

Review: Cholinergic Mechanisms and Epileptogenesis. The Seizures Induced by Pilocarpine: A Novel Experimental Model of Intractable Epilepsy

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ABSTRACT High-dose treatment with pilocarpine hydrochloride, a cholinergic muscarinic agonist, induces seizures in rodents following systemic or intracerebral administration. Pilocarpine seizures are characterized by a sequential development of behavioral patterns and electrographic activity. Hypoactivity, tremor, scratching, head bobbing, and myoclonic movements of the limbs progress to recurrent myoclonic convulsions with rearing, salivation, and falling, and status epilepticus. The sustained convulsions induced by pilocarpine are followed by widespread damage to the forebrain. The amygdala, thalamus, olfactory cortex, hippocampus, neocortex, and substantia nigra are the most sensitive regions to epilepsy-related damage following convulsions produced by pilocarpine. Spontaneous seizures are observed in the long-term period following the administration of convulsant doses of pilocarpine.

Developmental studies show age-dependent differences in the response of rats to pilocarpine. Seizures are first noted in 7-12 day-old rats, and the adult pattern of behavioral and electroencephalographic sequelae of pilocarpine is seen in 15-21-day-old rats. During the third week of life the rats show an increased susceptibility to the convulsant action of pilocarpine relative to older and younger animals. The developmental progress of the convulsive response to pilocarpine does not correlate with evolution of the brain damage. The adult pattern of the damage is seen after a delay of 1-2 weeks in comparison with the evolution of seizures and status epilepticus. The susceptibility to seizures induced by pilocarpine increases in rats aged over 4 months.

The basal ganglia curtail the generation and spread of seizures induced by pilocarpine. The caudate putamen, the substantia nigra, and the entopeduncular nucleus govern the propagation of pilocarpine-induced seizures.

The antiepileptic drugs diazepam, clonazepam, phenobarbital, valproate, and trimethadione protect against pilocarpine-induced convulsions, while diphenylhydantoin and carbamazepine are ineffective. Ethosuximide and acetazolamide increase the susceptibility to convulsant action of pilocarpine. Lithium, morphine, and aminophylline also increase the susceptibility of rats to pilocarpine seizures.

The pilocarpine seizure model may be of value in designing new therapeutic approaches to epilepsy.

INTRODUCTION

The term epilepsy gathers together diverse convulsive disorders that have in common a sudden, transient, and recurrent interruption of the function of the central nervous system characterized by abnormal motor, sensory, autonomic, and psychic phenomena (Janz, 1985). The etiology of human epilepsy has been a subject of intensive research for many years. Although several attempts to understand mechanisms of epileptogenesis have been made, no satisfactory unifying theory has yet

been advanced within which pathological phenomena underlying epilepsy can be understood.

The search for knowledge of the basic mechanisms of epilepsies and for new therapeutic approaches requires

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the use of animal models of seizures (Purpura et al., 1972; Bruni and Albright, 1984; Meldrum, 1986). Basic research on the mechanisms and treatment of epilepsy has been performed in the rodent and in primate models of convulsions (Purpura et al., 1972; Meldrum, 1986). The seizures produced by chemical or electrical stimulation are classically divided into myoclonic and generalized clonic/tonic, and it is possible to differentiate between antiepileptic drugs preferentially active against either type (Albright and Burnham, 1980; Bruni and Albright, 1984; Meldrum, 1986). For the therapy of human seizure disorders, no antiepileptic drug has been introduced that is inactive against pentylenetetrazol-induced seizures or convulsions elicited by maximal electroshock (Janz, 1985). Nevertheless, the relevance of pentylenetetrazol- and electroshock-induced seizures to human disease has not been established (Meldrum, 1986).

The kindling of seizures is generated by repeated electrical or chemical stimulation of sensitive brain regions, e.g., the amygdala, the hippocampus, or the cortex in rodents and primates (Girgis, 1981; Goddard, 1967; Vosu and Wise, 1975; Wasterlain et al., 1986). Research with the kindling model has contributed to an understanding of seizure generation and spread, and to an elucidation of the mechanisms of action of antiepileptic drugs (Mc Namara, 1986); however, questions continue to arise on the relevance of this seizure model to human forms of epilepsy (Engel and Cahan, 1986).

Systemic and intracerebral microinjections of the excitotoxin kainic acid (KA) in rats and mice may result in convulsions and epilepsy-related brain damage (Ben-Ari et al., 1980, 1981; Cavalheiro et al., 1982; Kleinrok et al., 1980; Schwob et al., 1980). Morphological sequelae of limbic seizures induced by KA are proposed to be reminiscent of human temporal lobe epilepsy (Ben-Ari, 1985; Ben-Ari et al., 1980). Nevertheless, the efficacy of this preparation in the development of antiepileptic drugs has been hampered by a narrow margin of safety between seizures and mortality (Fuller and Olney, 1981).

The doubtful relevance of experimental models of seizures relative to human forms of epilepsy justifies the search for new animal models that mimic pathological features of human seizure disorders as closely as possible and are more useful for studying mechanisms and therapeutic approaches to human disease.

In this overview we intend to present the creation of a novel experimental model of epilepsy based on intractable seizures induced in rodents following systemic administration of a cholinergic muscarinic agonist, pilocarpine, and to critically review a potential experimental use of this approach in an effort toward better understanding of epileptogenesis. The pioneering research on cholinergic mechanisms in epileptogenesis has already been surveyed (Maynert et al., 1975), allowing us to give more emphasis to developments achieved in the last decade.

DISCOVERY OF CHOLINERGICALLY MEDIATED SEIZURES AND BRAIN DAMAGE IN RODENTS Acetylcholine in epilepsy

The relation of cholinergic mechanisms to epilepsy was suspected by neurologists by the turn of the 19th century (Langley, 1901; Langley and Kato, 1915). The convulsant potential of acetylcholine (ACh) and physostigmine was experimentally demonstrated in the elec-

trocorticogram as early as the late thirties (Sjöstrand, 1937; Miller et al., 1938; Chatfield and Dempsey, 1943; Brenner and Merritt, 1942). Brenner and Merritt (1942) suggested that ACh may be primarily involved in human convulsive disorders. This view was experimentally justified by Forster (1945), who showed that intracisternal injections of ACh in cats resulted in motor seizures and electrical discharges in the cerebral cortex.

The debate on the involvement of cholinergic mechanisms in human and experimental epilepsy was dominated for more than the following four decades by a lack of consensus on the specific contribution of ACh to seizure disorders (Snead, 1983a). However, significant experimental contributions by Echlin (1959), Grossman (1963), Krnjevic (1969), Girgis (1981), Snead (1983a,b), and Wasterlain (Wasterlain et al., 1986) continuously placed ACh into a conceptual framework underlying the epileptogenesis. Studies of chronically isolated monkey cortex demonstrated an increased sensitivity to locally administered ACh, which suggested an epileptogenic potential of ACh in the cortex (Echlin, 1959). Grossman (1963) reported sustained seizures following the placement of crystalline ACh or carbachol into the basolateral amygdala of cats. Snead and associates (Cohen et al., 1981; Snead, 1983b) characterized behaviorally and electrographically sustained seizures induced by carbachol following intracerebroventricular administration in rats. Goddard (1969), Vosu and Wise (1975), and Wasterlain and associates (Wasterlain and Jonec, 1983; Wasterlain et al., 1986) reported on chemical kindling with carbachol in rats, while Girgis (1981, 1985) repeatedly stressed the participation of muscarinic cholinergic mechanisms in amygdala kindling in rabbits and primates.

The development of extracellular and intracellular recording techniques stimulated research into the cellular mechanisms of epilepsy (Goldensohn and Purpura, 1963). Evidence from electrophysiological experiments reinforced the contention that ACh may be involved in cellular processes underlying epilepsy (Dichter and Ayala, 1987). It has been shown that muscarinic cholinergic excitation in the brain occurs as a result of a reduced voltage-dependent and Ca-dependent K^+ conductance and is mediated by voltage-dependent Ca^{2+} and Na^+ conductances (Benardo and Prince, 1982). This action of ACh favours the inward movement of Ca^{2+} and Na^+ into neurons, which may be responsible for membrane depolarization and relevant for epileptic events (Pumain et al., 1983).

Early clinical observations in humans showed that the cerebrospinal fluid of epileptic patients with frequent seizures contains ACh (Tower and McEarchern, 1949). Elevated activity of the ACh catabolizing enzyme acetylcholinesterase in actively epileptic human cerebral cortex was reported in the late forties (Pope et al., 1947; Tower and Elliott, 1952). More recently, an elevated activity of ACh synthetic enzyme choline acetyltransferase was detected in epileptic cortex obtained from patients subjected to anterior temporal lobectomy for intractable seizures (Kish et al., 1988; Nadi et al., 1987).

This historical perspective should not give the impression that the cholinergic mechanisms have always been regarded as important for epileptogenesis. Until recently this was certainly not the case, and it still remains a minority viewpoint. The development of this field over the last century has been hallmarked by an

