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Calcium Antagonist Binding in Cat Brain Tolerant to Electroconvulsive Shock

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BOLGER, G. T., B. A. WEISSMAN, J. BACHER AND L. ISAAC. *Calcium antagonist binding in cat brain tolerant to electroconvulsive shock.* PHARMACOL BIOCHEM BEHAV 27(2) 217–221, 1987.—Cats subjected to daily (25–30 days) electroconvulsive shock (ECS) demonstrated an elevation of their electroconvulsive threshold or tolerance to ECS. [^{3}H] Nitrendipine binding was measured to brain regions from non-tolerant (sham shocked) and ECS tolerant cats 24 hr following the last shock. ECS produced a significant increase (45%) in the density of [^{3}H] nitrendipine binding sites in the cerebral cortex and a significant decrease (33%) in the apparent affinity of [^{3}H] nitrendipine in the cerebellum. No changes in binding were observed in the hippocampus. The effects of ECS were also investigated in the rat, an animal not displaying tolerance to repeated ECS. [^{3}H] Nitrendipine binding to rat brain was measured 10 min and 24 hr following one shock (acute) or ten shocks delivered transauricularly once daily (chronic). Twenty-four hours following chronic ECS, there was a significant increase (19%) and decrease (11%) in the density, but no change in the apparent affinity of [^{3}H] nitrendipine binding sites in the cerebral cortex and hippocampus respectively. No significant change in [^{3}H] nitrendipine binding was observed in rat cerebellum 24 hr following chronic ECS. There were no changes in [^{3}H] nitrendipine binding in the cerebral cortex and hippocampus 10 min and 24 hr following acute ECS. These results indicate that ECS can alter [^{3}H] nitrendipine binding to calcium channel linked dihydropyridine binding sites in the central nervous system. It is suggested that changes in [^{3}H] nitrendipine binding in the cat cerebellum may be involved in the development of tolerance to ECS.

Calcium antagonists Dihydropyridine [^{3}H] Nitrendipine Calcium channels Electroconvulsive shock

ECS has been widely used as an experimental model for studying the generalized convulsive state [36]. Several investigators have observed significant changes in the neuropharmacologic profile of the rat central nervous sytem (CNS) following acute or chronic ECS. These changes were most prominent in the cerebral cortex and hippocampus and included alterations in neurotransmitter release, receptor binding [1, 5, 18, 20, 24, 29, 35, 39] and synaptic protein turnover [5,16]. Current research suggests that calcium influx into the neuron is a contributing factor in the genesis of various types of experimental seizures [25]. The dihydropyridines (DHP) encompassing both calcium channel antagonist (i.e., nifedipine, nimodipine) and calcium channel activator (i.e., Bay K 8644) structures have been shown to possess marked neuromodulatory properties consistent with an action at neuronal calcium channels (for review see [34]). Bay K 8644 has been shown to increase neuronal calcium currents [28] depolarization evoked Ca^{2+} uptake into synaptosomes [37,40], neurotransmitter release [26, 37, 39] and phosphatidyl inositol turnover [20]. These effects of BAY K 8644 were blocked by DHP calcium antagonists, an indication that they are likely mediated through high affinity neuronal DHP binding sites [14,23].

Both BAY K 8644 and nimodipine have been shown to promote and attenuate seizure activity respectively, in a number of animal models. BAY K 8644 induced a behavioural syndrome in mice that was characterized by ataxia, ptosis, Straub tail and limb clonus/tonus [3]. Intravenous infusion of BAY K 8644 in the conscious dog resulted in self-limited grand-mal like seizures [31]. Nimodipine has been shown to block retrograde amnesia, but not convulsions in rodents following ECS and was able to prevent behavioural and electroencephalographic disturbances elicited by total cerebral ischemia in cats [16]. Furthermore, nimodipine arrested electroencephalographic seizures induced by ischemia, pentylenetetrazol and bicuculline in rabbits [25].

