, or "active" antagonists such as β-carboline-3-carboxylic acid ethyl ester. These results suggest that the benzodiazepine receptor is involved in both the affective and physiological manifestations of anxiety, and that the administration of β-carboline-3-carboxylic acid ethyl ester to monkeys may provide a reliable and reproducible animal model of human anxiety.*

The brain contains specific receptor sites for benzodiazepines that are functionally (and perhaps structurally) coupled to a recognition site for γ-aminobutyric acid and a chloride ionophore (*1*). Both direct and indirect evidence suggests that this "supramolecular receptor complex" mediates the pharmacological actions of benzodiazepines and of many structurally unrelated compounds that share common pharmacological properties with the benzodiazepines (*2*). Whether the benzodiazepine receptor complex has a physiological role in the absence of an exogenous ligand (that is, a drug) has been a source of considerable speculation, particularly regarding its function in the pathophysiology of anxiety and related disorders (*3*).

A study by Braestrup *et al.* (*4*) demonstrating that the ethyl ester of β-carboline-3-carboxylic acid (β-CCE) possesses a high affinity for the benzodiazepine receptor stimulated investigations of the pharmacological actions of this and related compounds. Although previous studies suggest that β-CCE antagonizes the pharmacological actions of diazepam and related benzodiazepines in rodents, with no apparent behavioral actions by itself (*5*), our data show that in primates β-CCE elicits a profound behavioral and physiological syndrome reminiscent of "fear" or "anxiety." Furthermore, both the behavioral and physiological effects of β-CCE are blocked by prior treatment with the specific benzodiazepine receptor antagonist Ro 15-1788 (*6*). The benzodiazepine receptor may not only be involved in the "anxiolytic" actions of benzodiazepines, but may also play a pivotal role in both the pathogenesis of anxiety and its pathophysiological sequelae in humans.

Adult male rhesus monkeys (*Macaca mulatta*) weighing 7 to 9 kg were restrained in chairs under ketamine anesthesia and allowed to adapt to this condition for at least 24 hours before drug administration. Previous behavioral and neuroendocrine studies with "chair-adapted" rhesus monkeys have validated this procedure for measuring stress-related hormones (*7*). All animals were fitted with femoral venous catheters, which were kept patent during the experiment by a slow infusion of sterile 0.9 percent NaCl. The animals were administered β-CCE (2.5 mg/kg) intravenously in vehicle (*8*). A control infusion of the vehicle alone was carried out 2 hours before the β-CCE infusion. The animals either received β-CCE alone or were treated with the benzodiazepine receptor antagonist Ro 15-1788 (5 mg/kg, intravenously) 20 minutes before β-CCE administration. In other experiments selected animals were treated with diazepam (1 to

0036-8075/82/1224-1332$01.00/0

2 mg/kg, intravenously) before receiving β-CCE.

The animals were videotaped during the course of the experiment and their behavior was rated by an investigator who had no knowledge of the protocol. Blood pressure and pulse were monitored automatically at 5-minute intervals with a Dinamap Research Monitor (model 1245, Applied Medical Research). Three milliliters of blood were drawn at 20-minute intervals and centrifuged immediately at 4°C. The plasma was frozen on dry ice and stored at −80°C. Plasma cortisol was measured by radioimmunoassay (New England Nuclear), and plasma epinephrine and norepinephrine were measured by high-performance liquid chromatography with electrochemical detection (*9*).

β-Carboline-3-carboxylic acid ethyl ester elicited dramatic behavioral changes in all monkeys to which it was administered. Their behavior was easily distinguishable from that of vehicle-treated animals, which showed no observable changes. Table 1 lists the salient behaviors observed over a 2-hour period directly following infusion of β-CCE. The first changes noted were increased vigilance and marked piloerection. Within 30 seconds the monkeys became agitated, as manifested by struggling in the chair, increased rotation of the head and neck, and constant shifting of position. Significant increases in vocalization, defecation, urination, and penile erection were observed within minutes. Behavioral agitation reached a peak about 45 to 60 minutes after the infusion. During this peak there was marked "picking" behavior, so intense in some animals that it resulted in bleeding. In all animals there were alternating periods of immobility. Although all the animals had free access to food (Purina Monkey Chow) and water, none of the β-CCE–treated animals ate or drank during the first hour after infusion. In contrast, vehicle-treated animals regularly consumed food and water during the same period. During the peak of their behavioral response to β-CCE, two animals appeared sedated and somnolent, although, as in the other animals, their heart rate and blood pressure were significantly elevated throughout the observation period. The behavioral effects of β-CCE were no longer apparent 2 hours after the infusion. Despite some individual variations in the behavioral effects of β-CCE, the general pattern was remarkably consistent.

