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The basal ganglia, the deep prepyriform cortex, and seizure spread: Bicuculline is anticonvulsant in the rat striatum

(epilepsy/γ-aminobutyric acid/chemoconvulsant agents/seizure propagation)

LECHOSLAW TURSKI*†, ESPER A. CAVALHEIRO‡, LINEU S. CALDERAZZO-FILHO‡, ZUNER A. BORTOLOTTO‡, THOMAS KLOCKGETHER§, CHRYSANTHY IKONOMIDOU*, AND WALDEMAR A. TURSKI*

*Department of Pharmacology, Medical School, Jaczewskiego 8, PL-20-090 Lublin, Poland; ‡Department of Neurology and Neurosurgery, Laboratory of Experimental Neurology, R. Botucatu 862, CEP-04023 Sao Paulo, SP, Brazil; and §Department of Neurology, University of Tübingen, Liebermeisterstrasse 18-20, D-7400 Tübingen, Federal Republic of Germany

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ABSTRACT **The γ-aminobutyric acid antagonist, bicuculline methiodide (BMI), induces myoclonic seizures in rats when injected into the deep prepyriform cortex at concentrations lower than those that induce convulsions from the amygdala, hippocampus, or neocortex. This observation prompted the suggestion that the deep prepyriform cortex was responsible for seizure generation regardless of the neurotransmitter and neuronal circuits involved. Bilateral intrastriatal application of BMI protects rats against seizures induced by (*i*) local application of BMI into the deep prepyriform cortex and (*ii*) systemic application of bicuculline, pilocarpine (a cholinergic agonist), or kainic acid (a glutamate receptor agonist). The region of the striatum sensitive to the previously unknown anticonvulsant action of BMI is located in the immediate vicinity of the deep prepyriform cortex and is 100–150 times more sensitive to the *anticonvulsant* action relative to the sensitivity of the deep prepyriform cortex to the *convulsant* action of BMI. These data suggest a powerful γ-aminobutyric acid-dependent gating role of the basal ganglia in determining the seizure threshold in the forebrain. This argues against the suggestion that the deep prepyriform cortex plays a crucial role in the generation of seizures following systemic administration of convulsants. The discovery of an anticonvulsant action of BMI in the rat striatum contradicts the γ-aminobutyric acid theory of epilepsy, which implies that deficits in the γ-aminobutyric acid-mediated inhibition in the central nervous system lead to the emergence of seizures.**

The search for morphological substrates of seizures in the brain has been the major goal of epilepsy research over the last three decades. The function of the basal ganglia in the spread of seizures has received considerable attention since the discovery of anticonvulsant activity of drugs that enhance γ-aminobutyric acid (GABA)-mediated inhibition in the substantia nigra (1). The entopeduncular nucleus has been subsequently identified as another site where GABA agonists act to block seizures (2). The anatomical background for these actions was the finding that pathways linking the striatum and either substantia nigra or entopeduncular nucleus regulate the seizure threshold in the forebrain (3). Clinical studies in humans and lesion studies in primates and rodents have described a role for the striatum in determining seizure threshold (3–5). However, the actions and interactions of different striatal transmitters possibly involved in modulating seizure spread have not been identified. We recently discovered that microinjections of nanomole amounts of the excitatory amino acid *N*-methyl-D-aspartate into the striatum of rats suppressed amygdala kindling (6) and pilocarpine-induced seizures (3). The anticonvulsant action of *N*-methyl-D-aspartate in the striatum was reversed by blocking GABA-mediated inhibition in the substantia nigra pars reticulata or in the entopeduncular nucleus (3). This finding suggested that activation of striatal GABAergic efferent pathways may induce an anticonvulsant action.

GABA is reported to be localized in striatal interneurons or in collaterals of striatal output neurons (7). The GABAergic interneurons innervate GABA-containing efferent pathways (8) and regulate the activity of striatal output neurons (9). Thus, the inference emerges that a GABA/GABA balance in the striatum participates in the fine regulation of the seizure threshold in the forebrain.

The deep prepyriform cortex is believed to subserve a triggering function for different types of convulsions and may be crucial to the generation of convulsant-induced seizures (10). Therefore, we have compared the convulsant actions of bicuculline methiodide (BMI) following its application into the deep prepyriform cortex with its anticonvulsant actions after bilateral microinjections into the rat striatum.