While it appears that the DHPs can modulate the seizure

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state in a number of animal models, there is no data concerning the effects of seizures on the properties of DHP binding sites in the CNS. The cat provided us with an interesting animal model to study the effects of ECS on neuronal DHP binding sites. Chronic ECS in cats results in a diminished response or tolerance to the convulsive effects of applied current [9,10]. Furthermore, it was observed that cerebrospinal fluid taken from the ventricular space of a cat made tolerant to ECS could raise the ECS shock threshold of a naive animal [17] implying that a humoral factor(s) was responsible for mediating the ECS susceptibility of the cat. We have now characterized the effects of ECS on the neuronal DHP binding site labeled with [^{3}H] nitrendipine in the cat, following tolerance to chronic ECS, and in the rat, an animal model which does not demonstrate tolerance to chronic ECS. Significant changes in [^{3}H] nitrendipine binding in the cerebellum and cerebral cortex of the cat and in the cerebral cortex and hippocampus of the rat were observed 24 hr following chronic ECS. These findings indicate that seizures produced by ECS alter the properties of neuronal DHP binding sites. Furthermore, it is suggested that changes in DHP binding sites in the cat cerebellum may be a consequence of, or in part mediate, the development of tolerance to ECS.

METHOD

Administration of ECS

Male mongrel cats were housed individually in a room with constant humidity, temperature and a 12 hour light-dark cycle. Techniques for the surgical implanting of the stimulating electrodes and for delivering of the seizure generating shock are described in detail elsewhere [17]. Convulsive thresholds were determined daily as previously described [17].

Male albino rats (Sprague-Dawley, Veterinary Resources Branch, NIH, Bethesda, MD; 200–250 g) housed three per cage with free access to food and water were maintained on a 12 hr light/dark cycle. ECS was delivered transauricularly, through clips attached to the pinna of each ear. Maximal seizures were elicited by a 60 Hz alternating current of 50 mA delivered for 0.5 sec. Experimental animals received either a single shock on one day (acute ECS) or a single shock each day for ten days (chronic ECS; [5, 14, 19, 21, 35, 39]). Sham animals were handled in the same manner as experimental animals but received no shock.

Neuropharmacology

Twenty-four hours following the last ECS, cats were decapitated and their brains rapidly removed and placed into ice cold 50 mM tris HCl buffer, pH 7.4 at 22°C. [^{3}H] Nitrendipine binding was studied in four brain regions. These were isolated by blunt dissection and included (1) the proximal cortex, which was the area directly under the stimulating electrodes (motor cortex); (2) the distal cortex, which included an area 1 cm caudal to the proximal cortex (occipital cortex); (3) the hippocampus; and (4) the cerebellum. For studies involving rats, sham or shocked animals were decapitated either 10 min or 24 hr following acute or chronic ECS. Their brains were rapidly removed and placed into ice cold 50 mM tris-buffer. [^{3}H] Nitrendipine binding was studied in the cerebral cortex and hippocampi which were isolated from the cerebrum by blunt dissection. Individual cat or rat brain regions were weighed and placed into 50 vols of 50 mM tris buffer at 4°C. The tissue was disrupted using a Brinkmann Polytron (setting 5, 15 sec). The homogenate was centrifuged at 20,000 × g for 20 min and the brain membranes resuspended in 100 vols of 50 mM tris buffer. [^{3}H] Nitrendipine binding was assayed in a total volume of 2 ml consisting of 1 ml membrane resuspension (0.3–0.5 mg protein), 0.9 ml of 50 mM tris buffer and 0.1 ml of radioligand. Nonspecific binding was determined in the presence of 10 μM nifedipine. All binding experiments were carried out in borosilicate glass tubes under subdued light for an incubation period of 1 hr at 25°C. Binding was terminated by rapid filtration under vacuum on Whatman GF/B glass fibre filters followed by 2×5 ml washes of iced cold Tris buffer using a Brandel Cell Harvester (Brandel Co., Gaithersburg, MD). The radioactivity of the filters was determined by liquid scintillation counting (Ready-Solve MP, Beckman, Beckman Model LS 330 liquid scintillation counter). Specific binding was expressed as the difference between total binding and nonspecific binding.

