References and Notes

1. R. S. Bar, P. Gorden, J. Roth, C. R. Kahn, P. DeMeyts, *J. Clin. Invest.* **58**, 1123 (1976); M. B. Davidson and S. A. Kaplan, *ibid.* **59**, 22 (1977).
2. J. R. Gavin, III, J. Roth, D. M. Neville, Jr., P. DeMeyts, D. N. Buell, *Proc. Natl. Acad. Sci. U.S.A.* **71**, 84 (1974); F. C. Kosmakos and J. Roth, *58th Annual Meeting of the Endocrine Society, San Francisco* (1976), p. 69; J. N. Livingston, B. J. Purvis, D. H. Lockwood, *Nature (London)* **273**, 394 (1978); W. G. Blackard; P. S. Guzelian, M. E. Small, *Endocrinology* **103**, 548 (1978).
3. J. M. Amatruda, J. N. Livingston, D. H. Lockwood, *Science* **188**, 264 (1975); R. I. Misbin, J. P. O'Leary, A. Pulkkinen, *ibid.* **205**, 1003 (1979); V. Smith and M. DiGirolamo, *Clin. Res.* **28**, 406A (1980).
4. Y. Broer, P. Freychet, G. Rosselin, *Endocrinology* **101**, 236 (1977).
5. J. N. Livingston, P. Cuatrecasas, D. H. Lockwood, *Science* **177**, 626 (1972).
6. T. Huecksteadt, F. Kosmakos, D. Mott, *Diabetes* **29**, 155 (1980).
7. R. F. Kletzien, M. W. Pariza, J. E. Becker, V. R. Potter, F. R. Butcher, *J. Biol. Chem.* **251**, 3014 (1976).
8. J. N. Cech, R. B. Freeman, Jr., J. F. Caro, J. M. Amatruda, *Biochem. J.* **188**, 839 (1980).
9. S. Jacobs, K.-J. Chang, P. Cuatrecasas, *Science* **200**, 1283 (1978); J. N. Livingston, J. M. Amatruda, D. H. Lockwood, *Am. J. Physiol.* **234**, E484 (1978).
10. J. F. Caro and J. M. Amatruda, *Am. J. Physiol.*, in press.
11. J. N. Livingston, P. A. Gurny, D. H. Lockwood, *J. Biol. Chem.* **252**, 560 (1977).
12. G. Scatchard, *Ann. N.Y. Acad. Sci.* **51**, 660 (1949).
13. P. Cuatrecasas, *Biochemistry* **12**, 1312 (1973).
14. C. R. Kahn and K. Baird, *J. Biol. Chem.* **253**, 4900 (1978).
15. D. H. Lockwood and L. E. East, *ibid.* **249**, 7717 (1977); M. P. Czech and J. N. Fain, *Endocrinology* **87**, 191 (1970); J. N. Livingston and B. J. Purvis, *Am. J. Physiol.* **238**, E267 (1980); F. A. Karlsson *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **76**, 809 (1979); C. R. Kahn, K. Baird, J. S. Flier, D. B. Jarett, *J. Clin. Invest.* **60**, 1094 (1977).
16. E. G. Bligh and W. J. Dyer, *Can. J. Biochem. Physiol.* **37**, 911 (1959).
17. J. F. Caro and J. M. Amatruda, *Metabolism* **29**, 732 (1980).
18. R. J. Mans and G. D. Novelli, *Arch. Biochem. Biophys.* **94**, 48 (1961).
19. G. L. Miller, *Anal. Chem.* **31**, 964 (1959).
20. P. Cuatrecasas, *Proc. Natl. Acad. Sci. U.S.A.* **68**, 1264 (1971).