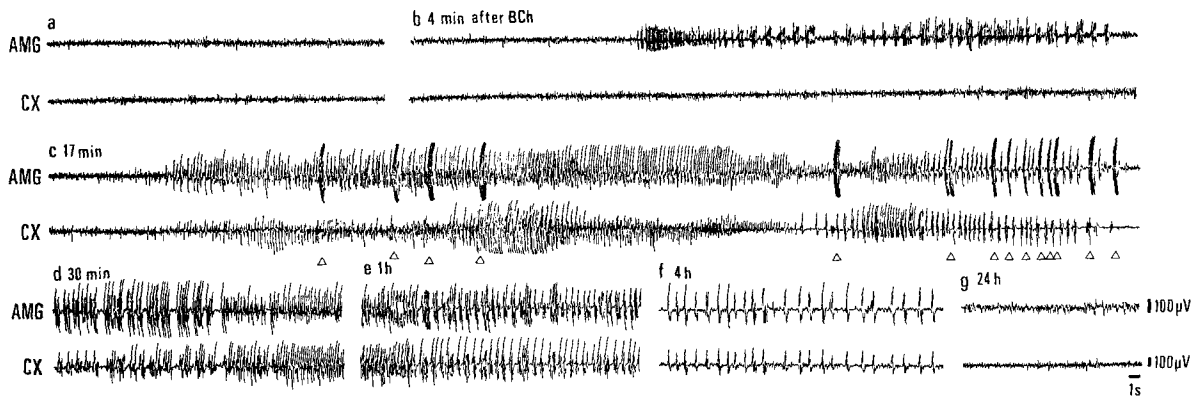


Fig. 1. Electrographic recordings demonstrating convulsant activity of bethanechol chloride (BCh) in the rat amygdala. BCh, 0.1 μ mol, was microinjected into the amygdala bilaterally. a: Predrug control recordings. b: High-voltage spiking and bursts of polyspike registered in the amygdala 4 min after the injection of BCh. c: Electrographic seizure registered 17 min after the microinjection of BCh. d-f: Electrographic activity registered 30 min to 4 h after microinjection of BCh during status epilepticus. g: The EEG returns to the predrug patterns 8–24 h after the injection of BCh. Δ , wet dog shakes; AMG, amygdala; CX, cortex. Methods: Male Wistar rats, 220–280 g in weight, were anesthetized with sodium pentobarbitone, 50 mg/kg i.p., and mounted in a stereotaxic frame. For injections into the amygdala, guide cannulae were chronically implanted. Rapid-setting methyl methacrylate was used to secure the guide cannulae to the skull by means of stainless steel anchor screws. After surgery animals were allowed to recover for a period of 3–5 days. The injections into the

amygdala were performed bilaterally in unanesthetized rats with an injection cannula attached by a polyethylene tube to a 2- μ l Hamilton syringe. The drugs were delivered in a volume of 0.5 μ l at a rate of 0.1 μ l/min. For depth recordings, bipolar twisted electrodes (tip diameter 100 μ m, interelectrode distance 500 μ m) were stereotactically positioned in the dorsal hippocampus and/or amygdala, deep prepiriform cortex, and nucleus accumbens and anchored to the skull with dental acrylic. Surface recordings were led from jewellers screws positioned bilaterally over the occipital cortex. An additional screw placed in the frontal sinus served as a reference (indifferent) electrode. Signals under investigation were amplified by a Beckmann model RM polygraph (time constant 0.03 s, high cut-off filter 15). EEG recordings were made continuously for periods ranging from 6 to 10 h following injection of drugs. Additional recordings were made between 10–12, 20–24 and 36–48 h. The correct location of the implanted deep electrodes was controlled histologically in cresyl violet-stained serial sections.

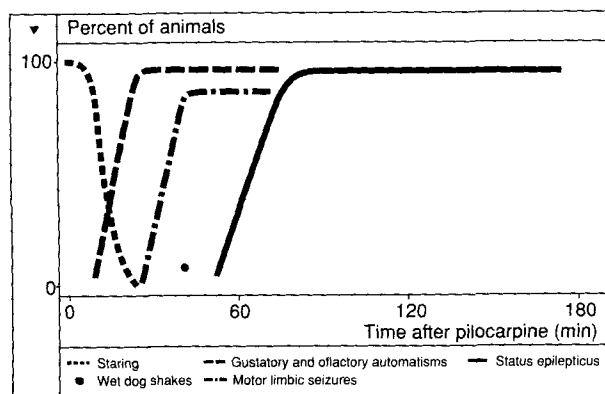


Fig. 2. Time course of behavioral patterns, seizures, and status epilepticus occurring during a 180-min observation period following the intraperitoneal injection of 380 mg/kg of pilocarpine in N-methylscopolamine (1 mg/kg s.c.; 30 min) pretreated rats ($n = 38$).

initial enthusiasm and endeavor followed by a long period of neglect and discount. The involvement of cholinergic mechanisms in epilepsy has been depreciated largely because anticholinergic drugs are not effective anticonvulsants (Maynert et al., 1975). Although the muscarinic agonists pilocarpine and arecoline lower the threshold for electroshock-induced seizures (Chen et al., 1968) and acetylcholinesterase inhibitors increase the susceptibility of rodents to chemical convulsions induced by pentylenetetrazol, picrotoxin, and strychnine (Karczmar, 1967), muscarinic agonists such as atropine and

scopolamine do not affect seizures produced by common convulsant drugs (Maynert et al., 1975).

Muscarinic cholinergic agonists

Recent interest in the relation of cholinergic mechanisms to epilepsy began when the occurrence of sustained seizure activity accompanied by widespread damage to the forebrain was shown in rats and mice after intraamygdaloid (Olney et al., 1983; Turski et al., 1983d) or systemic (Turski et al., 1983b, 1984) injections of high doses of muscarinic cholinergic agonists.

A chance discovery that atropine reverses some of the behavioral effects produced by KA when administered into the rat brain ventricle (Kleinrok and Turski, 1979, 1980) prompted us to microinject carbachol and bethanechol intracerebroventricularly in rats. We have found that both agents reproduce the behavioral effects elicited by KA (Turski et al., 1981, 1982). Consequently, we selected bethanechol, a potent muscarinic agonist resistant to acetylcholinesterases and devoid of nicotinic activity, for further investigations. We microinjected bethanechol into the amygdaloid complex and hippocampus, and found that this drug produces a seizure-related behavioral syndrome and brain damage (Turski et al., 1983a,d), which can be antagonized by coadministration of scopolamine or atropine (Turski et al., 1983d). Behavioral alterations include akinesia, tremor, gustatory and olfactory automatisms, wet dog shakes, and mild-to-severe limbic seizures culminating in long-lasting status epilepticus (Turski et al., 1983a,d). Morphological analysis of the brains from such animals reveals widespread damage to the hippocampus, thalamus,

TABLE I. Arterial blood gases, pH, and glucose in the course of sustained seizures after exposure of rats to pilocarpine¹

	pH	p _a CO ₂ (mm Hg)	p _a O ₂ (mm Hg)	Glucose (mg/ml)
Control (n = 6)	7.45 ± 0.02	33.6 ± 1.1	82.5 ± 3.2	1.09 ± 0.08
Pilocarpine (380 mg/kg; n = 7)	7.53 ± 0.02***	23.4 ± 0.9*	93.3 ± 3.7**	1.83 ± 0.13**

¹Samples of arterial blood were collected 2 h after i.p. injection of pilocarpine (380 mg/kg). The pH, arterial oxygen, carbon dioxide, and glucose were determined using automated diagnostic procedures. Shown are the means ± SEM of six or seven determinations. Statistical significances were calculated by means of Student's *t* test.

**P* > 0.05.

***P* > 0.01.

****P* > 0.001.

amygdala, olfactory cortex, neocortex, and substantia nigra (Turski et al., 1983d). Electrographic monitoring showed sequential development of seizure activity, which started in the amygdala and spread to the hippocampus and cortex (Fig. 1). These observations consequently led us to administer systemically pilocarpine and arecoline in rats and mice (Turski et al., 1983b,d). High-dose treatment with pilocarpine, 300–400 mg/kg (ED₅₀ 284 mg/kg; 247–326), or arecoline, 350–500 mg/kg (ED₅₀ 326 mg/kg; 288–369), induces sequential behavioral (Fig. 2) and electrographic changes (Fig. 3A) indicative of sustained epileptic activity in both species (Turski et al., 1983b,d, 1984). This treatment results in widespread damage to the forebrain in both rats and mice (Turski et al., 1983b,d, 1984). The damage to the brain detected following pilocarpine seizures in rats occurs in the absence of hypotension, hypoxemia, and hypoglycemia (Fujikawa et al., 1986). The arterial blood chemistries during pilocarpine seizures reflect hyperventilation with alkalosis and hypocarbia, while oxygen and blood glucose are maintained above normal values (Table I). Scopolamine prevents but does not interrupt seizures produced by pilocarpine (Turski et al., 1983b, 1984). Precipitous reduction in body weight, ranging from 10% to 20% on the third day after administration, occurs in rats subjected to the convulsant action of pilocarpine (Fig. 4). Predrug body weight is regained between the 5th and 7th day postinjection (Fig. 4). The lethality in such rats registered during a 24-h observation period reached 27.4% (17/62; male Wistar rats). Temporal analysis of lethality shows that 4.8% of animals die within 1 h postinjection (3/62), whereas 11.3% die after 6 h (7/62). Thus the majority of deaths occurs between 1 and 24 h following pilocarpine administration in the course of status epilepticus. Snead and associates (Jope et al., 1986) reported lethality of up to 85% in rats (male Sprague-Dawley) subjected to high-dose treatment with pilocarpine.

Electrographic analysis of seizures induced by pilocarpine in rats and mice shows sequential development of pathological activity in the forebrain (Turski et al., 1983b,d, 1984). The hippocampus is activated earlier, in comparison with the amygdala and cortex (Fig. 3A). In contrast to this observation, Clifford and colleagues (1987) have reported that electrographic activity in high-dose pilocarpine and lithium-pilocarpine seizures in rats originates from ventral forebrain in the vicinity of the ventral pallidum and/or nucleus accumbens. These find-

ings are at variance with our recent observations in rats with high-dose pilocarpine seizures indicating that epileptic activity in the nucleus accumbens is registered with considerable delay relative to similar changes in the hippocampus and amygdala, and with early observations in cats with physostigmine seizures suggesting a hippocampal origin of convulsions (Bokums and Elliott, 1968).

Metabolic activity during the course of sustained seizures induced by pilocarpine shows differential changes in the glucose utilization in the forebrain as measured with the use of [¹⁴C]2-deoxyglucose technique 45 min after the first episode of forelimb clonus (Clifford et al., 1987). Increases in glucose utilization were reported in several hippocampal subfields, dentate gyrus, globus pallidus, ventral pallidum, amygdala, substantia nigra, ventrobasal and mediodorsal thalamus, lateral septum, pyriform cortex, olfactory tubercle, frontal and visual cortex, and caudate putamen (Clifford et al., 1987). The intensity of glucose utilization in these regions exceeded control values by three- to sevenfold (Clifford et al., 1987).

Ultrastructural analysis of the cell damage associated with pilocarpine-induced convulsions demonstrates the characteristic type of cytopathology described following protracted seizures (Clifford et al., 1987). At the electron microscopic level, pilocarpine seizures induce massive swelling of dendrites and neuronal cell bodies (or vacuolar condensation) and dilatation of astroglial elements, axons remaining relatively spared (Clifford et al., 1987; Olney et al., 1986). This pattern of cytopathology resembles the excitotoxic reaction of the brain to glutamate (Olney, 1986).