MATERIALS AND METHODS

The subjects were male Wistar rats weighing 250–300 g. The rats were anesthetized i.p. with sodium pentobarbital (Nembutal; Ceva, Watford, U.K.; 50 mg/kg) and implanted with guide cannulae directed towards the deep prepyriform cortex or the striatum. The microinjections were made in the frontal plane at AP +4.0 (11), which corresponds to the anatomical definition of sites in the deep prepyriform cortex sensitive to the convulsant action of BMI (10). The microinjections into the deep prepyriform cortex and striatum were performed bilaterally in unanesthetized rats. No rats were used for more than one pair of injections. Drugs were delivered into the deep prepyriform cortex and striatum in a volume of 0.25 μl at a rate of 0.1 μl/min. BMI (Pierce) was administered into the deep prepyriform cortex in doses of 10, 20, 50, and 100 pmol and into the striatum in doses of 0.1, 0.2, 0.5, and 1 pmol. The microinjections of BMI in the striatum were followed 15 min later by i.p. injection of bicuculline (4 mg/kg). BMI in the dose of 1 pmol was also administered into the striatum 15 min before microinjection of BMI (100 pmol) into the deep prepyriform cortex, pilocarpine hydrochloride (Sigma; 380 mg/kg, i.p.) or kainic acid (Sigma; 12 mg/kg, i.p.). Methylscopolamine nitrate (Sigma; 1 mg/kg) was administered s.c. 30 min before injection of pilocarpine to limit peripheral toxic effects (3). The dose of BMI required to induce or block seizures in 50% of rats (ED_{50}) was determined

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Abbreviations: GABA, γ-aminobutyric acid; BMI, bicuculline methiodide.

†Present address: Department of Neuropsychopharmacology, Schering AG, Sellerstrasse 6-8, P.O. Box 650311, D-1000 Berlin 65, F.R.G.

in four experiments with different doses. The criterion used to indicate a convulsive response to (*i*) BMI was the myoclonic seizure defined by Piredda and Gale (10), which occurs within 60 min after microinjection into the deep prepyriform cortex, and (*ii*) bicuculline (4 mg/kg) was a clonic/tonic seizure within 30 min after i.p. injection. The incidence of seizure response (probit transformed percentages) was plotted versus the logarithm of the dose of BMI administered into the deep prepyriform cortex or striatum. The ED_{50} and the confidence limits were estimated by fitting the data by linear regression analysis.

For electroencephalographic recordings (Beckman model RM polygraph; time constant, 0.03 sec; high cut-off filter, 15 Hz) bipolar twisted electrodes (tip diameter, 100 μm; interelectrode distance, 500 μm) were positioned in the dorsal hippocampus (3). Surface recordings were made from screws positioned bilaterally over the occipital cortex. The correct location of the implanted deep electrodes was histologically verified in serial sections stained with cresyl violet.

For morphological examination of the brains by light microscopy 3–10 days after administration of pilocarpine or kainic acid, rats were anesthetized with an overdose of sodium pentobarbital and perfused with a fixative containing 10% acetic acid, 10% formaldehyde, and 80% methanol. The brains were allowed to fix *in situ* at 4°C for 24 hr and then were removed and processed for paraffin embedding. Subsequently, serial coronal sections of the whole brain were cut 10 μm thick, and every 10th section was mounted on a glass slide and stained with cresyl violet or by the Fink and Heimer technique (12).

RESULTS

Microinjections of BMI into the deep prepyriform cortex triggered myoclonic seizures from a topographically restricted area (AP +4.0) with an ED_{50} of 37 pmol (26–52) (n = 23). None of four rats injected with 10 pmol of BMI showed seizures (0/4), whereas one of six rats receiving 20 pmol had myoclonic convulsions (1/6). Two of six animals treated with 50 pmol of BMI developed convulsions (2/6), and all seven animals injected with 100 pmol exhibited seizures (7/7).