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Anticonvulsants Specific for Petit Mal Antagonize Epileptogenic Effect of Leucine Enkephalin

Abstract. *The anticonvulsants ethosuximide, sodium valproate, and trimethadione that are specific for petit mal epilepsy abolished in rats the electrical seizure activity and behavioral abnormalities produced by leucine enkephalin, whereas phenobarbital and phenytoin had no effect. The dose-response curve for naloxone against seizure activity induced by leucine enkephalin was the same as that in γ-hydroxybutyrate-induced petit mal. These data indicate that the epileptic properties of leucine enkephalin are petit mal-like and raise the possibility of involvement of enkephalinergic systems in petit mal epilepsy.*

Leucine enkephalin is a pentapeptide that occurs naturally in brain and is presumed to be one of the natural ligands for opiate receptor in brain (*1*). In 1977 Urca and colleagues (*2*) reported that methionine enkephalin, a natural ligand for the opiate receptor differing in its structure from leucine enkephalin only in the NH_2-terminal amino acid, produced epileptiform activity when introduced into the lateral ventricle of rats. Since then similar activity has been demonstrated for leucine enkephalin (*3*) and for β-endorphin (*4*), a larger opiate peptide. This paroxysmal activity can be aborted by the specific opiate antagonist naloxone, but not by other anticonvulsants used in generalized convulsive seizures (*4*). Conversely, naloxone has been ineffective in a number of generalized and partial seizure models in animals as well as complex partial seizure disorders in humans (*5*); however, we demonstrated that naloxone was effective in aborting petit mal seizures induced in animals by γ-hydroxybutyrate (*6, 7*). We now report experiments on the efficacy of several anticonvulsants against seizure activity induced by leucine enkephalin in the rat. We found that the paroxysmal abnormalities produced by the leucine enkephalin were selectively aborted by anticonvulsants specific for petit mal. Furthermore, dose-response studies with naloxone showed that doses in excess of 4 mg/kg were required to block this action of leucine enkephalin.

We implanted epidural electrodes in male Sprague-Dawley rats (200 to 300 g) under pentobarbital anesthesia to record the electrocorticogram (ECoG). An indwelling cannula was also implanted stereotactically in the lateral ventricle. The ECoG was quantitated by an automated frequency analysis system based on the zero crossing method (*8*). Recordings were made with the animals moving freely in clear plexiglass chambers. Cannula position was confirmed histologically after completion of the experiments.

Animals were rested for 7 days after surgery. Then 100 μg of leucine enkephalin in 15 μl of lactated Ringer solution was injected intraventricularly while the ECoG was continuously monitored. Animals that showed a characteristic electrical response to the enkephalin were then assigned to either an anticonvulsant or a control group, and drug experiments were begun 7 days after the initial enkephalin injection.

The anticonvulsants used were ethosuximide, trimethadione, sodium valproate, diazepam, clonazepam, phenobarbital, and phenytoin. The dosages of each drug and the time elapsed from injection of the anticonvulsant drug to enkephalin administration are shown in Table 1. Control and drug-treated animals were paired for all experiments with the control receiving the vehicle of the drug. All drugs except leucine enkephalin were administered intraperitoneally. Once the anticonvulsant or vehicle was administered and the response to leucine enkephalin determined, the animal groups were interchanged, and the experiment was repeated after a 7-day rest period. We performed single and multiple dose anticonvulsant drug studies. For the multiple dose experiments, the anticonvulsant drugs or their vehicles were administered once daily for 7 days and the response to leucine enkephalin was determined; the animals were then rested for 1 week, control and drug-treated groups were interchanged, and the experiment was repeated. In addition to the anticonvulsant drug experiments outlined above, a dose-response curve was ascertained for naloxone against leucine enkephalin-induced seizure activity, with doses of naloxone ranging from 0.1 to 10 mg/kg.

Leucine enkephalin produced a consistent dramatic paroxysmal electrical response within the first 60 seconds of administration with changes lasting up to 6 minutes (Fig. 1). The animals were immobile during all these paroxysms and showed occasional myoclonic jerks. The enkephalin-induced paroxysms recorded on the ECoG were reflected in the frequency analysis system by an increase in the 3- to 6-Hz band.