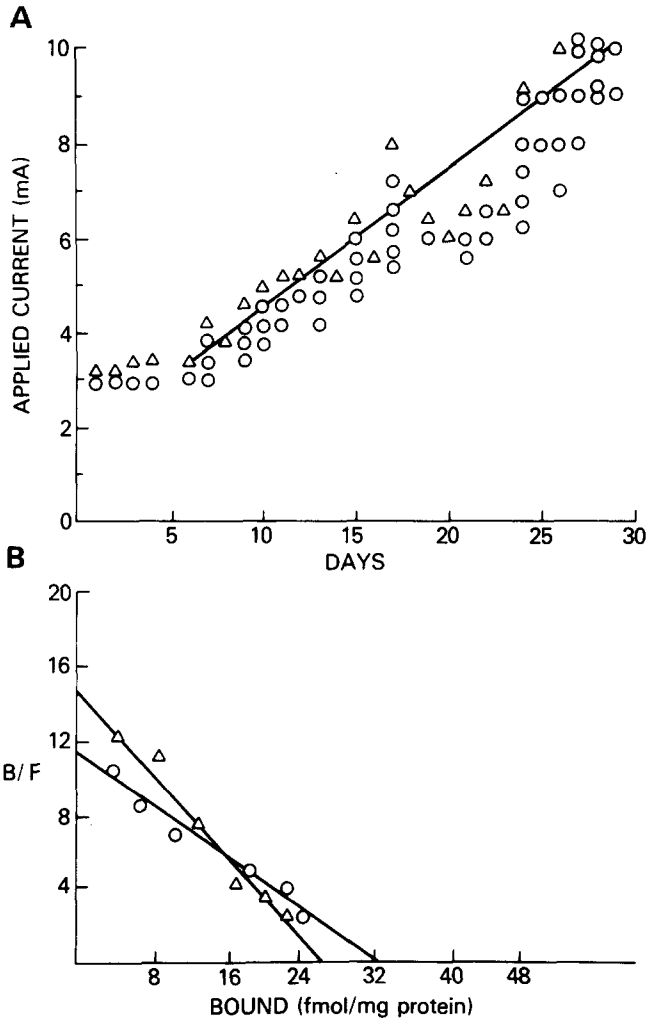

FIG. 1. (A) Electroconvulsive threshold determinations in a representative cat. The (○) represents the electrical current intensity delivered to an animal that did not result in generation of major convulsion. The (Δ) denotes the electrical current intensity delivered to an animal that lead to a loss of posture and generation of tonic clonic convulsive activity lasting for 1 to 2 min. (B) Scatchard plot of specific [^{3}H] nitrendipine binding to cerebellar membranes from cats that were sham shocked (○) or made tolerant (Δ) to ECS. The results are from a representative experiment repeated five times with similar results (Table 1).

TABLE 1

[^{3}H]NITRENDIPINE BINDING IN CAT BRAIN FOLLOWING TOLERANCE TO REPEATED ECS

Brain Region	K_d (pM)	B_{max} (fmol/mg protein)
Proximal Cortex		
Sham	190 ± 14	114 ± 3
ECS	190 ± 4	121 ± 14
Distal Cortex		
Sham	192 ± 20	130 ± 11
ECS	198 ± 25	189 ± 22*
Hippocampus		
Sham	204 ± 27	140 ± 7
ECS	200 ± 24	126 ± 9
Cerebellum		
Sham	293 ± 26†	32 ± 1
ECS	195 ± 25	28 ± 3

[^{3}H]Nitrendipine binding (35–100 pM) was studied 24 hr following the last ECS treatment. The K_d and B_{max} values were obtained by Scatchard analysis of [^{3}H]nitrendipine (35–1000 pM) binding and are presented as the mean ± S.E.M. of 4–5 cats. *Significantly different from distal cortex (sham) and proximal cortex (sham and ECS) $p<0.05$; †Significantly different from cerebellum (sham) and K_d values for remaining brain regions (sham and ECS) $p<0.05$; ANOVA, SPSS-X version.

TABLE 2

[^{3}H]NITRENDIPINE BINDING IN RAT BRAIN REGIONS 24 HOURS FOLLOWING CHRONIC ECS

Brain Region	K_d (pM)	B_{max} (fmol/mg protein)
Cortex		
Sham	268 ± 38	117 ± 8
ECS	295 ± 45	139 ± 4†
Hippocampus		
Sham	156 ± 14	197 ± 8
ECS	129 ± 6	176 ± 5*
Cerebellum		
Sham	277 ± 40	58 ± 5
ECS	240 ± 47	61 ± 6

The values are presented as the mean ± S.E.M. of 6–11 experiments. The K_d and B_{max} values were determined by Scatchard analysis of [^{3}H]nitrendipine (35–1000 pM) binding. *Significantly different from sham control, *$p<0.05$, †$p<0.02$, unpaired (two tailed) t-test.

Protein Determinations

Protein was determined by the modified Lowry method of Miller [27].

MATERIALS

[^{3}H] Nitrendipine was obtained from New England Nuclear (Boston, MA) at a specific activity of 72–80 Ci/mmol and stored in the dark at −20°C. Nifedipine was obtained from Pfizer (Groton, CT). All other materials were obtained from standard commercial sources.