In the long-term period following pilocarpine seizures, in rats and mice that survived status epilepticus, spontaneous recurrent seizures are detected (Fig. 3B). The first spontaneous seizure is usually observed 5–10 days after pilocarpine-induced status epilepticus. These seizures resemble stage 4/5 amygdala kindling and continue to appear for several weeks following status epilepticus (Turski et al., 1983c).

Olney and his associates have observed a structural analogy between dipiperidinoethane and oxotremorine (Olney et al., 1980, 1982; Olney, 1983, 1985). Dipiperidinoethane has been found to mimic the seizure-related brain damage of KA in rats when administered systemically but not when placed in the amygdala (Olney et al., 1980). This finding prompted the inference that an

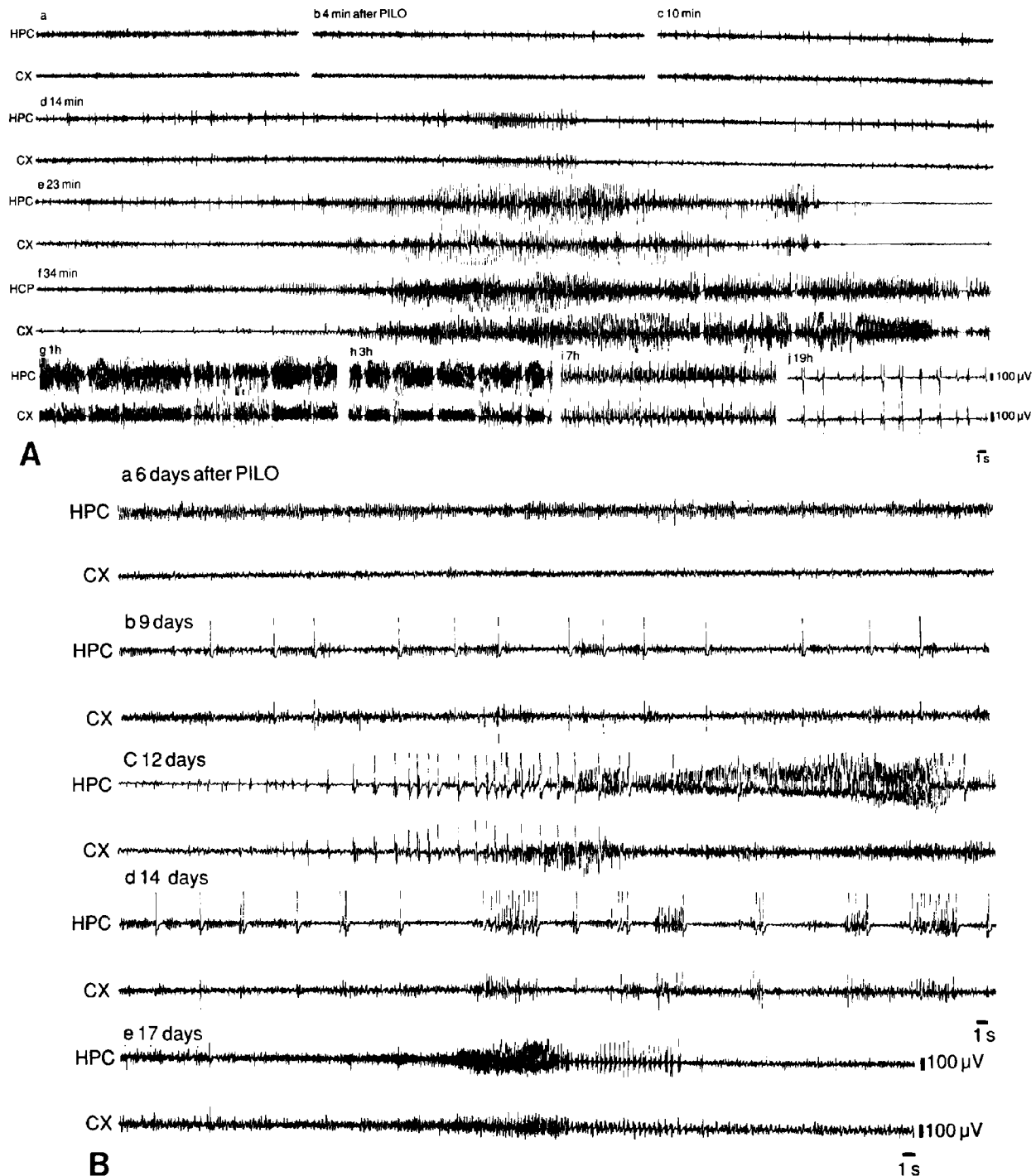


Fig. 3. A: The evolution of seizure activity produced by pilocarpine hydrochloride (PILO) in rats. PILO, 380 mg/kg, was administered i.p. in rats pretreated with N-methylscopolamine, 1 mg/kg s.c. a: Predrug control recordings. b: Significant theta rhythm and isolated spikes in the hippocampus and low-voltage fast activity in the cortex registered 4 min after PILO. c: High-voltage spiking is initially registered in hippocampal recordings, while low-voltage fast activity prevails in the cortex. d: Spiking becomes synchronized in the hippocampus and cortex 10–20 min after PILO. e: An electrographic seizure registered 23 min after PILO. The waxing of the seizure is faster in the hippocampus. f: Paroxysmal activity becomes highly synchronized in all recordings. g, h: Electrographic activity during the status epilepticus 1 and 3 h after PILO. i, j: By 6–24 h, the paroxysmal activity slowly abates

and the EEG progressively normalizes and returns to predrug patterns within 48–72 h after PILO. HPC, hippocampus; CX, cortex. B: Spontaneous recurrent seizures registered in a rat that survived status epilepticus induced by pilocarpine, 380 mg/kg. The development of seizures and status epilepticus is shown in A. a: Unchanged activity 6 days following administration of PILO. b, d: Interictal periods registered 9 and 14 days after PILO. High-voltage spiking activity and bursts of polyspikes are registered in the hippocampus. Isolated spikes are less frequently registered in the cortex. c, e: Spontaneous electrographic seizures registered 12 and 17 days after the administration of PILO. The waxing of the seizure is usually faster and the waning longer in the hippocampus (c).

TABLE II. Effect of antiepileptic and other drugs on seizures induced by pilocarpine¹

Drug	Time (min)	ED ₅₀ (mg/kg)	No.
Rats			
Pilocarpine hydrochloride	—	284 (247–326)	22
Arecoline hydrochloride	—	326 (288–369)	26
Anticonvulsive activity			
Clonazepam	30	0.35 (0.25–0.49)	19
Diazepam	30	0.76 (0.44–1.33)	25
Phenobarbital	30	23.4 (18.5–29.6)	17
Trimethadione	60	179 (116–277)	24
Valproate sodium	15	286 (202–405)	21
Diphenylhydantoin	60	> 200	12
Carbamazepine	60	> 50	18
Scopolamine hydrochloride	30	0.26 (0.08–0.71)	21
Baclofen	30	4.28 (2.69–6.82)	16
2-Chloradenosine	10	4.51 (2.98–6.69)	18
Mefenamic acid	45	26 (22–30)	22
Ibuprofen	45	> 100	11
Indomethacin	45	> 10	23
Apomorphine hydrochloride	5	> 10	15
LY-171555	30	> 5	8
SCH-23390	60	> 5	9
Proconvulsive activity			
Ethosuximide	30	196 (141–272)	21
Acetazolamide	60	505 (332–766)	31
Morphine hydrochloride ²	30	11.5 (8.0–16.5)	33
Aminophylline ²	30	38.5 (28.4–52.2)	21
SKF-38393	30	0.81 (0.45–1.47)	19
Haloperidol	60	3.0 (2.0–4.3)	18
Sodium salicylate	45	103 (60–174)	28
Phenylbutazone	45	59 (50–70)	24
Prazosin	30	> 10	5
Lithium chloride ²	24 h	1.38 (1.19–1.6) ³	18
Caesium chloride ²	24 h	> 3 ³	8
Rubidium carbonate ²	24 h	> 3 ³	8
Mice			
Pilocarpine hydrochloride	—	246 (210–288)	32
Anticonvulsive activity			
Diazepam	30	0.56 (0.21–1.46)	21
Phenobarbital	30	13.2 (6.7–26.1)	19
Trimethadion	60	243 (165–361)	23
Valproate sodium	15	360 (344–378)	20
Diphenylhydantoin	60	> 100	16
Carbamazepine	60	> 50	8
Proconvulsive activity			
Ethosuximide ²	30	469 (358–613)	19
Morphine hydrochloride	30	58.4 (46.0–74.1)	28
Aminophylline	30	35.8 (24.5–52.3)	48
Lithium chloride	1 h/24 h	> 3 ³	18

¹The drugs were administered systemically (i.p. or s.c.) to adult male Wistar rats or Swiss S mice at the time indicated before the injection of pilocarpine, 380 mg/kg (anticonvulsant test), or 200 mg/kg (proconvulsant test). The table data represent anticonvulsive/proconvulsive potency expressed as ED₅₀ values (and their 95% confidence limits) estimated from probit-log dosage regression curves. The criterion used to indicate convulsive response was status epilepticus defined as continuous seizures [stage 4/5 according to Racine (1972)] persisting for a period of at least 30 min before spontaneous termination. No., number of animals.

²Pilocarpine, 100 mg/kg, was used as a trigger of seizures.

³mEq/kg.

active metabolite is responsible for dipiperidinoethane neurotoxicity and led to the development of an oxidized derivative, dipiperidinoethane-di-N-oxide (Olney et al., 1982; Olney, 1983). This compound induced brain damage syndrome when placed in the amygdala in rats, had a structural analogy to the muscarinic agonist oxotremorine (Olney et al., 1982, 1986), and selectively inhibited acetylcholinesterase activity in the brain (Baron et al., 1985). Consequently, Olney and his associates have administered cholinergic agents, e.g., carbachol, pilocarpine, oxotremorine, physostigmine, and neostigmine, into the rat amygdala and found that these compounds produce seizure-related brain damage (Olney et al., 1983,

1986). This group also discovered the ability of lithium to lower the threshold for seizures and seizure-associated brain damage produced by pilocarpine in rats (Honchar et al., 1983; Olney et al., 1986). The potentiation by lithium of lethality induced by cholinomimetics was first reported by Samples and colleagues (Samples et al., 1977) and later by Davis and Hatoum (1980). Our group confirmed the supra-additive interaction of lithium and pilocarpine in rats, but no such interaction was seen in mice (Table II). A treatment with rubidium and caesium was also ineffective in potentiating convulsant action of pilocarpine in both species (Table II).