In the single dose studies (Table 1) ethosuximide and sodium valproate completely prevented the electrical and behavioral responses to the enkephalin in all animals. Trimethadione was completely effective in abolishing responses in three animals and lessened the duration and voltage of the discharge in three. Diazepam was completely effective in three animals and ineffective in three animals. Phenobarbital and phenytoin had no effect on the activity; however, clonazepam changed the pattern of

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Table 1. Effect of anticonvulsants on leucine enkephalin-induced seizure activity. Data show the time elapsed between anticonvulsant and enkephalin administration. The number of experiments in single and multiple dose studies in which leucine enkephalin seizures were abolished or attenuated are listed.

Drug	Dose (mg/kg)	Time (min)	Number of experiments					
			Single dose			Multiple doses		
			Total	Abolished	Attenuated	Total	Abolished	Attenuated
Ethosuximide	300	60	6	6		6	6	
Trimethadione	300	45	6	3	3	6	6	
Sodium valproate	300	60	6	6		6	6	
Clonazepam	1	30	8	0	0	6	0	0
Phenobarbital	40	60	8	0	0	6	0	0
Diazepam	10	15	6	3	0	6	0	0
Phenytoin	40	60	6	0	0	6	0	0

the paroxysms such that single, high-voltage spikes occurred at a frequency of 1 Hz for 8 to 10 minutes after leucine enkephalin administration. The animal was immobile and unresponsive during these paroxysms.

In the drug experiments with multiple doses (Table 1), ethosuximide, sodium valproate, and trimethadione completely prevented the leucine enkephalin-induced activity in all animals; clonazepam showed the same effect as in the single dose experiments; and phenobarbital, phenytoin, and diazepam were ineffective. The dose-response experiments with naloxone demonstrated that this drug blocked the electrical and behavioral responses to enkephalin at doses greater than 4 mg/kg, whereas doses of 0.1 to 3 mg/kg had no effect.

Ethosuximide, trimethadione, and sodium valproate are the anticonvulsants most specific for petit mal now used in this form of epilepsy (*9*). Phenobarbital, diazepam, and clonazepam are less specific in their clinical effects, and phenytoin is ineffective against petit mal seizures (*10*). The antagonism of ethosuximide, trimethadione, and sodium valproate to the leucine enkephalin-induced seizure activity suggests a specific sensitivity of this activity to drugs directed against petit mal seizures.

Rats treated with leucine enkephalin are similar in some ways to those that have received γ-hydroxybutyric acid (GHB), a substance which induces petit mal-like seizure activity in animals (*11*). An increase in activity of the 3- to 6-Hz frequency band of the ECoG is observed with both compounds (*12*); the ECoG and behavioral effects of both are effectively treated with anticonvulsant drugs specific for petit mal (*13*) and with naloxone (*6*); the dose-response curve for naloxone against the electrical paroxysms induced by both compounds is the same (*6*); and the behavior of the animals treated with either substance is characterized by stupor, immobility, and myoclonic jerks. A major difference between the animals treated with leucine enkephalin or with GHB is that the electrical paroxysms and behavioral abnormalities induced by GHB last much longer and are continuous (*8, 11, 12*) whereas the leucine enkephalin-induced changes are short-lived and characterized by two paroxysmal bursts followed by single spiking. The paroxysms produced by both compounds are only partially similar in appearance to the specific electroencephalogram changes seen in human petit mal epilepsy (*7*).

The relatively high dose of naloxone required to block the epileptic effect of leucine enkephalin suggests that this property of the enkephalins differs in its site of action from the analgesic effect produced by these compounds. There is anatomic evidence for such separate sites of action (*14*). Pharmacologic evidence for disparate sites of action of the opiates has been developed by Martin and colleagues (*15*) who postulated the presence of three types of receptors in brain. These receptors have been named for the drugs that gave rise to the distinction: μ (morphine), κ (ethylketocyclazocine), and σ (SKF 10,047). There