Concomitant with the behavioral effects produced by β-CCE was an immediate sharp increase in heart rate and blood pressure (Figs. 1A and 2A). There was also a highly significant increase in plasma cortisol (Fig. 1B), which peaked later than the maximum behavioral and cardiovascular changes. Plasma epinephrine and norepinephrine were also significantly elevated (Fig. 2B) within 20 minutes of β-CCE administration (*10*). Treatment with the specific benzodiazepine receptor antagonist Ro 15-1788 (5 mg/kg) 20 minutes before β-CCE administration completely blocked the behavioral and physiological changes pro-

Table 1. Behavioral effects of β-CCE in the rhesus monkey. Behaviors 1 through 3 were rated by an investigator from video recordings taken before and after β-CCE or vehicle administration. Behaviors 4 through 9 were assessed during the actual experiments. All items were rated for up to 2 hours. Values are numbers of animals exhibiting the specific behavior.

Behavior	Vehicle (N = 7)	β-CCE (N = 7)
1. Struggling in chair	0	7
2. Increased head and body turning	0	7
3. Immobility (> 5 seconds)	1	7
4. Bleeding caused by scratching	0	2
5. Eating and drinking	7	0
6. Distress vocalization	0	6
7. Defecation and urination*	0	7
8. Penile erection*	0	7
9. Sedation	1	2

*Behavior noted immediately after infusion.

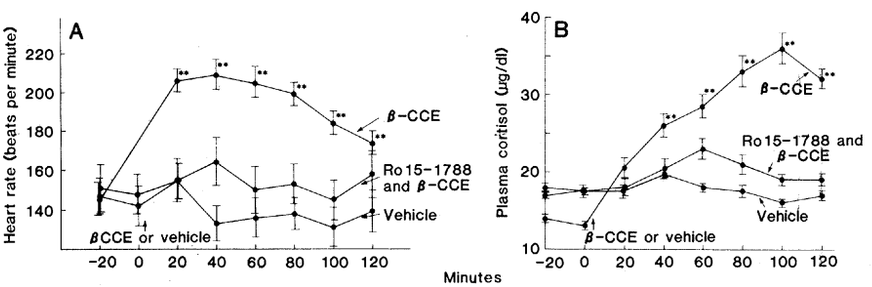

Fig. 1. Effects of β-CCE and Ro 15-1788 on (A) mean heart rate (N = 4) and (B) plasma cortisol (N = 5) in rhesus monkeys. Ro 15-1788 (5 mg/kg) was administered 20 minutes before β-CCE (2.5 mg/kg). A repeated measures analysis of variance for values obtained before and after infusion shows the time factor to be significant for β-CCE administration only (P = .012 and .008 for pulse and plasma cortisol, respectively). No significant changes in heart rate or plasma cortisol were observed in vehicle-treated animals or animals treated with Ro 15-1788 before receiving β-CCE. Tukey's multiple comparison test for comparing values obtained before and after infusion shows heart rate and cortisol to be significantly elevated (P < .01) after β-CCE administration only. Asterisks indicate significant differences from control values.

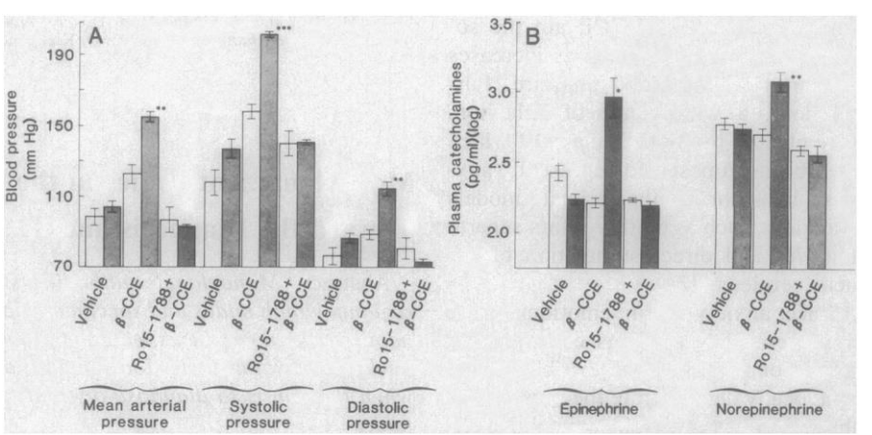

Fig. 2. Effects of β-CCE and Ro 15-1788 on (A) mean blood pressure (N = 4) and (B) plasma catecholamines (N = 5) in rhesus monkeys. Open bars represent values obtained 20 minutes before injection and shaded bars represent values obtained 20 minutes after infusion. Symbols: (★) P < .05, (★★) P < .02, and (★★★) P < .001 (repeated measures analysis of variance comparing values obtained before and after treatment). Values for plasma catecholamines are logarithmically transformed for statistical and illustrative purposes. Absolute values for plasma catecholamines are given in (*10*).

