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CARBAMAZEPINE INHIBITS ELECTROCONVULSIVE SHOCK-INDUCED INOSITOL TRISPHOSPHATE (IP_3) ACCUMULATION IN RAT CEREBRAL CORTEX AND HIPPOCAMPUS

Robert E. Vadnal and Nicolas G. Bazan*

LSU Eye Center, Louisiana State University Medical Center
School of Medicine, New Orleans, Louisiana 70112

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Summary: Carbamazepine is used to treat manic-depressive disorder, and is also an anticonvulsant. Rats were injected with this drug 90 min prior to this experiment, when mild inhibition of convulsions took place. Intraventricular injections of 14 uCi [^{3}H]myoinositol were made 20-24 hrs prior to the experiment. Ninety min after intraperitoneal injection of carbamazepine or vehicle, rats were given electroconvulsive shock or sham procedure and sacrificed 30 sec later. Incorporation of radiolabel into inositol lipids and inositol phosphates was analyzed in cerebral cortex and hippocampus. Carbamazepine's effects on the brain inositol lipid cycle, studied here for the first time, showed 1) enhanced labeling in the polyphosphoinositides (carbamazepine-ECS groups showed increases of about 40% in PIP_2); 2) decreased [H]IP_1 levels; and 3) inhibition of ECS-induced [^{3}H]-IP_3 accumulation.

Electroconvulsive shock (ECS) transiently alters cell signal-transduction systems as well as second messengers, including the inositol lipid cycle (1-8). This cycle is a major second messenger system which is linked to a number of neurotransmitters/neuromodulators including the cholinergic (ACh), serotonergic (5-HT_2), noradrenergic (alpha-1), thyrotropin-releasing hormone (TRH), and platelet activating factor (PAF) (7-9). After receptor activation, two second messengers are produced: diacylglycerol (DG), which is involved in activation of protein kinase C (10); and inositol 1,4,5-trisphosphate, which is involved in the release of calcium from intracellular stores (11). Using an in vivo model, we recently demonstrated that ECS produces a significant increase in the accumulation of [^{3}H]-IP_3 in rat cerebrum, and that pretreatment with lithium attenuates this effect (1). Since this result is not readi-

*Reprint requests to: NG Bazan, LSU Eye Center, 2020 Gravier St, New Orleans, Louisiana 70112.

Abbreviations: PI, phosphatidylinositol; PIP, phosphatidylinositol 4-phosphate; PIP_2, phosphatidylinositol 4,5-bisphosphate; GPI, glycerolphosphatidylinositol; IP_1, inositol monophosphate; IP_2, inositol bisphosphate; IP_3, inositol trisphosphate.

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ly explainable by the inhibitory effect of lithium on myoinositol-1-phosphatase (12), which results in an increase in myoinositol-1-phosphate (13), it has been suggested that lithium may elicit a new effect at the plasma membrane on the cell signal transduction system itself (1).

Lithium's therapeutic effect in the treatment of manic-depressive disorder has been related to its inhibitory effect on the inositol lipid cycle (13-15). Because carbamazepine 1) is an alternative medication to lithium in the treatment of manic-depressive disorder, 2) possesses a clinical profile similar to that of lithium, and 3) is a potent anticonvulsant drug used in the treatment of complex partial seizures of temporal lobe epilepsy (16-19), we have explored the effects of the drug on the inositol lipid cycle. Our studies demonstrate that ECS produces increases in $[^3H]$-IP_3 in rat cerebral cortex and hippocampus. Pretreatment with carbamazepine completely inhibited these effects, and did not result in increased levels of $[^3H]$-IP_1, as seen with lithium (1,22). Moreover, carbamazepine pretreatment demonstrates a tendency to decrease $[^3H]$-IP_1 levels. This report is the first to demonstrate effects of carbamazepine on the inositol lipid cycle.

MATERIALS AND METHODS

Animal Preparation: Male Sprague-Dawley rats (220-280 g) were prelabeled by bilateral intracerebroventricular injection of 7 uCi of $[^3H]$-myoinositol (15 Ci/mmol, American Radiolabeled Chemicals), 20-24 hrs prior to sacrifice under light ether anesthesia. Rats were kept overnight and given access to food and water ad libitum under a regular light-dark cycle. Rats were pretreated with carbamazepine (50 mg/kg) or vehicle (ethanol) intraperitoneally 90 minutes prior to either ECS or sham procedure. This time point was selected after pretreatment of rats with carbamazepine because, although the drug inhibited seizures after 30 min, only a mild inhibition was observed after 90 min. ECS was produced using a Grass S48 Stimulator (Grass Medical Instruments, Quincy, MA) using stainless steel scalp electrodes with a stimulation rate of 155 cps, 120 Volts, 750 ms duration. Rats were sacrificed 30 sec after real or sham ECS by high-power head-focussed microwave irradiation for 1.5 sec (6.5 KW, 2450 MH_z; Cober Electronics, Stamford, CT), and immersed immediately in ice water.