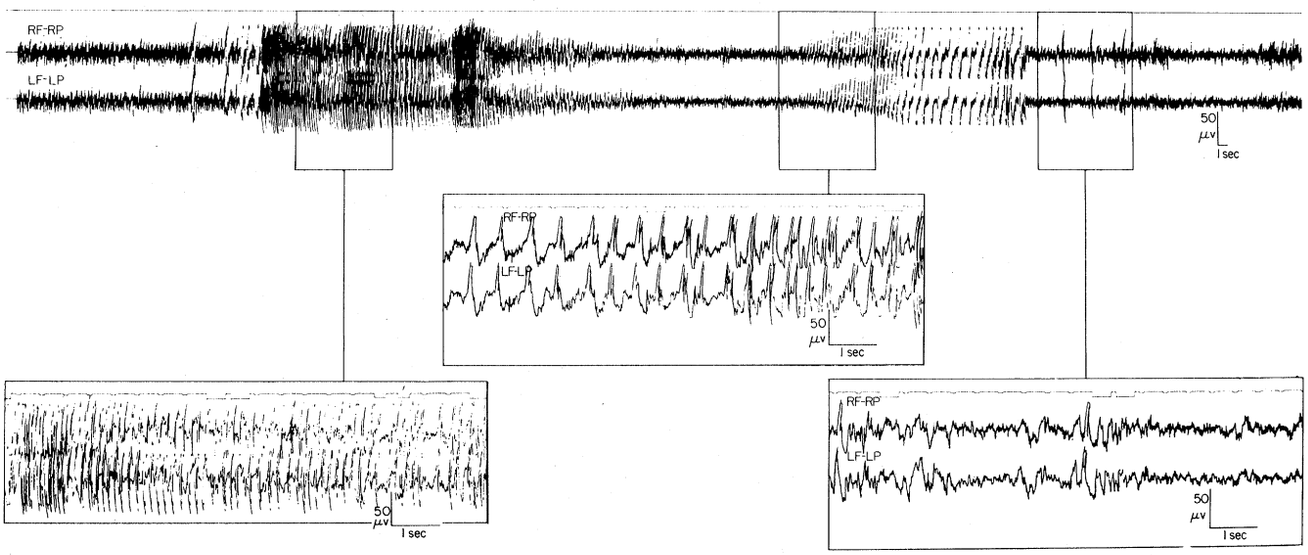

Fig. 1. Electrocorticogram (ECoG) changes produced by 100 μg of leucine enkephalin injected intraventricularly. The insets show a faster time trace. *RF* and *RP* are right frontal and parietal leads; *LF* and *LP* are left frontal and parietal leads. The animal was immobile during this paroxysmal electrical activity. The first change in the ECoG occurred within 1 minute of administration and was a paroxysm of high-voltage spikes at a frequency of 7 to 9 Hz lasting 30 to 40 seconds. This tapered off to 2- to 3-Hz slow-wave activity which was followed by 20 to 25 seconds of low-voltage fast activity. A few seconds of 1-Hz high-voltage slow-wave activity then built up to 25 to 30 seconds of a second paroxysm of spike and slow-wave activity followed by 15 to 20 seconds of low-voltage fast activity. Finally, a prolonged period of high-voltage single spikes occurred at a rate of one paroxysm per 5 seconds.

is some biochemical evidence for such opiate receptor heterogeneity (*16*), although the presence of κ receptors in rat brain has recently been questioned (*17*). The μ receptor is thought to be responsible for the analgesic actions of the opiates and requires the lowest doses of naloxone for reversal. Hence the dose-response curve of naloxone against leucine enkephalin–induced seizures would indicate that some receptor other than the μ receptor is responsible for the epileptogenic action of this substance. The enkephalin-induced electrical seizures and behavioral abnormalities that are overcome by anticonvulsants specific for petit mal indicate possible involvement of enkephalinergic systems in petit mal epilepsy.