RESULTS

ECS

Six cats whose electroconvulsive threshold was elevated by repeated ECS and six cats which served as sham shock controls were used in these studies [17]. The following behaviours were observed in cats 1–2 min following ECS: initially a downward head jerk followed by twitching of the face and ears and trembling of the upper extremities (subconvulsive behaviours), subsequently culminating in loss of posture with tonic and clonic movements (convulsive behaviour) which were frequently associated with urination and salivation [17]. Tolerance to ECS (see Fig. 1a) was indicated when there was an increase in the applied current to elicit convulsant behavior. Figure 1a illustrates the effects of daily ECS on the electroconvulsive threshold. Previous work has shown that subconvulsant stimulation does not influence the electroconvulsive threshold [11] and that the rate of increase in threshold is directly related to the number of convulsions per day providing the total number of seizures each day does not exceed four [11]. The rate of increase in threshold (Fig. 1, 0.25 mA/day) is consistent with previous data [17] in which the animals experienced two convulsions per day. As the threshold approached 10 mA, the animals responded only with subconvulsive behaviour. At 24 hr following attainment of the maximum ECS threshold (23–30 days) for the stimulator (10 mA), the animals were killed for neurochemical measurements as continued stimulation without evoking major motor convulsions would have lead to a decrease in the threshold via the "kindling" mechanism of Goodard [12].

In contrast to cats, chronic ECS in rats does not result in the development of tolerance [5, 14, 21, 29, 35, 39]. The seizures following ECS consisted of a short period of initial tonic extension usually followed by clonus. The typical seizure lasted for 20–25 sec and was followed by 2–5 min of postictal lethargy.

[^{3}H] Nitrendipine Binding

[^{3}H] Nitrendipine binding in cat cerebral cortex, hippocampus and cerebellum was measured in sham shocked (non-tolerant) and ECS tolerant cats (Table 1). The apparent affinity (K_d) and B_{max} (maximun binding site capacity) values obtained for [^{3}H] nitrendipine binding in the various brain regions of sham shocked cats are in good agreement with those obtained in other species [7, 13, 23]. The K_d of [^{3}H] nitrendipine was however, significantly higher in the cerebellum than in other cat brain regions. In ECS tolerant cats, there was a significant increase (45%) in the B_{max}, but no change in the K_d of [^{3}H] nitrendipine in the distal cerebral cortex. In addition, the K_d of [^{3}H] nitrendipine but not the B_{max} significantly decreased (33%) in the cerebellum (Fig. 1b). There was no significant change in [^{3}H] nitrendipine binding in the proximal cortex and hippocampus (Table 1) of cat brain.

[^{3}H] Nitrendipine binding was investigated in the hippocampus and cortex of rat brain at short (10 min) and long (24 hr) time periods following acute and chronic ECS (Table 2). This time course was chosen since previous studies have shown that neurotransmitter and benzodiazepine receptor binding in the rat are differentially affected by ECS dependent on the protocol followed and the time of experimental observation [1, 19, 24, 30, 39].

Twenty-four hours following chronic ECS in the rat, there

was a significant increase (19%) and significant decrease (11%) in the B_{max} but not the K_d of [^{3}H] nitrendipine in the cerebral cortex and hippocampus respectively. However, there was no change in [^{3}H] nitrendipine binding in the cerebellum 24 hr following chronic ECS in the rat. [^{3}H] Nitrendipine binding in rat cerebral cortex and hippocampus was not affected 10 min and 24 hr following acute ECS and 10 min following chronic ECS.

DISCUSSION

Recent studies suggest that calcium influx into the neuron is a critical factor in the genesis of seizures [25]. Calcium fluxes are mediatory in the evolution of intrinsic burst potential in subpopulations of neurons [32,33]. The disinhibition of this activity results in the generation of paroxysmal depolarizing shifts in the neuronal membrane potential, a major contributor to the characteristic interictal "spike" observed in electroencephalographic recordings during seizures. Thus, drugs which block the entry of calcium into neuronal tissue (i.e., phenytoin) [15] are useful in the control of seizure activity.