Electrographic characterization of lithium/pilocarpine seizures has shown that spiking activity appears concurrently in the amygdala, dorsal hippocampus, cortex, and caudate-putamen and rapidly progresses to status epilepticus (Jope et al., 1986; Morrisett et al., 1987b).

The lethality in lithium/pilocarpine seizures reached 90–95% within 24 h (male Sprague-Dawley rats) (Jope et al., 1986). Venugopal and Persinger (1988) reported that systemic administration of acepromazine between 0 and 1 h after administration of pilocarpine in lithium-pretreated rats ensures the survival and reduces the lethality to less than 5%.

The pattern of enhanced glucose utilization and the type of cytopathology at the electron microscopic level reported for seizures induced by lithium/pilocarpine are identical with those observed in rats subjected to high-dose treatment with pilocarpine (Clifford et al., 1987).

Acetylcholinesterase inhibitors

Much effort has also been focused on the convulsant action of cholinesterase inhibitors. The irreversible acetylcholinesterase inhibitors and organophosphates soman, sarin, and VX (O-ethyl-S-(2-diisopropylaminoethyl)-methylphosphonothioate) induce in rats convulsions that resemble those elicited by systemic or intra-amygdaloid injections of cholinomimetics (Koelle, 1981; McDonough et al., 1983; McLeod et al., 1984; McLeod, 1985). Lemerrier and his colleagues (1983), McLeod and associates

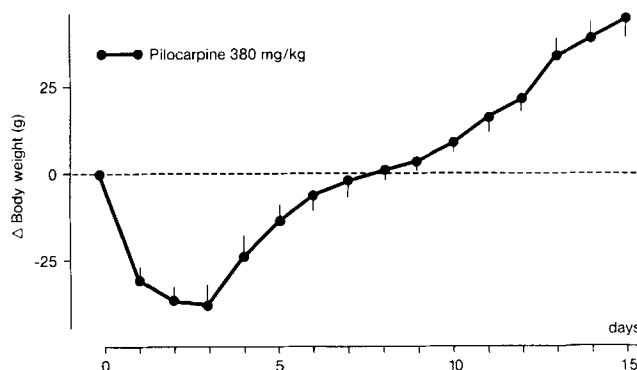


Fig. 4. Time course of changes in the body weight of rats after exposure to convulsant action of pilocarpine at a dose of 380 mg/kg. Male Wistar rats (200–230 g; mean weight 214.5 ± 7.9 g; $n = 7$) received s.c. injection of N-methylscopolamine (1 mg/kg) 30 min prior to the i.p. administration of pilocarpine. Predrug body weights were measured 1 h prior to the injection of pilocarpine or saline, and these measurements served as a reference for the determination of changes in the weight in daily intervals over 15 days. The changes in the body weight are expressed as differences $\Delta \pm$ SEM between weights before and after pilocarpine administration.

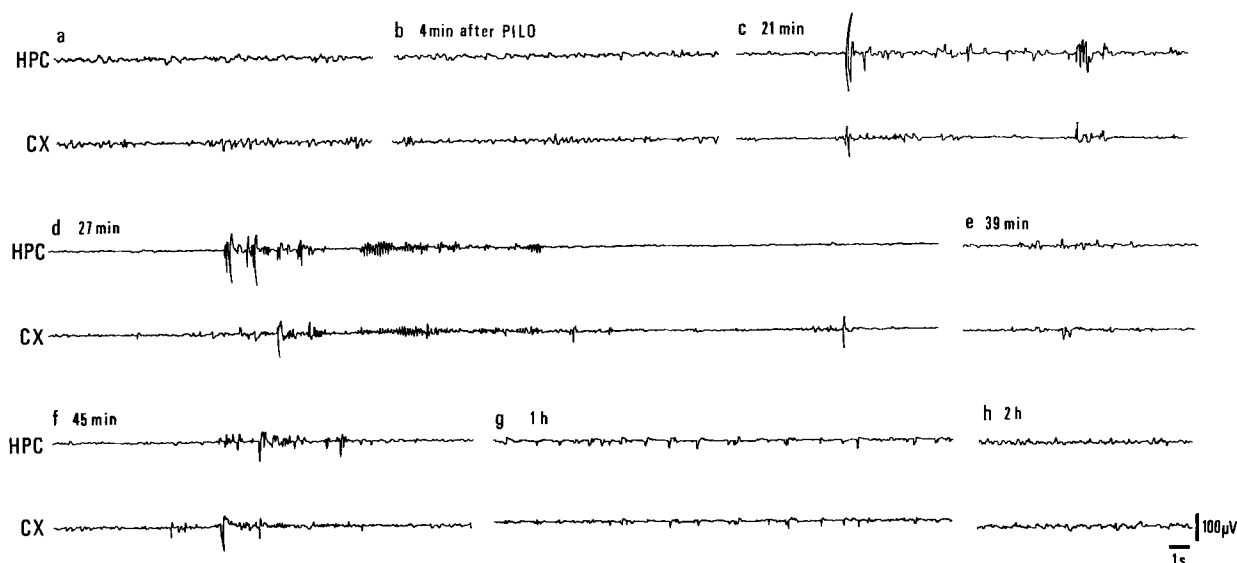


Fig. 5. Electrographic activity recorded in 3 day-old rats after systemic administration of pilocarpine (PILO), 380 mg/kg. a: Predrug control recordings. b: Unchanged activity 4 min following the administration of PILO. c–g: Recordings illustrating electrographic events 20–60 min after PILO. Isolated spikes or polyspikes are infrequently registered in the hippocampus or cortex. h: Two hours after the injection of PILO, the EEG returns to the predrug pattern (a). HPC, hippo-

campus; CX, cortex. Methods: For surgery, animals aged less than 30 days were anesthetized with ether, while animals aged 31–90 days received a chloral hydrate/pentobarbital mixture. Bipolar and monopolar electrodes (tip diameter 100 μ m) were chronically implanted under stereotaxic guidance in the right and left sensorimotor cortex and in the hippocampus (Cavalheiro et al., 1987).

(1984), and Samson and coworkers (1984) have described neuropathological sequelae of seizures induced by soman in rats. These observations were followed by detailed reports on brain pathology in rats subjected to soman convulsions with the aid of light and electron microscopy, and muscarinic receptor autoradiography (Churchill et al., 1985; McLeod, 1985; Samson et al., 1985; Pazdernik et al., 1985a). The rats that had experienced sustained convulsions induced by soman show a consistent pattern of irreversible neuronal degeneration in the brain including the hippocampus, amygdala, thalamus, pyriform cortex, neocortex, and substantia nigra (Lemerrier et al., 1983; McLeod et al., 1984; McLeod, 1985; Samson et al., 1984). The damage to the brain detected following soman seizures occurs in normoxemic rats (Clement and Lee, 1980; Lynch et al., 1985; McDonough et al., 1983). Blood p_aO_2 , p_aCO_2 , glucose concentration, or Na^+ and K^+ levels were normal at the initiation of soman seizures (Clement and Lee, 1980), while p_aO_2 and plasma glucose were maintained within or above normal ranges in the course of soman-induced status epilepticus (Green et al., 1984; Lynch et al., 1985; McDonough et al., 1983). Weight loss in the range of 20–30% consistently occurred in rats that had convulsions after exposure to soman (Churchill et al., 1985). The lethality following soman seizures ranged from 60% to 100% (male Sprague-Dawley rats) (McLeod et al., 1984). Microinjections of VX into the amygdala of naive rats or soman into the amygdala of lithium-pretreated rats also led to sustained limbic convulsions and seizure-related brain damage (McDonough et al., 1987). The seizures induced by soman in rats and monkeys were prevented but not interrupted by atropine (Lipp, 1968; 1972; McDonough et al., 1987).

The changes in local cerebral glucose utilization induced by soman seizures resemble but are not identical to those described for either pilocarpine or lithium/pilocarpine convulsions (McDonough et al., 1983; Pazdernik et al., 1985b; Samson et al., 1984). The pattern of enhanced glucose utilization detected in the course of pilocarpine, lithium/pilocarpine, or soman seizures overlaps the pattern of brain damage seen following sustained seizures induced by either drug (Clifford et al., 1987; Turski et al., 1983b,d). These experimental data justify an inference that ACh triggers seizure activity in susceptible brain regions.

Generation of convulsions, spread of seizures, and brain damage associated with epilepsy: acetylcholine versus glutamate

The similarity of cytopathology following pilocarpine, lithium/pilocarpine, and soman seizures to the tissue reaction to glutamate does not mean that the cholinergic system has excitotoxic potential (Clifford et al., 1987; Samson et al., 1985). It is rather believed that the cholinergic system may play a role in triggering and maintaining seizure activity (Olney, 1986). An excessive synaptic release of glutamate leads rapidly to depolarization block and breakdown of membranes (Bradford and Peterson, 1987; Olney et al., 1986). A synaptic release of ACh provides a sustained drive that keeps neurons firing (as in status epilepticus) without breaking down the membrane (Olney et al., 1986). Such a hypothesis sees the function of the cholinergic system in the maintenance of sustained seizure activity that drives excitatory mechanisms responsible for neuronal damage (e.g., glutamate/aspartate) (Olney, 1983; Olney et al., 1986). This hypothesis infers that anticholinergic drugs

may be active in prevention of sustained seizures but may not be useful for seizure arrest. Thus the lack of anticonvulsant activity of anticholinergic agents in chemical models of seizures is not necessarily a drawback for postulating a key role for ACh in epileptogenesis. Experimental support for this hypothesis comes from kindling studies in rodents. Atropine and scopolamine retard the development of kindling in rats undergoing electrical stimulation of the amygdala in a dose-related manner (Arnold et al., 1973; Albright et al., 1979; Cain et al., 1987, 1988; Corcoran, 1986; Westerberg and Corcoran, 1987), but they do not block established kindled seizures (Corcoran et al., 1974; Westerberg and Corcoran, 1987). Atropine also inhibits the development of chemical kindling of amygdala in rats with carbachol, but does not prevent established seizures in carbachol-kindled animals (Wasterlain and Jonec, 1983). Consistent with this finding is the observation that subconvulsant doses of pilocarpine facilitate the development of status epilepticus in amygdala-kindled rats in response to suprathreshold stimulation (Buterbaugh et al., 1986). Therefore an inference emerges that the cholinergic system functions to establish neuronal networks necessary for spatial spread of seizures required for either amygdala kindling or status epilepticus to develop (Buterbaugh et al., 1986; Corcoran, 1986). This mechanism may play a role in the overall process of seizure generation and may be important in clinical epilepsy.