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References and Notes

1. H. W. Kosterlitz and J. Hughes, *Adv. Biochem. Psychopharmacol.* **18**, 31 (1978); J. Hughes and H. W. Kosterlitz, *Br. Med. Bull.* **33**, 157 (1977); J. Hughes, T. W. Smith, H. W. Kosterlitz, L. A. Fothergill, B. A. Morgan, H. R. Morris, *Nature (London)* **258**, 577 (1975).
2. G. Urca, H. Frenk, J. C. Liebeskind, A. N. Taylor, *Science* **197**, 83 (1977).
3. H. Frenk, G. Urca, J. C. Liebeskind, *Brain Res.* **147**, 327 (1978).
4. S. J. Henriksen, F. E. Bloom, F. McCoy, N. Ling, R. Guillemin, *Proc. Natl. Acad. Sci. U.S.A.* **75**, 5221 (1978).
5. H. Frenk, J. Engle, R. F. Ackermann, Y. Shavit, J. C. Liebeskind, *Brain Res.* **167**, 435 (1979); M. E. Corcoran and J. A. Wada, *Life Sci.* **24**, 791 (1979).
6. O. C. Snead and L. J. Bearden, *Neurology* **30**, 832 (1980).
7. Petit mal seizures consist of brief lapses of consciousness associated with staring and a characteristic electroencephalogram pattern of 2.5- to 3.5-Hz spike and wave discharges. There may be associated motor movements. This type of epilepsy typically occurs in children [S. Livingston, I. Tones, L. L. Pauli, R. V. Rider, *J. Am. Med. Assoc.* **194**, 277 (1965); J. K. Penry, R. J. Porter, F. E. Dreifuss, *Brain* **98**, 427 (1975); E. Neidermeyer, *Epilepsia* **6**, 250 (1965)].
8. L. J. Bearden, O. C. Snead, C. H. Healey, V. G. Pegram, *Electroencephalogr. Clin. Neurophysiol.* **49**, 181 (1980).
9. T. R. Browne, F. E. Dreifuss, P. R. Dyken, D. J. Goode, J. K. Penry, R. J. Porter, B. G. White, P. T. White, *Neurology* **25**, 515 (1975); H. J. Villarveal, B. J. Wilder, L. J. Willmore, A. W. Baumann, E. J. Hammond, *ibid.* **28**, 886 (1978); E. B. Kirsten and E. P. Schoener, *Neuropharmacology* **11**, 591 (1972).
10. H. Gasteaut, J. Roger, H. Lob, in *Anticonvulsant Drugs*, vol. 2, *International Encyclopedia of Pharmacology and Therapeutics*, J. Mercier, Ed. (Pergamon, New York, 1973), p. 535; J. A. Vida, Ed., *Anticonvulsants* (Academic Press, New York, 1977); J. Aicardi, *Therapie* **30**, 161 (1975); J. J. Coatsworth and J. K. Penry, in *Antiepileptic Drugs*, D. M. Woodbury, J. K. Penry, R. P. Schmidt, Eds. (Raven, New York, 1972), p. 87.
11. M. Godschalk, M. R. Dzoljic, I. L. Bonta, *Eur. J. Pharmacol.* **44**, 105 (1977); W. D. Winters and C. E. Spooner, *Int. J. Neuropharmacol.* **4**, 197 (1965); R. J. Marcus, W. D. Winters, K. Mori, *Int. J. Neuropharmacol.* **6**, 175 (1967); R. J. Marcus, W. D. Winters, E. Hultin, *Neuropharmacology* **15**, 29 (1976); O. C. Snead, R. K. Yu, P. R. Huttenlocher, *Neurology* **26**, 51 (1976); O. C. Snead, *ibid.* **28**, 636 (1978).
12. O. C. Snead and L. J. Bearden, *Neuropharmacology* **19**, 47 (1980).
13. M. Godschalk, M. R. Dzoljic, I. L. Bonta, *Neurosci. Lett.* **3**, 145 (1976); O. C. Snead, *Neurology* **28**, 643 (1978); *ibid.*, p. 1173.
14. H. Frenk, B. C. McCarty, J. C. Liebeskind, *Science* **200**, 335 (1978).
15. W. R. Martin, C. G. Eades, J. A. Thompson, R. E. Huppler, P. E. Gilbert, *J. Pharmacol. Exp. Ther.* **197**, 517 (1976); P. E. Gilbert and W. R. Martin, *ibid.* **198**, 66 (1976).
16. H. W. Kosterlitz and F. M. Leslie, *Br. J. Pharmacol.* **64**, 607 (1978); K. Chang and P. Cuatrecasas, *J. Biol. Chem.* **254**, 2610 (1979); J. A. H. Lord, A. A. Waterfield, J. Hughes, H. W. Kosterlitz, *Nature (London)* **267**, 495 (1977); D. Della Bella, F. Casacci, A. Sassi, *Adv. Biochem. Psychopharmacol.* **18**, 271 (1978).
17. J. M. Hiller and E. J. Simon, *Eur. J. Pharmacol.* **60**, 389 (1979).
18. We thank Abbott Laboratories, Parke, Davis & Co., Eli Lilly & Co., Roche Laboratories, and Endo Laboratories for supplying the drugs used in this study, and H. Parker for technical assistance. This work was supported in part by grant 1K07 NS 00 484-01 from the National Institute of Neurological and Communicative Disorders and Stroke.