By contrast, the anticonvulsant potential of BMI in the striatum was addressed by means of seizures induced by systemic administration of bicuculline. Bicuculline (4 mg/kg) consistently induced clonic/tonic seizures and postseizure death. Microinjections of BMI into the striatum (AP +3.0–+4.0) (11) protected rats against seizures produced by bicuculline with an ED_{50} of 0.3 pmol (0.17–0.48) (n = 27). Microinjection of 0.1 pmol of BMI had no effect on bicuculline seizures in five rats (5/5). With 0.2 pmol, two of six rats (4/6) and with 0.5 pmol, seven of eight rats (1/8) were protected from seizures induced by bicuculline. One pmol of BMI blocked seizures and lethal toxicity induced by bicuculline in all eight rats tested (0/8). All rats receiving saline into the striatum and then bicuculline (4 mg/kg) died in the course of seizures within 10–20 min (5/5) (Table 1).

The 1-pmol dose of BMI suppressed both the behavioral and electrographic seizures induced by bicuculline (Fig. 1). This dose of BMI was therefore selected to challenge the convulsant action of BMI in the deep prepyriform cortex and that of pilocarpine and kainic acid following i.p. administration. Microinjection of 1 pmol of BMI into the striatum protected all eight rats tested from seizures produced by microinjection of 100 pmol of BMI into the deep prepyriform cortex 15 min later (0/8) (Table 1; Fig. 2). Electroencephalographic monitoring showed that microinjection of BMI into the striatum suppressed the generation of seizures in the cortex and hippocampus after BMI injection into the deep prepyriform cortex. Microinjection of saline into the striatum did not affect the convulsant action of BMI in the deep prepyriform cortex (5/5) (Table 1).

Table 1. Anticonvulsant effect of BMI (1 pmol) in the striatum against seizures elicited from deep prepyriform cortex by BMI (100 pmol) and against seizures induced by systemic administration of bicuculline, pilocarpine, and kainic acid

	Control	BMI (1 pmol; striatum)
BMI (100 pmol; DPC)		
Incidence of myoclonus	5/5	0/8
Seizure score	4	0*
Bicuculline (4 mg/kg)		
Incidence of myoclonus	5/5	0/8
Seizure score	5	0*
Pilocarpine (380 mg/kg)†		
Incidence of myoclonus	9/9	0/5
Seizure score	5	1*
Kainic acid (12 mg/kg)		
Incidence of myoclonus	5/5	0/5
Seizure score	4	1*

BMI (100 pmol) was infused into the deep prepyriform cortex over a period of 2.5 min. BMI (1 pmol) was microinjected into the striatum 15 min before infusion of BMI into the deep prepyriform cortex or systemic administration of convulsants. Rats were observed for 30 min–6 hr after the injection of convulsants, and the score of seizures was recorded using a 5-point scale: 0, no convulsive activity; 1, gustatory movements; 2, tremor, hind limb extension, and mild forelimb clonus; 3, severe forelimb clonus/wet dog shakes; 4, rearing and severe clonus; 5, rearing, severe clonus with falling or whole body jerks. Median values are recorded.

*Significantly different from control group (Mann–Whitney U test for nonparametric grouped data, $\alpha < 0.05$).

†Methylscopolamine nitrate (1 mg/kg) was administered s.c. 30 min before pilocarpine to limit peripheral toxic effects (3). DPC, deep prepyriform cortex.

BMI (1 pmol) injected into the striatum was also anticonvulsant in rats subjected 15 min later to systemic injection of pilocarpine, 380 mg/kg (0/5) or kainic acid, 12 mg/kg (0/5) (Table 1). Electroencephalographic monitoring and morphological analysis revealed neither seizure activity in the hippocampus and cortex nor seizure-related brain damage typically observed in rats receiving saline in the striatum and then pilocarpine or kainic acid.

DISCUSSION

BMI is 20–50 times more potent as a convulsant in the deep prepyriform cortex than in the amygdala or hippocampus (10, 13). This high sensitivity of the deep prepyriform cortex was used as evidence for Piredda and Gale (10) to suggest this region was the site from which seizures are generated after systemic administration of convulsants. However, their experimental protocol did not extend to the paradoxical anticonvulsant activity of bicuculline reported here. A clear-cut anticonvulsant activity of BMI in the striatum in four different experimental models of epilepsy presented in this study shows that the view of Piredda and Gale (10) does not apply to the focal application of chemoconvulsants into the brain.