McGeer and McGeer (1986) postulate that overstimulation of cholinergic pathways may itself result in the destruction of cholinceptive neurons that receive such projections. This hypothesis is based on: 1) the protective action of scopolamine upon the epileptic activity and distant damage seen following placement of excitotoxins KA or folic acid in the substantia innominata and rostral pontine tegmentum (McGeer et al., 1983a,b, 1984); 2) the distribution of the distant damage in the field of cholinergic projection arising from injected areas (McGeer and McGeer, 1986); and 3) decreases in the number of cholinergic receptors in regions undergoing neuropathological tissue reaction (Wong et al., 1983). These findings imply that cholinergic pathways may be of importance in mediating epileptic activity and epilepsy-related brain damage. The postulate, however, that ACh released from excessively firing presynaptic terminals may itself be neurotoxic or may reach concentrations that are neurotoxic seems less likely since Sloviter and Dempster (1985) showed that perfusion of the hippocampus with high doses of ACh does not produce a neurotoxic effect. In contrast, glutamate and aspartate reproduce in hippocampus all aspects of epilepsy-related damage seen in the brains of human epileptics and in animals subjected to experimental seizures (Sloviter and Dempster, 1985). This is in line with the view that ACh induces seizures without producing direct neurotoxicity (Olney et al., 1986).

DEVELOPING RAT BRAIN AND SUSCEPTIBILITY TO SEIZURES INDUCED BY PILOCARPINE

The mechanisms of epileptogenesis and seizure spread throughout the developing central nervous system are beginning to be explored. The several forms of epilepsy in infants and young children are clinically less varied than in adults (Aicardi, 1986). Statistical analysis of seizure onset in childhood epilepsy challenges the hy-

pothesis that the mean age for the first complex partial seizure varies from 3 to 7 years (Ounsted et al., 1966). Clinical experience indicates that although the probability of complex partial seizures is higher in children after the age of 3 years (Aicardi, 1985; Gastaut and Broughton, 1972), this form of epilepsy is also seen in younger children (less than 3 years of age), and the clinical expression of such seizures in infants does not seem to be different from that seen in older children (Watanabe et al., 1987). Febrile convulsions occur in children only after the age of 5–6 months and are common up to 4–5 years of age; thereafter the probability decreases with age (Addy, 1985; Aicardi, 1986).

Experimental studies in rodents also report on age-dependency in susceptibility to chemically and electrically induced convulsions. Newborn rats are less susceptible to KA (Albala et al., 1984) and tungstic acid (Yamauchi et al., 1976) and are less resistant to electrical stimulation of the amygdala (Gilbert and Cain, 1982). In addition, rats at the age of 3–12 days are also more resistant to the convulsant action of pilocarpine in comparison with older animals (Fig. 5). Interestingly, an enhanced susceptibility to pilocarpine has been detected between the second and fourth week of life (Cavalheiro et al., 1987). This observation parallels developmental studies with KA in rats, in which pups 15–18 days old showed the lowest threshold for seizures, while in older pups (33–37 days of age) the susceptibility to seizures was reduced (Albala et al., 1984).

Another crucial issue relates to the development of seizure-related cell death in the immature epileptic brain. Clinical experience has left much uncertainty as to whether complex partial seizures in children lead to brain pathology (Aicardi, 1986). Morphological analysis of the forebrain in 1–18-day-old rats subjected to systemic injection of KA shows no epilepsy-related brain damage, although 14–18-day-old animals develop severe seizures (Albala et al., 1984). The development of epilepsy-related cell death in the brains of 14–28-day-old rats subjected to convulsant doses of pilocarpine also does not correlate with the appearance of seizures or status epilepticus. Furthermore, the rats, at this age, that underwent status epilepticus following pilocarpine and developed seizure-related damage in the brain show differences in the extent and topography of morphological alterations relative to those known from neuropathological analysis of brains from mature rats (Turski et al., 1983b,d; Cavalheiro et al., 1987). The substantia nigra and lateral thalamic nucleus show no morphological changes following status epilepticus in developing rats (Cavalheiro et al., 1987). The adult pattern of damage to the brain is seen first in 5–6-week-old rats. Meldrum and his associates (Patel et al., 1987a) investigated susceptibility of adult and senescent rats to the convulsant action of pilocarpine. They observed a similar response to pilocarpine in 1–3-month-old rats, while in 4–7-month-old rats significant decrease of the seizure threshold was detected (Patel et al., 1987a). The apparent age dependency in the susceptibility of rats to pilocarpine seizures provides further evidence for a distinction in the sensitivity of the developing, mature, and senescent brain to convulsions.

SEIZURES PRODUCED BY Pilocarpine: EFFECT OF ANTIEPILEPTIC DRUGS

Modern research into the mechanisms of human diseases bases on animal models (Dichter and Ayala, 1987).

The experimental models that reproduce several components of human epilepsy are used to study mechanisms underlying seizures and are utilized for screening potential anticonvulsant drugs (Porter and Nadi, 1987; Purpura et al., 1972). The epilepsies are highly heterogeneous disorders with transient and recurrent disruptions of normal brain function extending throughout the human life. This implies that neither acute nor chronic models of seizures simulate human epilepsy (Fariello and Golden, 1985). Although the *in vivo* and *in vitro* models of epileptogenesis have significantly contributed to understanding major phenomena underlying epilepsy and to the creation of current management of seizures, it is important to recognize the inherent limits of those experimental approaches.

The validity of an experimental model of epilepsy is usually challenged by the profile of action of antiepileptic drugs (Cavalheiro and Lima, 1984; Meldrum, 1986). Pilocarpine induces in rats limbic seizures that become secondarily generalized and evolve into status epilepticus (Turski et al., 1983d). The seizures produced by pilocarpine in rats are prevented by systemically given diazepam, clonazepam, phenobarbital, valproate, and trimethadione, while diphenylhydantoin and carbamazepine are ineffective (Table II). Surprisingly, ethosuximide and acetazolamide lower the threshold for seizures produced by the drug (Table II). Similar profiles of anticonvulsant action are seen in mice treated with pilocarpine (Table II). Seizures induced by pilocarpine are also prevented by potential anticonvulsant drugs, 2-chloroadenosine and baclofen, and the nonsteroidal antiinflammatory drug, mefenamic acid (Table II) (Ikonomidou-Turski et al., 1988; Turski et al., 1985c, 1987b). This profile of action of antiepileptic drugs does not correlate with their action in other animal models of seizures (Bruni and Albright, 1984; Meldrum, 1986; Purpura et al., 1972; Turski et al., 1987d).

Diphenylhydantoin and carbamazepine, drugs of choice for the treatment of complex partial seizures in humans, are definitively ineffective after systemic administration against convulsions induced by pilocarpine (Turski et al., 1987d). Thus a protection against seizures elicited by pilocarpine in rodents is not an invariant prediction of anticonvulsant activity in human forms of epilepsy (Turski et al., 1987d). The severity of seizures and refractoriness to anticonvulsant treatment is also observed in lithium-treated rats subjected to systemic pilocarpine (Morrisett et al., 1987a). Diazepam, phenobarbital, and valproate are effective against seizures induced by pilocarpine in lithium-treated rats. Diphenylhydantoin and carbamazepine in extremely high doses (200 mg/kg and 100 mg/kg, respectively) have been found to prevent seizures produced by pilocarpine in rats subjected to lithium (Morrisett et al., 1987a). Neither treatment was found effective against pilocarpine-induced seizures in lithium-pretreated rats when introduced 1 h after administration of pilocarpine, during the course of status epilepticus (Morrisett et al., 1987a). The seizures induced by soman in rats are also resistant to anticonvulsant therapy (Lundy et al., 1978). Diazepam, clonazepam, phenobarbital, and valproate prevent the sequelae of sustained convulsions produced by soman in rats (Lipp, 1972; Lundy et al., 1978; Lundy and Shaw, 1983; Martin et al., 1985).

The seizures monitored after the systemic injection of pilocarpine reveal at most only drug-induced evolution

of intractable convulsions. These seizures may be related to the rapid evolution of hippocampal or amygdaloid kindling (Turski et al., 1987d). In fact, the efficacy of anticonvulsant drugs in preventing the development of amygdala kindling (Leviel and Naquet, 1977; Wada, 1977; Albertson et al., 1980; Albright and Burnham, 1980; Campos et al., 1980) resembles the antiepileptic potential observed against seizures produced by pilocarpine (Turski et al., 1987d). Benzodiazepines, diazepam, and clonazepam (Campos et al., 1980; Wise and Chinermann, 1974), phenobarbital (Wise and Chinermann, 1974), valproate (Leviel and Naquet, 1977), and trimethadione (Kretschmar and Teschendorf, 1985) retard the development of amygdala kindling in rats, while diphenylhydantoin (Racine et al., 1975) and carbamazepine (Post et al., 1986) afford no such activity.

The spontaneously recurrent seizures are registered during the long-term period following the systemic administration of pilocarpine in rats (Turski et al., 1983c). The efficacy of antiepileptic drugs against these seizures has not yet been established. Studies with antiepileptic drugs on spontaneous seizures monitored in the long-term after systemic pilocarpine may provide the necessary information for assessing the usefulness of the pilocarpine model in selecting antiepileptic drugs, and add to ongoing attempts to design better experimental approaches satisfactorily modeling the human condition.