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Perception of Numbers by Human Infants

Abstract. *Infants are capable of discriminating, representing, and remembering particular small numbers of items. A perceptual enumeration process called subitizing, present in 2-year-olds, probably underlies this capacity. This finding indicates that some number capacity is present before the onset of verbal counting, and it suggests that verbal counting may have precursors present during infancy.*

One view of cognitive developmental psychology is that the earliest phases of development must be characterized in terms of capacities as well as incapacities in order to understand the courses (both normal and abnormal) of later development (*1*). Much of the theoretical and empirical research on numerical development has, in the last decade, focused on very young children; we now know that 2- to 4-year-olds understand some basic number concepts (*2*). The presence of numerical abilities in very young children led to the question of the precursors of these abilities, which in turn led us to investigate whether infants have any basic numerical abilities. Our research was motivated by the belief that the ability to represent the numerical value of a set of items may be necessary for the development of an understanding of number. Two-year-olds use a rapid perceptual process (called subitizing) to distinguish among arrays containing fewer than four items (*3*). In this report, we present evidence that 22-week-old infants can also discriminate exact numbers of items. This raises the possibility that the young child's verbal counting abilities grow in part from the infant's numerical ability.

The focus of our study was to demonstrate skills for perceiving and representing specific small numbers of items. This focus differs from that of experiments demonstrating the ability of infants to discriminate stimulus arrays that greatly differ in number (such as 2 versus 8 versus 32 versus 128 items) (*4*). These discriminations treat numbers of items that massively exceed the range of those adults and children can subitize, since the adult subitizing range is one to four or five items (*5*). The bases of discriminations among large values are not known and may vary with different types of arrays (for example, dot size, the spatial distance separating adjacent dots, or surface area of the total array). The ability underlying these discriminations might be the same as that which allows adults to estimate the more numerous of two arrays differing substantially in number; however, such skills are clearly different from subitizing, and they cannot provide the same kind of information for later number development as the perception and representation of exact small numerical values.

Our experimental method used duration of first fixation in a standard habituation- dishabituation-of-looking procedure (*6*). The subjects were 72 normal, full-term infants with a mean age of 22 weeks (range, 16 to 30 weeks). The infants were first habituated to arrays containing a particular number of dots and were then presented a posthabituation (PH) array containing a different number of dots (Fig. 1). The small-number conditions ($2 \rightarrow 3$ and $3 \rightarrow 2$) were chosen because 2-year-olds can perceive and store the number of items of 2- and 3-dot arrays (*3*); both $2 \rightarrow 3$ and $3 \rightarrow 2$ were used because infants might prefer complexity. The large-number conditions ($4 \rightarrow 6$ and $6 \rightarrow 4$) were chosen as controls because 2-year-olds can not perceive (that is, subitize) arrays containing four or more dots (*3*). Since 4:6 maintains the same ratio as 2:3, large-number conditions to some extent control for discrimination based on physical cues such as differences in total contour.

We analyzed the data several ways, and each analysis revealed the same pattern of results: dishabituation occurred when the number of items was small but

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