Extraction and Isolation of Phosphoinositides: The cerebral cortex and hippocampus were dissected and homogenized in 20 vols of chloroform:methanol (2:1 v/v). The phosphoinositide extraction and TLC method were done as previously reported (1), except that the solvent system used was chloroform:acetone: methanol:glacial acetic acid:water (80:30:26:24:14 v/v). Lipid phosphorus was determined according to the method of Rouser et al (20). Authentic standards were used for PIP_2, PIP, and PI, and the lipids were visualized using iodine staining. Scintillation counting was done using 5 ml of Redi-Value per sample, and counted by H-number.

Analysis of Inositol Phosphates: An aliquot of the water-soluble phase of the acidified chloroform-methanol extraction was used for inositol phosphate determination, based on the elution system described by Berridge et al (21): 1) 5 mM myoinositol; 2) 0.60 Na formate/5 mM disodium tetraborate; 3) 0.15 M ammon. formate/0.1 M formic acid; 4) 0.5 M ammon. formate/0.1 formic acid; 5) 1.0 M ammon. formate/0.1 M formic acid; 6) 1.2 M ammon. formate/0.1 M formic acid. Authentic standards of [^{3}H]-myoinositol-1,4,5-phosphate and [^{3}H]-myoinositol-1,3,4,5-phosphate (New England Nuclear) were used to define the [^{3}H]-IP_3 and [^{3}H]-IP_4 peak areas. The resin used was 2 ml of Bio Rad AG1 X8, 200-400 mesh, formate form. Scintillation counting was done with 4.5 ml of Beckman Redi-Value per sample and counted by H-number.

Statistical Analysis: Statistical analysis was done using ANOVA.

RESULTS AND DISCUSSION

ECS showed a tendency to enhance the [^{3}H] myoinositol labeling in polyphosphoinositides in both cerebral cortex and hippocampus. PI in cerebral cortex, on the other hand, showed a diminished labeling by the precursor under these conditions (Table 1). This may reflect PI hydrolysis in the cerebral cortex during convulsions, in addition to enhanced turnover in PIP and PIP_2.

Table 1. Effects of electroconvulsive shock and/or carbamazepine on incorporation of [^{3}H]-myoinositol into rat cerebral cortex and hippocampus 20-24 hrs prior to sacrifice

Condition	[^{3}H]-PI	[^{3}H]-PIP	[^{3}H]-PIP_2
CEREBRAL CORTEX			
Control	4829 ± 49	548 ± 23	560 ± 32
ECS	4243 ± 137**	541 ± 19	602 ± 29
Carbamazepine	4890 ± 87	631 ± 16*	653 ± 39
Carbamazepine-ECS	5163 ± 186 a,**	666 ± 40** a,**	777 ± 54* a,**
HIPPOCAMPUS			
Control	2266 ± 45	166 ± 6	233 ± 11
ECS	2227 ± 44	181 ± 10	254 ± 13
Carbamazepine	2352 ± 35	185 ± 10	253 ± 13
Carbamazepine-ECS	2118 ± 42*	234 ± 14** a,**	321 ± 30** a,*

Values are dpm/100 mg lipid phosphorus ± S.E.M. (n = 7-9 rats/group); *$p < .05$, **$p < .005$; statistical analysis was done using ANOVA. Values are corrected to total label incorporated; a, refers to carbamazepine-ECS compared to ECS.

Carbamazepine pretreatment showed a tendency towards increased incorporation of [^{3}H]-myoinositol in the polyphosphoinositides (Table 1) and, when followed by ECS, led to increases of approximately 40% in PIP_2 of cerebral cortex and hippocampus. The carbamazepine-ECS treated rats, when compared with the ECS-treated rats, also showed statistically significant increases in PIP and PIP_2. This enhanced labeling may result from an ECS-induced activation of the inositol lipid cycle (1,6,8), and from an inhibitory effect of carbamazepine on the G protein-phospholipase C regulatory system. Lithium also resulted in a tendency towards increased incorporation of [^{3}H]-myoinositol in the brain polyphosphoinositides (1).

