Neuroscience 134 (2005) 233–246

BIPHASIC ACTIONS OF TOPIRAMATE ON MONOAMINE EXOCYTOSIS ASSOCIATED WITH BOTH SOLUBLE *N*-ETHYLMALEIMIDE-SENSITIVE FACTOR ATTACHMENT PROTEIN RECEPTORS AND Ca^{2+}-INDUCED Ca^{2+}-RELEASING SYSTEMS

M. OKADA,[a]* S. YOSHIDA,[a,b] G. ZHU,[a] S. HIROSE[c] AND S. KANEKO[a]

[a]*Department of Neuropsychiatry, School of Medicine, Hirosaki University, Hirosaki 036-8562, Japan*

[b]*Institute of Neuroscience and Molecular Biology, Shibata IRIKA, Hirosaki 036-8084, Japan*

[c]*Department of Pediatrics, Fukuoka University, Fukuoka 814-0180, Japan*

Abstract—To explore the pharmacological mechanisms of topiramate (TPM), we determined the effects of TPM on monoamine (dopamine and serotonin) exocytosis associated with *N*-ethylmaleimide-sensitive factor attachment protein receptors and Ca^{2+}-induced Ca^{2+}-releasing systems, including inositol-triphosphate receptor and ryanodine receptor in freely moving rat pre-frontal cortex using *in vivo* microdialysis. During resting stage, Ca^{2+} output from endoplasmic reticulum Ca^{2+} store via inositol-triphosphate receptor regulates syntaxin-associated monoamine exocytosis mechanism, whereas during neuronal hyperexcitable stage, Ca^{2+} output via ryanodine receptor regulates synaptobrevin-associated monoamine exocytosis mechanism. Basal monoamine releases were increased and decreased by therapeutically relevant and supratherapeutic concentration of TPM, respectively. The therapeutic-relevant concentration of TPM increased Ca^{2+}-evoked release concentration-dependently; however, its stimulatory effect was attenuated in the supratherapeutic range. The K^+-evoked releases were reduced by TPM concentration-dependently (from therapeutic to supratherapeutic ranges). The therapeutic-relevant concentration of TPM-induced elevation of basal release was reduced by cleavage with syntaxin and inhibition of inositol-triphosphate receptor predominantly, by cleavage with SNAP-25 and synaptobrevin weakly, but not by ryanodine receptor inhibitor. The therapeutic-relevant concentration of TPM-induced elevation of Ca^{2+}-evoked release was reduced by cleavage with syntaxin and inositol-triphosphate receptor inhibitor selectively. The therapeutic-relevant concentration of TPM-induced reduction of K^+-evoked monoamine release was abolished by cleavage with synaptobrevin, but was not affected by cleavage with SNAP-25 or synaptobrevin. The stimulatory effect of ryanodine receptor agonist on K^+-evoked monoamine release was reduced by TPM, whereas that of inositol-triphosphate receptor agonist was not affected by TPM. Therefore, these results indicate that the combination of the effects of TPM on exocytosis mechanisms associated with SNARE and Ca^{2+}-induced Ca^{2+}-releasing systems, enhancement of inositol-triphosphate receptor/syntaxin and inhibition of ryanodine receptor/synaptobrevin in pre-frontal cortex, may be involved in clinical actions of TPM.

Key words: CICR, SNARE, exocytosis, microdialysis, topiramate.

Recently, a number of new generation antiepileptic drugs have been introduced therapeutically, including lamotrigine, gabapentin, levetiracetam, topiramate (TPM) and zonisamide, which have proved highly effective against various types of epileptic seizures and have less troublesome side effects or adverse drug interactions than other established antiepileptic drugs (Cramer et al., 2001). Following clinical trials, these new generation antiepileptic drugs have become available for drug therapy of epilepsy, either singly or in combination with established antiepileptic drugs; however, the precise mechanism of antiepileptic action of these new generation antiepileptic drugs is still uncertain (Cramer et al., 2001). More recent studies show that new generation antiepileptic drugs have efficacy in non-epileptic neurological and psychiatric disorders: neuralgia (Zvartau-Hind et al., 2000), eating disorder (McElroy et al., 2003), and post-traumatic stress disorder (Berlant and van Kammen, 2002).