duced by β-CCE (Figs. 1 and 2). The antagonist had no significant behavioral or physiological actions when administered alone (Fig. 1). Diazepam (1 to 2 mg/kg) also markedly attenuated the behavioral and physiological effects of β-CCE.

β-Carboline-3-carboxylic acid ethyl ester possesses a high affinity for brain benzodiazepine receptors (*4*), antagonizes many of the pharmacological actions of benzodiazepines (*5*), and does not elicit overt changes in behavior in rodents when administered alone. However, in a more sensitive test of social interaction (*11*), this compound exerted an action opposite that of the benzodiazepines, which has been interpreted as "anxiogenic." These results suggest that at least three distinct classes of drugs are capable of binding to the benzodiazepine receptor. Drugs of the first class, historically called agonists, produce anxiolytic and anticonvulsant effects. An example is diazepam. The second class comprises antagonists with no intrinsic activity at moderate doses, such as Ro 15-1788 and CGS-8216 (*12*). The third class includes "active" antagonists, such as β-CCE and related β-carboline esters. Our data suggest that drugs of the first two classes effectively antagonize the pharmacological actions of the third (*12*).

In the rhesus monkey β-CCE elicits a profound elevation in the concentrations of circulating stress-related hormones, such as cortisol and the catecholamines epinephrine and norepinephrine. Increases in plasma cortisol and catecholamines are associated with anxiety in humans (*13*) and with experimental anxiety (such as conflict behavior) in animals (*14*). Benzodiazepines decrease stress-induced elevations in cortisol and catecholamines in both animals and humans (*15*). Concomitant with the endocrine changes elicited by β-CCE are the somatic manifestations, such as increases in heart rate and blood pressure. Furthermore, a wide range of behaviors were elicited by β-CCE, many of which have been proposed to represent "anxious" behavior in other primate models of anxiety, such as mother-infant separation (*16*) and direct stimulation of the locus ceruleus (*17*).

Since anxiety is an emotional state characterized by feelings of impending danger or fear, it is impossible to unequivocally demonstrate the presence or absence of anxiety in any animal. Nevertheless, the endocrine, somatic, and behavioral effects of β-CCE are reminiscent of changes observed in anxious patients and in animals and humans exposed to anxiety-provoking or stressful situations (*13–17*). The blockade of the behavioral and physiological actions of β-CCE by the benzodiazepine receptor antagonist Ro 15-1788 strongly suggests that the actions of β-CCE are mediated through the benzodiazepine receptors. Thus, administration of β-CCE to animals may represent a reliable and reproducible model of human anxiety and, as such, could be valuable in studying the postulated role of anxiety and stress in a variety of human diseases, including cardiovascular, ulcerative, and neoplastic disorders (*18*). Taken together, our results suggest that the benzodiazepine–γ-aminobutyric acid receptor complex not only mediates the pharmacological actions of the benzodiazepines, but also subserves the affective and physiological expression of anxiety.