NEUROCHEMICAL CONSEQUENCES OF SEIZURES INDUCED BY PILOCARPINE

Studies of neurotransmitter function in the course of seizures induced by pilocarpine in lithium-treated rats have resulted in demonstrating a decrease of the concentration of ACh in hippocampus, cortex, and striatum immediately prior to initiation of convulsions (Jope et al., 1987). This initial drop in the concentration of ACh was followed by an unprecedented rise in the concentration of ACh in the cortex and hippocampus but not in the striatum (Jope et al., 1987). The concentration of ACh in the cortex and hippocampus increased over time, concurrent with the progress of status epilepticus (Jope et al., 1987). The peak concentrations in the cortex and hippocampus reached 461% and 304% of control values, respectively, after 2 h of continuous seizures (Jope et al., 1987) and represent the highest concentrations of ACh measured in the brain after any treatment (Jope et al., 1987). This peak elevation of ACh in the cortex and hippocampus was followed by a decline monitored after the third hour of status epilepticus (Jope et al., 1987). In the striatum the concentration of ACh remained depressed during the first hour of status epilepticus and then normalized but did not rise significantly above control concentrations (up to 115% after 2 h of status epilepticus) (Jope et al., 1987). The choline concentrations also increased during status epilepticus and reached peak concentration in the cortex, hippocampus, and striatum after 2 h of sustained seizures (Jope et al., 1987). A steady increase over time in the choline concentration was observed in both the cortex and hippocampus, whereas the increase in striatum was much less consistent (Jope et al., 1987). Although *in vivo* cortical release of ACh was increased during initiation of seizures by pilocarpine and reached a plateau of about

160% of control values during status epilepticus, this was well below the level of synthesis (Joep et al., 1987). The data on accumulation and release of ACh show that although release of endogenous ACh was increased during generation of seizures and in the course of status epilepticus induced by lithium/pilocarpine treatment, the degree of increase in the *in vivo* concentration of ACh was considerably higher (Joep et al., 1987). The analysis of localization of ACh accumulated during status epilepticus induced by lithium/pilocarpine treatment showed that in the hippocampus cytoplasmic ACh increased while vesicular ACh remained unchanged (Joep et al., 1987). The cortical ACh was elevated in both subcellular fractions (Joep et al., 1987). The accumulated ACh was in a releasable compartment, since high K^+ concentration or ouabain increased the release of ACh from cortical slices taken from rats undergoing status epilepticus to a greater extent than from naive rats (Joep et al., 1987). The measurement of acetylcholinesterase activity in homogenates prepared from hippocampus and cortex of lithium/pilocarpine-treated rats after 2 h of status epilepticus did not reveal differences when compared with control animals (Joep et al., 1987). The high-affinity choline transport in synaptosomes from hippocampus, cortex, and striatum was unchanged in rats undergoing status epilepticus after lithium/pilocarpine treatment for 35 min as is $^{45}Ca^{2+}$ uptake into synaptosomes (Joep et al., 1986; Joep and Morrisett, 1986). The concentration of cGMP was markedly enhanced in the hippocampus, cortex, striatum, and cerebellum in the course of seizures induced by pilocarpine in lithium-treated rats (Joep and Morrisett, 1986).

A very similar profile of increases in the concentration of ACh in the cortex and hippocampus was shown in the course of seizures induced in rats by soman (Shih, 1982). In the cortex, maximal elevation was reached at 2 h (320% of control), and then slowly faded away and returned to normal after 8 h (Shih, 1982). In the hippocampus the concentration of ACh reached a plateau after 40 min (100% of control) and remained elevated for 16 h (Shih, 1982). The choline concentrations paralleled increases in the ACh concentration in both cortex and hippocampus; however, the duration of such changes was shorter (Shih, 1982). Transient elevations (30–60% of control) of ACh concentration following soman treatment were also observed in the cerebellum, midbrain, and brainstem (Shih, 1982). The striatum showed changes neither in the concentration of ACh nor in that of choline in the course of convulsions induced by soman (Shih, 1982). The concentration of cGMP was highly elevated in the cerebellum and striatum in rats (Lundy and Shaw, 1983), and in the cerebellum and cortex in mice undergoing soman seizures (Coult et al., 1979).

The analysis of changes in the concentration of ACh in the course of lithium/pilocarpine and soman seizures as well as analysis of ACh release during such seizures prompted the suggestion that the regulation of the synthesis of ACh during status epilepticus was broken (Joep et al., 1987). Status epilepticus apparently activated mechanisms overdriving the synthesis of ACh irrespective of its massive accumulation (Joep et al., 1987).

The γ -aminobutyrate (GABA) hypothesis of epilepsy was founded by Killam and Bain in 1957 and is one of the basic tenets of modern epileptology. This theory arose from observations accumulating over the previous three decades that: 1) drugs that impair GABAergic

transmission induce overt convulsions, while those that enhance GABAergic transmission are anticonvulsant (Meldrum, 1984; Ticku and Rastogi, 1986); 2) that impairment of GABAergic transmission contributes to experimental seizures (Löscher, 1986); 3) that GABAergic transmission in discrete brain regions modulates the propagation of seizures (Gale, 1985); and 4) that GABAergic transmission is impaired in human epilepsy (Lloyd et al., 1986). This theory serves currently to explain the mechanisms of generation of seizures and mechanisms of anticonvulsant action of several drugs (Meldrum, 1986). According to this theory, deficits in GABA-mediated inhibition are necessary for the emergence of seizures (Meldrum, 1984). Thus epilepsy may be attributed to deficits in GABA-mediated inhibition (Meldrum, 1975, 1982). In epileptic foci in human brain the activity of L-glutamate decarboxylase (GAD), the GABA-synthesizing enzyme, may be decreased or remain unchanged (Lloyd et al., 1986; Sherwin et al., 1984). Ribak and associates have shown that GAD-positive terminals disappear in epileptic foci in monkeys and rodents (Ribak, 1985; Ribak et al., 1982).

Early investigations on the role of GABA-mediated inhibition in the initiation of cholinergically mediated convulsions showed no alterations in the GABA concentration, GAD, or GABA-transaminase activity in the cerebellum, cortex, pons, and midbrain at the onset of soman-induced seizures in rats (Lundy et al., 1978). In the course of convulsions caused by the acetylcholinesterase inhibitor paraoxon, GABA concentration in the brain was decreased (Kar and Matin, 1972).

The assessment of the GAD activity in the brains of rats subjected to pilocarpine-induced seizures reveals deficits in regions undergoing morphological insult (Fig. 6). The substantia nigra was found to be the most af-

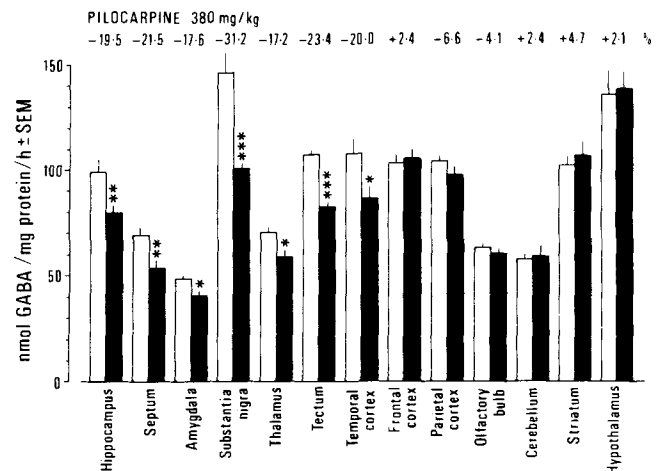


Fig. 6. Regional profile of the activity of L-glutamate decarboxylase (GAD) in the brains of rats subjected to convulsant action of pilocarpine, 380 mg/kg, determined 5–7 days after seizures. Open bars: saline-treated control rats. Filled bars: rats treated with pilocarpine, 380 mg/kg. The activity of GAD is expressed in nmol GABA/mg protein/h. The data are means $\pm$ SEM of 10–12 determinations. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$ vs. saline-treated rats (Student's *t* test). Methods: For the determination of GAD activity, the rats were decapitated, and their brains were rapidly removed and dissected. GAD was determined spectrophotometrically by the method of Lowe et al. (1958), as described by Turski et al. (1986b).

fects brain region, with GAD activity deficits approximating 30% 5–7 days after the injection of pilocarpine (Fig. 6). The tectum, septum, temporal cortex, hippocampus, amygdala/pyriform cortex, and thalamus also demonstrate marked (15–25%) deficits in GAD activity (Fig. 6) (Turski et al., 1986b). The topography of deficits in GAD activity matches well the distribution of the brain damage following seizures elicited by pilocarpine. This observation justifies an inference that profound dysfunction in GABA-mediated inhibition follows status epilepticus generated by pilocarpine.

The data presented show that the knowledge of neurochemical sequelae of pilocarpine-induced seizures is still scanty, and further studies of biochemical consequences of pilocarpine-induced seizures are urgently required.

FUNCTION OF THE BASAL GANGLIA IN SEIZURES INDUCED BY PILOCARPINE

Substantia nigra and entopeduncular nucleus

Almost three decades ago pioneering work of Hayashi (1952, 1953) indicated the function of basal ganglia in the control of seizure spread in primates. This important research has been largely ignored or misinterpreted within the following three decades and failed to stimulate further attempts toward precise identification of regions in the brain and pathways that curtail seizures. New interest in the function of basal ganglia in the spread and termination of seizures in the forebrain has been generated by the work of Gale and her associates, who discovered anticonvulsant action of drugs enhancing GABA-mediated inhibition in the substantia nigra (Iadarola and Gale, 1982). The most suggestive indication for engagement of the substantia nigra in the propagation of seizures was provided by microinjection studies showing an anticonvulsant action of muscimol, a GABA_A agonist, and γ -vinyl-GABA, an irreversible inhibitor of GABA transaminase, against electroshock- and pentylenetetrazol-induced seizures in rats (Iadarola and Gale, 1982). This finding was followed by a stream of literature showing similar anticonvulsant action of drugs increasing GABAergic transmission in the substantia nigra pars reticulata (SNR) against amygdala kindled, ethanol-withdrawal, fluorothyl-induced, and audiogenic seizures (Frye et al., 1983; Gonzales and Hettinger, 1984; Le Gal La Salle et al., 1983; Mc Namara et al., 1984; Moshe and Albala, 1984).