TPM is currently used as an additive treatment for adult patients with refractory partial and secondarily generalized seizures (Privitera and Twyman, 2002). It has also been found useful as adjunctive therapy in children, adolescents and young adults with partial-onset seizures or Lennox-Gastaut syndrome (Privitera and Twyman, 2002). A recent monotherapy study indicates that low-dose (100 mg/day) TPM is effective in treating newly diagnosed epilepsy (Privitera et al., 2003). The mechanisms of these wide antiepileptic spectrums of TPM have been considered to be modulated by the ion channels and ionotropic neurotransmitter receptors. Especially, electrophysiological studies have revealed several pharmacological properties, including a negative modulatory effect on the α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) and kainate subtypes of glutamate receptors (Skradski and

*Corresponding author. Tel: +81-172-395066; fax: +81-172-395067.
E-mail address: okadamot@cc.hirosaki-u.ac.jp (M. Okada).

Abbreviations: AdA, adenophostin A; AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid; BoNT, botulinum toxin; BoNT/A, botulinum toxin type A; BoNT/B, botulinum toxin type B; BoNT/C, botulinum toxin type C; CICR, Ca^{2+}-induced Ca^{2+}-releasing; HPLC, high performance liquid chromatograph; IP3R, inositol 1,4,5-trisphosphate receptor; MRS, modified Ringer's solution; pFC, pre-frontal cortex; RR, Ruthenium Red; RyR, ryanodine receptor; SNARE, soluble *N*-ethylmaleimide-sensitive factor attachment protein receptor; TPM, topiramate; VSCC, voltage-sensitive Ca^{2+} channel; wwbt, wet weight brain tissue; XeC, xestospongin C.

0306-4522/05$30.00+0.00
doi:10.1016/j.neuroscience.2005.03.045

White, 2000; Angehagen et al., 2004), mixed modulatory effects (usually positive) on some types of $GABA_A$ receptors (White et al., 1997; Herrero et al., 2002), negative modulatory effects on some types of voltage-gated Na^+ (Zona et al., 1997; McLean et al., 2000) and Ca^{2+} channels (Zhang et al., 2000), and a positive modulatory effect on at least one type of K^+ channel (Herrero et al., 2002; Russo and Constanti, 2004; Russo et al., 2004).

Recently, second-messenger pathways have been implicated as the targets of carbamazepine and valproate, but their mechanisms remain elusive (Berridge et al., 1989; Williams et al., 2002; Harwood and Agam, 2003). The phosphatidyl-inositol cycle plays an important role in intracellular Ca^{2+} signaling, endoplasmic reticulum associated Ca^{2+}-induced Ca^{2+}-releasing (CICR) (Williams et al., 2002; Harwood and Agam, 2003). Recently, the functional importance of multiple intraneuronal Ca^{2+} homeostatic system, including CICR, in the pathogenesis of epileptic seizure and neuronal damage associated with epilepsy has been demonstrated by several studies (Pal et al., 2000, 2001; Raza et al., 2001, 2004).

Release of synaptic vesicles containing neurotransmitters is triggered by influx of Ca^{2+} through voltage-sensitive Ca^{2+} channel (VSCC). The influx is induced by depolarization in nerve terminal and increases of intracellular Ca^{2+} from a basal level of 100 nM to more than 100 μM (Sollner et al., 1993; Rettig and Neher, 2002). In contrast, spontaneous neurotransmitter release can occur without this process (Emptage et al., 2001). It has been suggested that CICR can contribute to neurotransmitter exocytosis within neurosecretory tissue (Tse et al., 1997; Berridge, 1998; Rettig and Neher, 2002; Zhu et al., 2004a). We have demonstrated that therapeutically relevant concentrations of anticonvulsant mood stabilizers enhance basal monoamine release, which is regulated by both N-type VSCC and syntaxin-associated exocytosis mechanism, as well as inhibit K^+-evoked monoamine release, which is regulated by both P-type VSCC and synaptobrevin-associated exocytosis mechanism (Kawata et al., 2001; Murakami et al., 2001; Okada et al., 2002). At the same time, a line of evidence suggests that epileptic neuronal hyperexcitability stimulates not only VSCC and glutamate receptors but also CICR (Avoli et al., 1990; Parsons et al., 2001; Raza et al., 2001). Taken together with these previous evidences, the exocytosis mechanism associated with soluble *N*-ethylmaleimide-sensitive factor attachment protein receptors (SNARE) and CICR could be a key to further understanding of the antiepileptic and psychotropic mechanisms of TPM. Previously, the inhibitory effects of TPM on nicotine-induced elevation of dopamine release have been demonstrated (Schiffer et al., 2001). However, the effects of TPM on CICR-associated neurotransmitter exocytosis remained to be clarified. Therefore, the present study was designed to examine the involvement of the interaction between Ca^{2+}-associated intraneuronal signaling, containing SNAREs, CICRs and TPM on releases of dopamine and serotonin in rat pre-frontal cortex (pFC) region.

