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Amiodarone reduces depolarization-evoked glutamate release from hippocampual synaptosomes

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ABSTRACT

Decreased brain glutamate level has emerged as a new therapeutic approach for epilepsy. This study investigated the effect and mechanism of amiodarone, an anti-arrhythmic drug with antiepileptic activity, on glutamate release in the rat hippocampus. In a synaptosomal preparation, amiodarone reduced 4-aminopyridine-evoked Ca^{2+}-dependent glutamate release and cytosolic Ca^{2+} concentration elevation. Amiodarone did not affect the 4-aminopyridine-evoked depolarization of the synaptosomal membrane potential or the Na^{+} channel activator veratridine-evoked glutamate release, indicating that the amiodarone-mediated inhibition of glutamate release is not caused by a decrease in synaptosomal excitability. The inhibitory effect of amiodarone on 4-aminopyridine-evoked glutamate release was markedly decreased in synaptosomes pretreated with the $Ca_V2.2$ (N-type) and $Ca_V2.1$ (P/Q-type) channel blocker ω-conotoxin MVIIC, the calmodulin antagonists W7 and calmidazolium, or the protein kinase A inhibitors H89 and KT5720. However, the intracellular Ca^{2+}-release inhibitors dantrolene and CGP37157 had no effect on the amiodarone-mediated inhibition of glutamate release. Furthermore, amiodarone reduced the frequency of miniature excitatory postsynaptic currents without affecting their amplitude in hippocampal slices. Our data suggest that amiodarone reduces Ca^{2+} influx through N- and P/Q-type Ca^{2+} channels, subsequently reducing the Ca^{2+}-calmodulin/protein kinase A cascade to inhibit the evoked glutamate release from rat hippocampal nerve terminals.

1. Introduction

Glutamate is the main excitatory neurotransmitter in the central nervous system (CNS) of mammals, in which it acts through ionotropic and metabotropic glutamate receptors to regulate many physiological functions including cognition, learning, and memory (1,2). However, excessive glutamate release and activation of glutamate receptors eventually lead to calcium overload and subsequent cell death, and this glutamate neurotoxicity has been implicated in the pathogenesis of numerous brain disorders including epilepsy (3–5). A significant increase in the glutamate level has been observed in patients with epilepsy as well as in experimental models of epilepsy (6–8). Thus, reducing glutamate release may be a potentially crucial mechanism for antiepileptic effects. Several clinically used antiepileptic drugs, such as carbamazepine, phenytoin, lamotrigine, and gabapentin, have been reported to exhibit such a mechanism (9–11).

Abbreviations: $[Ca^{2+}]_C$, cytosolic free Ca^{2+} concentration; $DiSC_3(5)$, 3′,3′,3′-dipropylthiadicarbocyanine iodide; DL-TBOA, DL-threo-beta-benzyl-oxy aspartate; Fura-2-AM, fura-2-acetoxymethyl ester; GDH, glutamate dehydrogenase; HBM, HEPES buffer medium; BSA, bovine serum albumin; CGP37157, 7-chloro-5-(2-chlorophenyl)-1,5-dihydro-4,1-benzothiazepin-2(3H)-one; H89, N-[2-(p-bromocinnamylamino)-ethyl]-5-isoquinolinesulfonamide dihydrochloride; KT5720, hexahydro-10-hydroxy-9-methyl-1-oxo-9,12-epoxy-1H-diindolo-benzodiazocine-10-carboxylic acid; GF109203X, bisindolylmaleimide I; mEPSCs, miniature excitatory postsynaptic currents; CNQX, 6-cyano-7-nitroquinoxaline-2,3-dione; D-AP5, D(-)-2-amino-5-phosphonopentanoic acid; CNS, central nervous system.

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Amiodarone is an anti-arrhythmic drug that is commonly prescribed to patients with atrial fibrillation and ventricular arrhythmias (12,13). The pharmacologic profile of this drug in the heart is complex, involving inhibition of sodium and calcium channels, modulation of potassium outward current, and antagonistic effects on adrenergic receptors (14–16). In addition to its anti-arrhythmic effects, amiodarone has been shown to pass into the brain (17) and to increase the concentrations of inhibitory neurotransmitters in the rat medulla oblongata (18). Furthermore, amiodarone attenuates pentylenetetrazole- and caffeine-induced seizures in mice (19). Thus, amiodarone may have antiepileptic effects, but the underlying mechanism is unclear.

Considering the involvement of excessive glutamate release in the pathogenesis of epilepsy, the antiepileptic effect of amiodarone in experimental models may be associated with a decrease in glutamate release. However, according to our review of the literature, no information is available regarding the possible effect of amiodarone on glutamate release at the presynaptic level. Therefore, the present study investigated the effect and mechanism of amiodarone on glutamate release from rat hippocampal nerve terminals (synaptosomes), a system particularly suited for studying presynaptic events (20). We also used intact neurons in hippocampal slices to examine the effect of amiodarone on spontaneous-release events; miniature excitatory postsynaptic currents (mEPSCs) (21). In this study, the hippocampus was chosen because of it plays a crucial role in the pathogenesis of epilepsy (22), and neurodegeneration of this brain region has been attributable to glutamate (23).