The data presented here show that convulsions induced by BMI injection into the deep prepyriform cortex are blocked by BMI injection into the striatum. The doses of striatal BMI required to block seizures induced by BMI injected into the deep prepyriform cortex are ≈100 times lower than those that elicit convulsions in rats treated with saline in the striatum. If the argument of sensitivity is predictive for delineation of the primary convulsant site in the brain, then our observations contradict those of Piredda and Gale (10) made on the deep prepyriform cortex with the use of similar experimental techniques. Although it would be fascinating to learn from

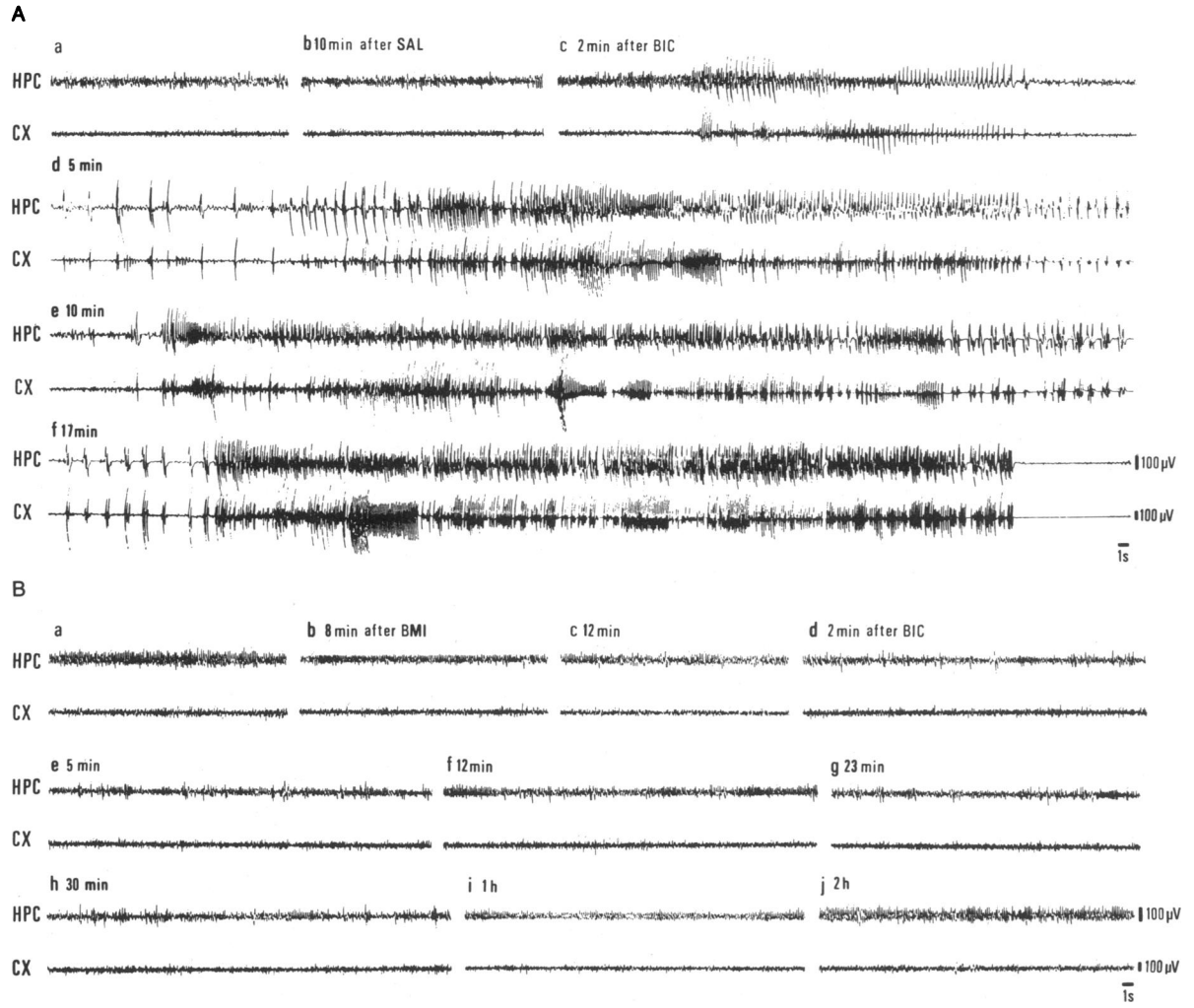

FIG. 1. Electrographic recordings from the hippocampus (HPC) and cortex (CX) demonstrating the effect of microinjection of saline (SAL) (*A*) and BMI (*B*) bilaterally, into the striatum on the convulsant action of bicuculline (BIC, 4 mg/kg) given i.p. 15 min later. (*A*) (Traces a) Predrug control recording. (Traces b) Unchanged records 10 min after microinjection of saline. (Traces c–f) Electrographic seizures registered 2, 5, 10, and 17 min after i.p. injection of BIC. (*B*) (Traces a) Predrug control recording. (Traces b and c) Unchanged records 8 and 12 min after microinjection of BMI (1 pmol) bilaterally into the striatum. (Traces d–h) Isolated spikes and low voltage fast activity registered up to 30 min after administration of BIC (4 mg/kg). (Traces i and j) Predrug pattern of activity registered in the electroencephalogram 30 min–1 hr after BIC administration.

which part of the brain the seizures arise, at the present time there is insufficient evidence implicating the deep prepyriform cortex as that region.

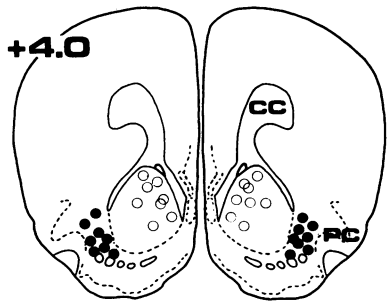

FIG. 2. Schematic reconstruction of injection sites of BMI in the deep prepyriform cortex and the striatum from serial coronal sections of perfused rat brain (from the atlas of Pellegrino *et al.*) (11). BMI (1 pmol) was microinjected bilaterally into the striatum 15 min before application of BMI (100 pmol) into the deep prepyriform cortex. ○, Sites in the striatum from which BMI (1 pmol) protects rats against seizures induced by BMI (100 pmol) from the deep prepyriform cortex. ●, Sites of injection of BMI (100 pmol) in the deep prepyriform cortex. CC, corpus callosum; PC, pyriform cortex.