EXPERIMENTAL PROCEDURES

All of the experiments described in this report conformed to the specifications of the Animal Research Committee and international guidelines on the ethical use of animals. Effort was made to minimize the number of animals used and their suffering. Male Wistar rats (Clea, Tokyo, Japan), weighing 250–300 g, were housed under conditions of constant temperature 22±2 °C with a 12-h light/dark cycle.

Chemical agents

The chemical agents used in this study included: the inositol 1,4,5-trisphosphate receptor (IP3R) agonist, adenophostin A (AdA: Sigma-Aldrich, St. Louis, MO, USA); the IP3R antagonist, xestospongin C (XeC: Wako Chemicals, Osaka, Japan); the ryanodine receptor (RyR) agonist, ryanodine (Calbiochem, San Diego, CA, USA); the RyR antagonist, Ruthenium Red (RR: Tokyo Chemicals, Tokyo, Japan); the synaptobrevin inhibitor, botulinum toxin (BoNT) type B (BoNT/B) (Wako Chemicals); the syntaxin inhibitor, BoNT/C (Wako Chemicals); the SNAP-25 inhibitor, BoNT/A (Wako Chemicals) and TPM.

Microdialysis system preparation

Each rat was placed in a stereotaxic frame and kept under halothane anesthesia (1.5% mixture of halothane and O_2 with N_2O). Before inserting the microdialysis probe, all rats used in this study were pre-treated with a microinjection of 0.3 μl modified Ringer's solution (MRS) with or without BoNTs (from 0.3 ng to 3 ng) (Murakami et al., 2001; Okada et al., 2001; Zhu et al., 2004b). A concentric I-type dialysis probe (0.22 mm diameter; 3 mm exposed membrane; Eicom, Kyoto, Japan) was implanted in pFC (A=+3.2 mm, L=0.8 mm, V=−5.5 mm relative to bregma) (Zhu et al., 2002), and the perfusion experiments were started 36 h after the rats had recovered from anesthesia (Murakami et al., 2001; Okada et al., 2001; Zhu et al., 2004b). The perfusion rate was always 1 μl/min, using MRS composed of (in mM): 145 Na^+, 2.7 K^+, 1.2 Ca^{2+}, 1.0 Mg^{2+}, 154.4 Cl^-, and buffered with 2 mM phosphate buffer and 1.1 mM Tris buffer to adjust the pH to 7.40 (Murakami et al., 2001; Okada et al., 2001; Zhu et al., 2004b). To study the effects of an increase in the extracellular Ca^{2+} (Ca^{2+}-evoked stimulation) or K^+ levels (K^+-evoked stimulation) on the extracellular monoamine level in pFC, MRS containing 3.4 mM Ca^{2+} (HCMRS) or 50 mM K^+ (HKMRS) was perfused for 20 min, respectively (Murakami et al., 2001; Okada et al., 2001; Zhu et al., 2004b). The ionic composition was modified and isotonicity was maintained by an equimolar decrease of Na^+ (Murakami et al., 2001; Okada et al., 2001; Zhu et al., 2004b). Each pre-frontal dialysate was injected every 20 min into a high performance liquid chromatograph (HPLC).

HPLC system preparation

The HPLC system used for determination of the extracellular levels of dopamine and serotonin was equipped with an electrochemical detector (ECD-300; Eicom) with pump (EP-300; Eicom) and a graphite carbon electrode set at +450 mV (vs an Ag/AgCl reference electrode). The analytical column (100×1.5 mm, internal diameter) was packed with Mightysil RP-18 (particle size, 5 μm) (gift from Kanto Chemicals, Tokyo, Japan) by Masis Inc. (Hirosaki, Japan). The mobile phase was composed of 0.1 M phosphate buffer containing 20% (v/v) methanol, 900 mg/l octansulfonic sodium, and 50 mg/l EDTA-Na_2; the final pH was 5.9, and the column temperature was maintained at 25 °C with the flow rate set at 200 μl/min (Murakami et al., 2001; Okada et al., 2001, 2002; Zhu et al., 2004b).