The seizures induced by pilocarpine have been also used to delineate the anatomical substrates essential for the generation of convulsions (DeSarro et al., 1986; Turski et al., 1986a,b,c). The threshold for seizures produced by pilocarpine is regulated by the degree of inhibition in the substantia nigra (Turski et al., 1986a,b). Focal microinjections into the substantia nigra of γ -vinyl-GABA increase, while microinjections of isoniazid, an inhibitor of activity of the GABA-synthesizing enzyme GAD, lower the threshold for seizures produced by pilocarpine (Turski et al., 1986a). This study was the first to show bidirectional changes in the seizure threshold following manipulation of the degree of GABA-mediated inhibition in the substantia nigra (Turski et al., 1986a). This work showed that anticonvulsant activity obtained from the substantia nigra is not limited to the control of motor expression of seizures but extends to the modulation of the threshold for seizures in the limbic system

(Turski et al., 1986a). Microinjections of γ -vinyl-GABA into the substantia nigra block the motor expression of limbic seizures and suppress electroencephalographic sequelae of seizures in the hippocampus and cortex (Turski et al., 1986a). Furthermore, the transplantation of GABAergic neurons from fetal striatal eminence into the substantia nigra reverses increased susceptibility to pilocarpine seizures in rats subjected to lesions of striatonigral pathway with ibotenate (Meldrum et al., 1988b). A discovery of the anticonvulsant action of the excitatory amino acid antagonists 2-amino-7-phosphonoheptanoate (AP7), an N-methyl-D-aspartate (NMDA) antagonist, and γ -D-glutamylaminomethylsulphonate (γ -D-GAMS), a preferential KA/quisqualate antagonist, when placed in the substantia nigra, against electroshock-induced seizures in rats has turned attention to the function of excitatory neurotransmission in the substantia nigra in the control of seizure spread (DeSarro et al., 1985). AP7 raises the threshold for seizures elicited by pilocarpine, while NMDA increases the susceptibility to pilocarpine-induced seizures, when placed in the substantia nigra (Turski et al., 1986c). The modulatory action of both AP7 and NMDA in the substantia nigra is topographically confined to the SNR (Turski et al., 1986c). This observation provided the first evidence for the SNR as a key site at which the anticonvulsant activity of drugs that impair excitatory neurotransmission is expressed (Turski et al., 1986c). γ -D-GAMS has also been identified as anticonvulsant in the substantia nigra against seizures induced by pilocarpine, whereas KA was found to lower the threshold for these seizures (De Sarro et al., 1986; Meldrum et al., 1988a). These investigators demonstrated, however, no anatomical dissociation between both parts of the substantia nigra, although they found that γ -D-GAMS and KA are less active in the SNC relative to SNR (DeSarro et al., 1986). The subsequent microinjection studies with the use of electroshock-induced seizures showed that opiates and substance P may be likewise involved in the regulation of the seizure threshold in the substantia nigra (Gale, 1985). Microinjections of high doses of morphine were found to increase the threshold for electrically induced seizures in rats (Garant and Gale, 1985). This action was naloxone-specific and could be reversed by this drug either following systemic administration or focal coinjection into the substantia nigra (Garant and Gale, 1985). Morphine administered systemically also protected rats against maximal electroshock (Foote and Gale, 1983). These observations prompted Gale and her associates to suggest that activation of opiate μ receptors in the substantia nigra has anticonvulsant potential (Gale, 1985). Microinjections of morphine, a preferential μ opiate agonist, into the substantia nigra lower the threshold for seizures elicited by pilocarpine, while naloxone, an opiate antagonist, displays anticonvulsant efficacy (Turski et al., 1986e). The action of morphine in the substantia nigra is topographically restricted to the SNR (Turski et al., 1986e). Other work has shown that systemic injection of morphine increases the susceptibility of rats to convulsions induced by pilocarpine (Turski et al., 1985a). The finding of the facilitatory action of morphine and weak anticonvulsant effect of naloxone in pilocarpine seizures parallels observations in KA-induced convulsions. Systemic administration of morphine enhances, while application of naloxone moderately reduces, convulsions and brain damage caused by KA seizures in rats (Fuller and Olney, 1979; Fuller et al., 1982). These

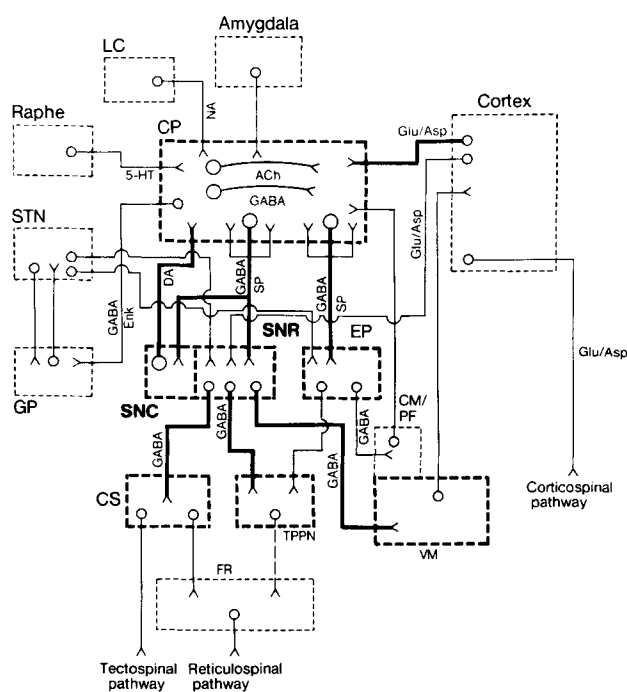


Fig. 7. The efferent and afferent pathways in the basal ganglia of the rat. ACh, acetylcholine; Asp, L-aspartate; CM/PF, centromedian and parafascicular nucleus thalami; CP, caudate putamen; CS, colliculus superior; DA, dopamine; Enk, enkephalin; EP, entopeduncular nucleus; FR, reticular formation; GABA, γ -aminobutyrate; Glu, L-glutamate; GP, globus pallidus; 5-HT, serotonin; LC, locus coeruleus; STN, subthalamic nucleus; NA, noradrenaline; SNC, substantia nigra pars compacta; SNR, substantia nigra pars reticulata; SP, substance P; TPN, tegmental pedunculopontine nucleus; VM, ventromedial thalamic nucleus.

apparently contrasting results indicate that different neuronal circuitries in the substantia nigra or elsewhere in the brain are involved in the propagation of either electroshock or limbic seizures (Clifford et al., 1987; Miller et al., 1987).

Chemical lesion of the substantia nigra with ibotenic acid was found to retard the development of pilocarpine-induced convulsions (Turski, 1987). This observation parallels those made with chemical or electrical lesions of substantia nigra in electroshock- and bicuculline-induced, and amygdala-kindled seizures (Gale, 1985; Iadarola and Gale, 1982), and contrasts with that made with electrolytic lesion of the nigra in audiogenic seizures (Garcia-Cairasco and Sabbatini, 1983).

These data establish that the substantia nigra serves as a relay station in the propagation of seizures and indicate that different neurotransmitters mediate anticonvulsant action in the substantia nigra. Furthermore, the antiepileptic drugs clearly affect the activity of neurons in the substantia nigra in rodents (Mereu and Biggio, 1983; Ross et al., 1982; Waszczak et al., 1986): benzodiazepines, barbiturates, valproate, and trimethadione depress the spontaneous activity of substantia nigra cells (Ross et al., 1982; Waszczak et al., 1986). These drugs are also active against seizures produced by pilocarpine (Table II) (Turski et al., 1987d). Midazolam and phenobarbital also prevent pilocarpine-induced seizures when focally microinjected into the rat substantia nigra (Turski et al., 1987a). It is not known whether this parallelism possesses physiological validity, but this observation offers an attractive possibility for suggesting that the substantia nigra is one of the important sites where

the antiepileptic action of drugs against pilocarpine seizures is expressed. The potent protective action of baclofen (Table II) against seizures induced by pilocarpine lends further support to the hypothesis of parallelism between anticonvulsant activity and depressant effect on the substantia nigra unit activity (Turski et al., 1987d; Waszczak et al., 1986), since it has been previously established that baclofen decreases activity of the substantia nigra neurons in rats following systemic application (Mereu and Biggio, 1983). These findings are further extended by the action of ethosuximide on seizures induced by pilocarpine. This anticonvulsant drug, clinically relevant in the treatment of absence epilepsy in humans, increases susceptibility of rats to the convulsant action of pilocarpine following systemic and intranigral microinjections (Turski et al., 1987a,d). Electrophysiological monitoring of the SNR cell activity in rats subjected to the systemic action of ethosuximide shows that this drug increases spontaneous activity of nigral cells (Waszczak et al., 1986). The anticonvulsant drugs that do not affect neuronal activity of the SNR, e.g., diphenylhydantoin and carbamazepine (Waszczak et al., 1986), do not affect convulsions produced by pilocarpine (Turski et al., 1987d).

Seizures produced by pilocarpine may also be attenuated by microapplication of the GABA agonist muscimol into the entopeduncular nucleus (EP) (Hosford and McNamara, 1988; Patel, 1988; Patel et al., 1986). This observation suggests that different output nuclei of the basal ganglia may govern the generation and spread of seizures in the forebrain (Patel et al., 1986; Turski et al., 1986a,c), indicating that the substantia nigra is not an exclusive site in the brain where antiepileptic drugs may act to govern spread of seizures (Millan et al., 1986; Patel et al., 1986, 1988).

Caudate putamen

The caudate putamen represents the largest receiving area of the basal ganglia (Fig. 7). This region transforms motor information coming from the cortex and conveys it to output nuclei, e.g., the substantia nigra, the EP, and the globus pallidus (DeLong and Georgopoulos, 1981). GABA is believed to serve as a major neurotransmitter in the striatal output pathways directed to the SNR and EP (Ottersen and Storm-Mathisen, 1984). An excitatory amino acid, glutamate or aspartate, is believed to subserve neurotransmitter function in the corticostriatal pathway (Fonnum, 1984). The dopaminergic nigrostriatal pathway complements the gross topographical organization of striatal pathways (McGeer et al., 1984).

Microinjection studies show that nmol quantities (ED_{50} 0.7 nmol (0.5–1.0)) of an excitatory amino acid, NMDA, applied into the caudate putamen block behavioral, electroencephalographic, and morphological sequelae of seizures induced by pilocarpine (Turski et al., 1987c). The anticonvulsant effect of NMDA in the caudate putamen is topographically confined to ventral and caudal parts of the region (Turski et al., 1987c).