A high anticonvulsant potential of BMI in the striatum suggests that GABA in this region regulates seizure threshold by acting as a gate allowing or disallowing activity changes in sets of output neurons. Electrophysiological analysis of striatonigral and striatoentopeduncular pathways shows that decreases in the GABAergic activity in the striatum increases GABAergic activity in the substantia nigra and entopeduncular nucleus (9). This implies that deficits in the GABA-mediated inhibition in the striatum induced by microinjection of BMI disinhibits GABA-mediated output pathways and enhances net inhibition in their respective targets, e.g. substantia nigra, pars reticulata, and entopeduncular nucleus. Previous microinjection studies have shown an anticonvulsant potential for the GABA agonist muscimol and GABA transaminase inhibitor γ-vinyl-GABA in the substantia nigra, pars reticulata, and entopeduncular nucleus in several seizure models in rodents (1, 3, 14). This may explain why BMI acting as a GABA antagonist is "paradoxically" anticonvulsant in the striatum.

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 GABA hypothesis of epilepsy was founded by Killam and Bain in 1957 (15) and is one of the basic tenets of modern

epileptology. This theory arose from observations that (*i*) drugs that impair GABAergic transmission induce overt convulsions, whereas those that enhance GABAergic transmission are anticonvulsant; (*ii*) impairment of GABAergic transmission contributes to experimental seizures; (*iii*) GABAergic transmission in discrete brain regions modulates propagation of seizures; and (*iv*) GABAergic transmission is impaired in human epilepsy (16, 17). This theory attempts to explain the mechanism of generation of seizures and the mechanism of action of several anticonvulsant drugs (16). According to this theory deficits in GABA-mediated inhibition are sufficient for the emergence of seizures (15, 17).

Our studies provide evidence that impairment of GABA-mediated inhibition in the striatum is anticonvulsant in four different seizure models. This finding shows that a deficiency in GABAergic transmission is not a sufficient prerequisite of seizures and argues against the overall validity of the GABA theory of epilepsy. This finding explicitly demonstrates that the GABA theory does not apply to the entire brain. Further research will expand our knowledge of the morphological structures and neuronal networks mediating the excitation/inhibition balance regulating seizure spread. In the meantime we may arrive at new interpretations of the functional role of GABA in epilepsy.

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