Carbamazepine decreased [^{3}H]-IP_1 in both cerebral cortex and hippocampus. This effect may reflect an inhibition of phospholipase C activity on phosphatidylinositol (PI), an inhibition of the IP_2-phosphatase, or a stimulation of the IP_1-phosphatase. This decrease in [^{3}H]-IP_1 is in marked contrast to the elevations seen with lithium pretreatment (1,22). Since carbamazepine and lithium are effective in the treatment of manic-depressive states, it is of interest that carbamazepine in vivo does not seem to inhibit myoinositol-1-phosphatase, as lithium does.

Electroconvulsive shock (ECS) increased the levels of [^{3}H]-IP_3 in rat cerebral cortex by 55% and in hippocampus by 48%, suggesting a marked stimulation of PIP_2 hydrolysis (Table 2). Carbamazepine pretreatment prior to ECS completely inhibited the ECS-induced accumulation of [^{3}H]-IP_3 in both cortex and hippocampus (Table 2). [^{3}H]-IP_4 (inositol tetrakisphosphate) was not detected using this in vivo system. The inhibition of ECS-induced [^{3}H]-IP_3 accumulation may be due to an inhibitory effect on membrane-bound cell signal transducing molecules; stimulation of PIP_2 phosphomonoesterase; stimulation of the IP_3 kinase; or stimulation of IP_3 phosphatase. The data suggests an effect at either the receptor, G protein, or phospholipase C level. Lithium was previously shown to attenuate the ECS-induced accumulation of [H]-IP_3.

Table 2. Changes in inositol phosphates in rat cerebral cortex and hippocampus after carbamazepine and/or electroconvulsive shock

Condition	[^{3}H]-GPI	[^{3}H]-IP$_1$	[^{3}H]-IP$_2$	[^{3}H]-IP$_3$
CEREBRAL CORTEX				
Control	519 ± 113	1856 ± 135	257 ± 15	387 ± 14
ECS	517 ± 119	2007 ± 95	453 ± 36**	598 ± 37**
Carbamazepine	475 ± 120	1581 ± 112	347 ± 50	385 ± 14
Carbamazepine-ECS	429 ± 57	1263 ± 195* a,*	313 ± 30 a,*	350 ± 48 a,**
HIPPOCAMPUS				
Control	472 ± 32	353 ± 21	119 ± 18	129 ± 9
ECS	410 ± 15	318 ± 17	158 ± 11	191 ± 10**
Carbamazepine	408 ± 24	290 ± 19*	117 ± 15	132 ± 5
Carbamazepine-ECS	458 ± 19	322 ± 11	151 ± 24	132 ± 11 a,**

Carbamazepine (50 mg/kg) or vehicle was injected IP 90 min prior to either ECS or sham. Animals were sacrificed 30 seconds after ECS or sham by head-focussed microwave (6.5 kw, 1.5 sec). Values are dpm/100 mg lipid phosphorus ± S.E.M. (n = 7-9 rats/group); $p < .05$, $^{**}p < .005$; statistical analysis was done using ANOVA. Values are corrected to total [^{3}H]-myoinositol incorporated; a, refers to carbamazepine-ECS compared to ECS; [^{3}H]-IP$_4$ was not detected.

Besides the similar therapeutic effects of carbamazepine and lithium, both drugs block stimulated release of norepinephrine (23), decrease GABA turnover (24), and inhibit adenylate cyclase stimulated by various ligands (25). The labeling of inositol phosphates (Table 2) suggests that carbamazepine does not inhibit myoinositol-1-phosphatase since [^{3}H]-IP$_1$ levels were actually reduced, and carbamazepine does not amplify the inositol phosphate in response to stimulation, as lithium does. Moreover, carbamazepine elicits an inhibition in [^{3}H]-IP$_3$ accumulation, probably interfering with cell membrane signal-transduction systems.

In summary, using an in vivo model, we have shown that ECS stimulates the inositol lipid cycle in rat cerebral cortex and hippocampus, and that carbamazepine completely inhibits the ECS-induced increases in [^{3}H]-IP$_3$. Previous work indicated that lithium attenuated the [^{3}H]-IP$_3$ response (1), sug-

gesting that lithium may elicit effects on the signal transduction mechanism in addition to inhibiting myoinositol-1-phosphatase (1). Carbamazepine did not increase [^{3}H]-IP_1, indicating a lack of effect on myoinositol-1-phosphatase. The effects reported here may underlie the basis of carbamazepine, or of lithium as recently suggested (1), as an effective treatment for manic-depressive disorders. Although the former is also an anticonvulsant drug, the present study was conducted with rats injected with carbamazepine 90 min prior to the experiment, a time at which mild inhibition of convulsions took place. As with lithium (1), carbamazepine may elicit effects at the cell signal transduction level, perhaps the G protein-phospholipase C system.

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