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References and Notes

1. J. F. Tallman, S. M. Paul, P. Skolnick, D. W. Gallager, *Science* **207**, 274 (1980); S. M. Paul, P. Marangos, P. Skolnick, *Biol. Psychiatry* **16**, 213 (1981).
2. P. Skolnick and S. M. Paul, *Int. Rev. Neurobiol.* **23**, 103 (1982).
3. S. M. Paul and P. Skolnick, in *Anxiety: New Research and Changing Concepts*, D. Klein and J. Rabkin, Eds. (Raven, New York, 1981), p. 215.
4. C. Braestrup, M. Nielsen, C. Olsen, *Proc. Natl. Acad. Sci. U.S.A.* **77**, 2288 (1980).
5. S. Tenen and J. Hirsch, *Nature (London)* **288**, 609 (1980); P. Cowen, A. Green, D. Nuh, I. Martin, *ibid.* **290**, 54 (1981).
6. W. Hunkeler, M. Mohler, L. Pieri, P. Polc, E. Bonetti, R. Cumin, R. Schaffner, W. Haefely, *ibid.* **290**, 514 (1981); P. Polc, J.-P. Laurent, R. Scherschlicht, W. Haefely, *Naunyn-Schmiedeberg's Arch. Pharmacol.* **316**, 317 (1981).
7. B. H. Natelson, N. Krasnejor, J. W. Holaday, *J. Comp. Physiol. Psychol.* **90**, 958 (1976); C. F. Farver, D. De Wied, P. T. K. Toivola, *J. Endocrinol.* **79**, 247 (1978).
8. All drugs were dissolved or suspended in 20 percent Emulphor (GAF Corp.) diluted 1:1 with 95 percent ethanol and in 80 percent sterile saline in a volume of 10 ml for β-CCE and 5 ml for Ro 15-1788. The dose of β-CCE used (2.5 mg/kg) was empirically chosen on the basis of (i) its reported affinity for the benzodiazepine receptor (≃ 1 n*M*) and (ii) recent studies from our laboratory demonstrating that β-CCE degrades very rapidly when incubated with rat plasma in vitro at 37°C [W. Mendelson, M. Cain, J. Cook, S. M. Paul, P. Skolnick, in *Beta-Carbolines and Tetraisoquinolines*, E. Usdin, J. Barchas, F. Bloom, Eds. (Liss, New York, 1982), p. 253].
9. D. S. Goldstein, G. Fuerstein, J. L. Izzo, I. J. Kopin, H. R. Keiser, *Life Sci.* **28**, 467 (1981).
10. Plasma epinephrine concentrations rose from 171 ± 23 to 1542 ± 505 pg/ml (means ± standard errors) and norepinephrine levels rose from 545 ± 109 to 1476 ± 467 pg/ml after β-CCE administration (Fig. 2).
11. S. File, in *Pharmacology of Benzodiazepines*, E. Usdin, P. Skolnick, J. F. Tallman, D. Greenblatt, S. M. Paul, Eds. (Macmillan, London, in press).
12. M. Schweri, M. Cain, J. Cook, S. M. Paul, P. Skolnick, *Pharmacol. Biochem. Behav.* **17**, 457 (1982); C. Braestrup, R. Schmiechen, G. Neef, M. Nielsen, E. N. Petersen, *Science* **216**, 1241 (1982).
13. E. J. Sachar, *Prog. Brain Res.* **32**, 36 (1970).
14. A. S. Lippa, E. N. Greenblatt, R. W. Pelham, in *Animal Models in Psychiatry and Neurology*, I. Hanin and E. Usdin, Eds. (Pergamon, New York, 1979), p. 279.
15. G. Lefur, F. Guilloux, N. Mitrani, J. Mizoule, A. Uzan, *J. Pharmacol. Exp. Ther.* **211**, 305 (1979); R. Lahiti and C. Barsuhn, *Psychopharmacology* **35**, 215 (1979).
16. S. J. Suomi, G. W. Kraemer, C. M. Baysinger, R. D. DeLizio, in *Anxiety: New Research and Changing Concepts*, D. F. Klein and J. Rabkin, Eds. (Raven, New York, 1981), p. 174.
17. D. E. Redmond and Y. H. Huang, *Life Sci.* **25**, 2149 (1979).
18. V. Riley, *Science* **212**, 1100 (1981); J. P. Henry and G. A. Santisteban, *Atherosclerosis* **14**, 203 (1971); J. W. Mason, in *Frontiers in Neurology and Neuroscience Research*, P. Seeman and G. M. Brown, Eds. (Univ. of Toronto Press, Toronto, 1974), p. 168.
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Monoclonal Antibodies in the Lymphatics: Toward the Diagnosis and Therapy of Tumor Metastases

Abstract. *Monoclonal antibodies subcutaneously injected into mice track to regional lymph nodes and specifically label target cells there. The lymphatic route of administration can be expected to provide much higher sensitivity, higher target-to-background ratio, faster localization, and lower toxicity than the intravenous route when the aim is to diagnose or treat tumor metastases or lymphoma in the lymph nodes.*

Monoclonal antibodies (*1*) have been touted as a modern incarnation of Paul Ehrlich's "magic bullet." With radionuclides attached, they appear clinically useful for gamma camera imaging of tumors (*2*). In the realm of therapy, they may be able to mobilize endogenous defenses or to direct an attached drug, toxin, or radionuclide to tumor cells (*3*). Thus far, monoclonal antibodies have

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