Apomorphine, a dopamine (DA) agonist, blocks seizures induced by pilocarpine when given into the caudate putamen in pmol amounts (ED_{50} 1.6 pmol (1.1–2.2)) (Turski et al., 1988a). This effect is topographically restricted to the rostral part of the caudate putamen, the nucleus accumbens, and the olfactory tubercle (Turski et al., 1988a). A possible physiological validity of this observation finds support in the finding of an anticon-

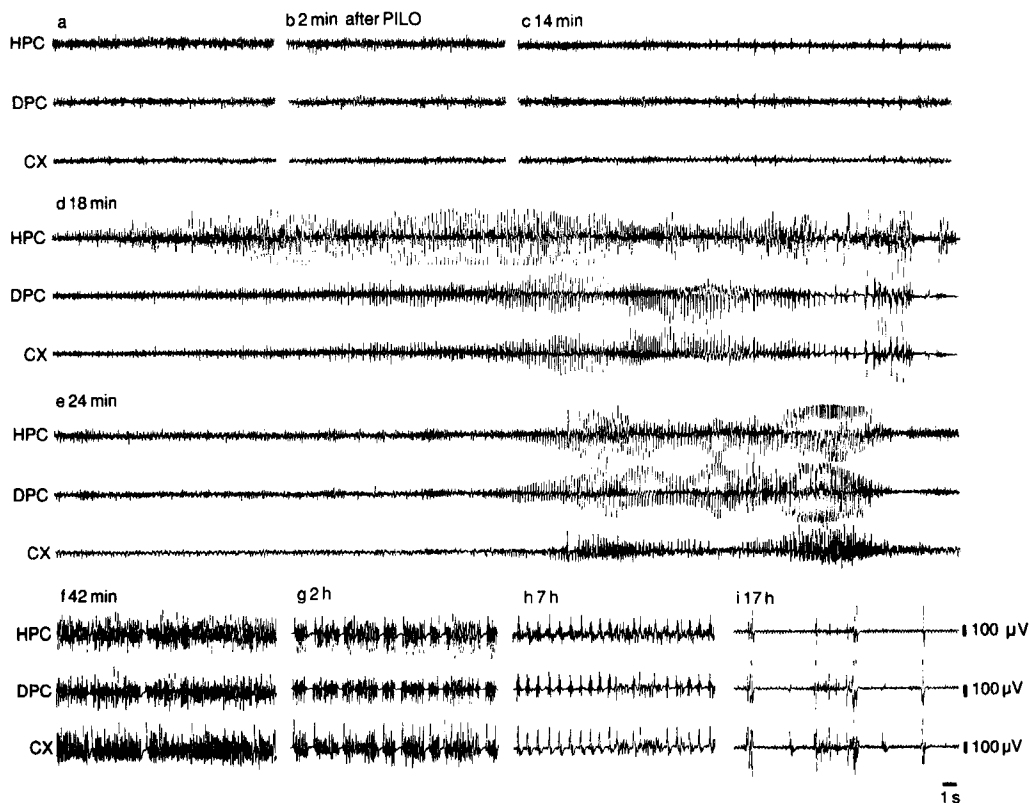


Fig. 8. Electrographic recordings from the hippocampus, deep prepiriform cortex, and cortex demonstrating the sequential development of seizure activity induced by pilocarpine (PILO) in rats. PILO, 380 mg/kg, was administered i.p. in rats pretreated with N-methylscopolamine, 1 mg/kg s.c. a: Predrug control recordings. b: Theta rhythm and low-voltage fast activity dominate recordings 2 min after PILO. c: High-voltage spiking is initially registered in the HPC and then becomes synchronized in all three recordings. d: The first electrographic seizure 18 min after the injection of PILO. The waxing of the seizure

is faster in the hippocampus. e: Highly synchronized evolution of a seizure registered 24 min after PILO. f, g: Electrographic activity recorded 42 min and 2 h after administration of PILO during status epilepticus. h: The paroxysmal activity slowly abates during the next 7–12 h. i: Isolated spikes and bursts of polyspikes with severely depressed background activity characterize the EEG 14–24 h following the administration of PILO. HPC, hippocampus; DPC, deep prepiriform cortex; CX, cortex.

vulsant effect of NMDA when placed in the SNC (Turski et al., 1988b). Bilateral microinjections of NMDA into the SNC protect against limbic seizures induced by pilocarpine with an ED_{50} of 105 pmol (80–138) (Turski et al., 1988b). The anticonvulsant effect of NMDA in the SNC is reversed by pretreatment with haloperidol, a DA antagonist, in the rostral caudate putamen (Turski et al., 1988b). The DA D_2 receptors may be involved in the transmission of anticonvulsant action in the striatum, since selective D_2 agonist LY-171555 [trans-(–)-4aR-4,4a,5,6,7,8,8a,9-octahydro-5-propyl- ^1H (or ^2H)-pyrazolo-3,4-g-quinoline monohydrochloride], and not a preferential D_1 agonist SKF-38393 [RS-1-phenyl-2,3,4,5-tetrahydro-(1H)-3-benzazepine-7,8-diol hydrochloride] displays anticonvulsant activity in the striatum (Turski et al., 1988a). Microinjection studies with DA agonists show that SKF-38393 (D_1 agonist) decreases the threshold for seizures induced by pilocarpine following administration in the substantia nigra, while LY-171555 has no effect on susceptibility to pilocarpine in this brain region (Turski et al., 1988a). Systemic administration of SKF-38393 was also found to trigger seizures in response to subconvulsant doses of pilocarpine (Table II).

A GABA_A antagonist, bicuculline methiodide, has a higher anticonvulsant potential than NMDA and apo-

morphine when given into the caudate putamen (Turski et al., 1986d). This drug prevents sequelae of seizures induced by pilocarpine with an ED_{50} of 94 fmol (45–195) (Turski et al., 1986d). Furthermore, an anticonvulsant effect of bicuculline is found throughout the entire caudate putamen and may be blocked by muscimol (Turski et al., 1986d).

The cholinergic neurotransmission has an important motor function in the rat striatum (McGeer et al., 1984). Microinjection studies show that application of scopolamine into the striatum also suppresses seizures induced by pilocarpine with an ED_{50} of 1 nmol (0.7–1.5) (Turski et al., 1987b).

A controversy surrounds the involvement of pathways and neurotransmitters in funneling the basal ganglia functions in the control of seizure spread. Involvement of the nigroreticular pathway is suggested by an anticonvulsant effect of precollicular transection in rats subjected to the convulsant action of bicuculline (Garant and Gale, 1987). There is no unequivocal evidence for the role of the tectum in the spread of seizures induced by pilocarpine (Turski et al., 1986b). The microinjection studies provide some evidence that pathways linking basal ganglia and reticular formation may regulate the threshold for seizures induced by pilocarpine (Patel et

al., 1988). Microinjections of AP7 and muscimol into the pedunclopontine nucleus provide suppression of pilocarpine-induced seizures (Patel et al., 1988). The evidence on the involvement of the thalamus in the spread of pilocarpine-induced seizures is provided by the observation that placement of AP7 in the mediodorsal nuclei blocked convulsant action of pilocarpine (Patel et al., 1988). Another study demonstrates that decreases of excitatory neurotransmission in the lateral habenula following microinjections of pmol quantities of AP7 suppress seizures induced by pilocarpine (Patel et al., 1988). This evidence argues for the role of pathways linking the basal ganglia output nuclei, e.g., SNR and EP with the brainstem in the propagation of seizures.

These observations lend support to the hypothesis that the basal ganglia and their output stations are engaged in the complex process of seizure propagation. The interplay between neurotransmitters in the basal ganglia and between pathways linking the basal ganglia, cortex, tectum, thalamus, and reticular formation detected by means of microinjection studies provide evidence: 1) that excitatory neurotransmission mediated via the NMDA subtype of glutamate receptors in the SNC and the striatum act to prevent emergence of seizures; 2) that dopaminergic neurotransmission in the striatum appears to have anticonvulsant potential that is mediated via D₂ subtype of receptors; 3) that a decrease in the GABA-mediated inhibition and cholinergically mediated excitation in the striatum elevates the threshold for seizures; 4) that striatonigral and striatoentopeduncular pathways modulate the threshold for seizures; 5) that excitatory neurotransmission mediated by NMDA and KA subtypes of glutamate receptors in the SNR and EP act to promote the seizures; 6) that a decrease in the GABA-mediated inhibition in the SNR and EP lowers the threshold for seizures; and 7) that pathways linking basal ganglia output nuclei with the tectum, the thalamus, and the reticular formation are essential for the expression of seizures.

THE DEEP PREPIRIFORM CORTEX AND PILOCARPINE-INDUCED SEIZURES

A topographically restricted region in the deep prepiriform cortex (DPC) in rats is suggested to be responsible for generation of seizures following systemic application of chemoconvulsants (Piredda and Gale, 1985). Microinjections of GABA agonist, muscimol, or γ -vinyl-GABA into the DPC suppress seizures induced by systemic application of bicuculline but are ineffective against maximal electroshock-induced seizures (Piredda et al., 1987). Seizures induced by pilocarpine are also suppressed by microinjections of muscimol into the DPC (Millan et al., 1986). The excitatory amino acid antagonists AP7 and γ -D-GAMS efficiently block pilocarpine-induced seizures following the application into the DPC (Meldrum, 1988a; Millan et al., 1986; Patel et al., 1988). The threshold for pilocarpine-induced seizures is lowered by microinjections of KA but not by placement of NMDA into the DPC (Millan et al., 1987). These observations show that both blockade of excitatory neurotransmission and activation of GABA-mediated inhibition in the DPC enhances the threshold for pilocarpine-induced seizures. Focal application of cholinomimetics, excitatory amino acids, or GABA antagonists into the DPC in rats leads to limbic seizures (Piredda

and Gale, 1985). The sensitivity of the DPC to convulsant action of cholinomimetics and GABA antagonists is higher relative to that of the amygdala and the hippocampus (Piredda and Gale, 1985; Turski et al., 1983d, 1985b). The high sensitivity of the DPC to cholinergic stimulation raises the question of whether DPC functions in the generation of pilocarpine-induced seizures following systemic administration of the drug. Electrographic monitoring of hippocampal, amygdaloid, cortical, and DPC activity following systemic injection of convulsant doses of pilocarpine shows that the DPC is activated with a delay in comparison with that observed from hippocampus and amygdala. The hippocampal recordings show the earliest paroxysmal alterations relative to the DPC and neocortex (Fig. 8). This finding may suggest that the DPC does not generate seizures following systemic administration of pilocarpine in rats, although this brain region definitely functions in the spread of pilocarpine-induced convulsions.

CONCLUSIONS

A defined characteristic of pilocarpine-induced convulsions offers a unique possibility for the recognition of brain regions, pathways, and neurotransmitters engaged in the spread of seizures and for testing potential antiepileptic drugs. Current progress in research on pilocarpine seizures warrants a belief that future research with this and other cholinergically mediated seizure models may help our quest to improve the life of epileptic patients.

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