2. Material and methods

2.1. Chemicals

Amiodarone, 4-aminopyridine, veratridine, bafilomycin A1, DL-*threo*-β-benzyloxyaspartate (DL-TBOA), ω-conotoxin MVIIC, dantrolene, 7-chloro-5-(2-chlorophenyl)-1,5-dihydro-4,1-benzothiazepin-2(3H)-one (CGP37157), D(−)-2-amino-5-phosphonopentanoic acid (D-AP5), bisindolylmaleimide I (GF109203X), N-[2-(p-bromocinnamylamino)-ethyl]-5-isoquinolinesulfonamide dihydrochloride (H89), and hexahydro-10-hydroxy-9-methyl-1-oxo-9,12-epoxy-1H-diindolo-benzodiazocine-10-carboxylic acid (KT5720) were purchased from Tocris Cookson (Bristol, UK). 3′,3′,3′-dipropylthiadicarbocyanine iodide [$DiSC_3(5)$], and fura-2-acetoxymethyl ester (Fura-2-AM) were purchased from Invitrogen (Carlsbad, CA, USA). Ethylene glycol bis (β-aminoethyl ether)-N,N,N′,N′-tetraacetic acid (EGTA), tetrodotoxin and all other reagents were purchased from Sigma–Aldrich (St. Louis, MO, USA). $DiSC_3(5)$, and Fura-2 were dissolved in dimethylsulfoxide, with a final concentration in the medium was ≤0.1% (v/v).

2.2. Animals

Adult (150–200 g) and 8- to 23-day-old male Sprague–Dawley rats were purchased from BioLASCO (Taiwan Co., Ltd, Taipei, Taiwan). Animals were housed under constant conditions of temperature (22 ± 1 °C) and relative humidity (50–70%) with a regular light–dark schedule (lights on from 7 am to 7 pm) and free access to food and water. Animal experiments were in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory, and were approved by the Institutional Animal Care and Use Committee at the Fu Jen Catholic University. All efforts were made to minimize animal suffering and to use a minimum number of animals necessary to produce reliable results.

2.3. Preparation of synaptosomes

Rats were decapitated, brains were quickly removed and the hippocampus dissected out at 4 °C. As previously described (24), synaptosomes were prepared from the hippocampus of adult rats. The final synaptosomal pellets were resuspended in HEPES buffer medium (HBM) with the following composition (mM): NaCl, 140; KCl, 5; $NaHCO_3$, 5; $MgCl_2{\cdot}6H_2O$, 1; Na_2HPO_4, 1.2; glucose, 10; HEPES, 10; pH 7.4. Protein concentration was then determined using the Bradford assay. Synaptosomes were centrifuged in the final wash to obtain synaptosomal pellets with 0.5 mg protein. Synaptosomal pellets were stored on ice and used within 4–6 h.

2.4. Synaptosomal glutamate release

Glutamate release was assayed by on-line fluorimetry as described previously (24). Synaptosomal pellets were resuspended in HBM (0.5 mg/mL) and preincubated at 37 °C for 1 h in the presence of 16 μM bovine serum albumin (BSA) to bind any free fatty acids released from synaptosomes during the preincubaion. A 1 ml aliquot was transferred to a stirred and thermostated cuvette containing 2 mM $NADP^+$, 50 units of glutamate dehydrogenase (GDH), and 1.2 mM $CaCl_2$, and the fluorescence of NADPH was followed in a Perkin-Elmer LS-55 spectrofluorimeter (PerkinElmer Life and Analytical Sciences, Waltham, MA, USA) at excitation and emission wavelengths of 340 and 460 nm, respectively. A standard of exogenous glutamate (5 nmol) was added at the end of each experiment and the fluorescence change produced by the standard addition was used to calculate the released glutamate as nanomoles glutamate per milligram synaptosomal protein (nmol/mg). Release values quoted in the text and depicted in bar graphs represent the levels of glutamate cumulatively released after 5 min of depolarization, and are expressed as nmol/mg/5 min. Data were accumulated at 2-s intervals and cumulative data were analyzed using Lotus 1-2-3.

2.5. Synaptosomal cytosolic Ca^{2+} concentration ($[Ca^{2+}]_C$)

$[Ca^{2+}]_C$ was measured with fura-2. Synaptosomes were resuspended (2 mg/mL) in HBM containing 16 μM BSA in the presence of 5 μM fura-2 and 0.1 mM $CaCl_2$, and incubated at 37 °C for 30 min. After fura-2 loading, the synaptosomes were pelleted and resuspended in HBM containing bovine serum albumin. A 1 ml aliquot was stirred in a thermostated cuvette containing 1.2 mM $CaCl_2$ and the fluorescence was monitored at excitation wavelengths of 340 and 380 nm (emission wavelength 505 nm). Data was collected at 2-s intervals and $[Ca^{2+}]_C$ (nM) was calculated using the equations described previously (25).

2.6. Synaptosomal plasma membrane potential

The plasma membrane potential was determined using a membrane-potential-sensitive dye, $DiSC_3(5)$ (26). Synaptosomes were resuspended in HBM and incubated in a stirred and thermostated cuvette at 37 °C in a Perkin-Elmer LS-55 spectrofluorimeter. After 3 min of incubation, 5 μM $DiSC_3(5)$ was added and allowed to equilibrate before the addition of 1 mM $CaCl_2$ after 4 min of incubation. 4-aminopyridine was then added to depolarize the synaptosomes for 10 min, and $DiSC_3(5)$ fluorescence was monitored at excitation and emission wavelengths of 646 and 674 nm, respectively. Cumulative data were analyzed using Lotus 1-2-3 and expressed in fluorescence units.

2.7. Slice preparation and electrophysiological recording

Hippocampal slices (300 μm) were prepared from 8- to 23-day-old male rats (n = 5), as described in detail previously (27, 28). The

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