ECS produced significant brain region specific changes in calcium channel linked DHP binding sites in the ECS tolerant cat and in the rat, 24 hr following ECS treatment. With the exception of cat cerebellum, the effects of ECS were manifested as changes in the B_{max} of [^{3}H] nitrendipine. The ability of ECS to produce changes in the density of neuronal [^{3}H] nitrendipine binding sites is consistent with changes in neurotransmitter receptor binding densities following ECS in rats. Twenty-four hours following chronic ECS there were increases in the density of benzodiazepine receptors (35%) in the frontal cortex [14], and α_1-adrenergic receptors (36%) in the cerebral cortex [39]. In contrast, there was a decrease in the density of cholinergic (13–15%) and β-adrenergic receptors (24–37%) in the cortex and hippocampus [19,21]. The observation that not all brain regions were affected similarly in the cat or rat implies that neuronal DHP binding sites are subject to a brain region specific regulation or as recent studies suggest, neuronal DHP binding sites possess different molecular properties in different brain regions [2,4].

The time course for the ECS mediated changes in [^{3}H] nitrendipine binding in the rat (24 hr following last ECS treatment) suggest they may occur secondarily to alterations in neurotransmitter/receptor function or neuronal protein homeostasis [5,16]. Consistent with the inability of acute ECS to affect [^{3}H] nitrendipine binding in rat brain, acute ECS did not affect [^{3}H] nitrendipine binding in the mouse cerebral cortex membranes (Weissman and Bolger, unpublished observations). The inability to detect significant changes in [^{3}H] nitrendipine binding 10 min following chronic ECS in rats, or in the proximal cortex of ECS tolerant cats, suggests that electrical current application in the brain may affect ECS elicited changes in [^{3}H] nitrendipine binding in a time and proximity dependent fashion.

The significance of changes in the B_{max} of [^{3}H] nitrendipine following ECS and their relationship to seizures is not readily apparent, particularly in light of the fact that a relationship between the density of neuronal DHP binding sites and calcium currents has not been established. However, chronic ethanol administration to rats resulted in both a decrease in the density of [^{3}H] nitrendipine binding and a decrease in Ca^{2+} uptake in rat striatum [22]. Thus, increases or decreases in the B_{max} of [^{3}H] nitrendipine following ECS may reflect corresponding changes in neuronal calcium influx. Changes in the density of DHP binding sites in the rat hippocampus and consequently calcium influx, may be of particular importance with respect to ECS. 6-Hydroxydopamine destruction of noradrenergic terminals in the rat hippocampus, increased both the B_{max} of [^{3}H] nitrendipine (Bolger, Basile and Skolnick, unpublished observation) and the severity of convulsions elicited by ECS [36]. Thus, chronic ECS may have reduced the density of hippocampal DHP binding sites, as a compensatory response to increased electrical or neuronal activity in the CNS.

In contrast to rats, cats exposed to chronic ECS developed tolerance to ECS [9,10] and demonstrated a significant decrease in the K_d of [^{3}H] nitrendipine in the cerebellum. Although the major effect of ECS on neurotransmitter receptors is to alter their maximum binding capacities, ECS has been reported to change the affinity of serotonin receptors 24 hr following chronic ECS [14]. Changes in the properties of calcium channel linked-DHP binding sites in the cat cerebellum might reflect changes in calcium mobilization and consequently GABAergic neurotransmission. The importance of calcium in GABA function [8] provides for a sensitive mechanism by which changes in calcium mobilization might produce an enhanced GABA release, and tolerance to the effects of ECS in the cat. Since a humoral factor(s) in the cerebrospinal fluid of the cat can mediate tolerance to ECS [17], it is possible that this factor may also be responsible for decreasing the K_d of [^{3}H] nitrendipine in the cat cerebellum. An endogenous tissue or humoral factor(s) in rat brain, particularly in the cerebellum, was shown to potentiate the decrease in the K_d of [^{3}H] nitrendipine mediated by the potent psychotomimetic, phencyclidine [2]. The increase in [^{3}H] nitrendipine binding in the distil cerebral cortex of the cat is likely not involved in the mediation of tolerance to ECS, since there also was an increase in [^{3}H] nitrendipine binding in rat cerebral cortex following ECS.

DHPs preferentially interact with "L-type" calcium channels, a subpopulation of neuronal calcium channels that have been identified electrophysiologically [28]. The inhibition of seizure activity by nimodipine [25] suggest that "L-type" channels and DHP binding sites play a crucial role in seizure etiology. In general, the inability of ECS to either produce no change or small changes in neuronal DHP binding sites is an important finding with respect to the potential development of antiseizure drugs active at these sites [25]. In the cat, modulation of the neuronal DHP binding site in the cerebellum either directly or indirectly (through humoral factors) by ECS, may in part mediate the development of tolerance to ECS. A better understanding of the relationship between neuronal DHP binding sites and seizures is clearly needed.

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