ABSTRACT

Objective: Oxidative stress is thought to be responsible for neurotoxicity through the generation of oxygen radicals. The aim of the current study was to determine the generation of superoxide radicals under depolarization conditions in rat striatal slices. We also investigated the effect of intracellular and extracellular calcium ion concentrations on superoxide radical formation.

Methods: Striatal slices were obtained using a vibroslicer and after incubation of slices in appropriate incubation media the formation of superoxide radicals was detected by the chemiluminescence technique using a luminometer.

Results: Depolarization conditions (50 mM-K+) were used to induce superoxide radical generation in striatal slices. Under depolarization conditions lactate dehydrogenase activity (LDH) was also increased. Incubation of striatal slices in calcium chelator EGTA and intracellular calcium release blocker ryanodine increased superoxide generation compared to depolarization conditions.

Conclusion: Under calcium ion deficiency conditions, increased superoxide generation may be related with the suppression of LTD in striatum. Suppression of free radical generation from different sources in the neuron may help to develop new therapeutical approaches and can prevent neurodegeneration in the striatum.

Key Words: Striatal slices, superoxide radical, chemiluminescence recording, calcium ion deficiency

INTRODUCTION

Basal ganglia is formed from a group of deep forebrain nuclei that play an important role in the regulation of motor behaviour. Striatum is the central processing region of basal ganglia. Disorders of striatum include Parkinson's and Huntington's diseases. Afferents to neostriatum from neocortex induce glutamatergic synaptic transmission (1,2).

Glutamate is an excitatory neurotransmitter which mediates its effect through the activation of ionotropic and metabotropic receptors (3,4). Metabotropic receptor activation is related with synaptic plasticity (5). Long-term potentiation (LTP) in hippocampal slices has been implicated with learning and memory (6), whereas long-term depression (LTD) of synaptic activity has been observed following high frequency activation of synapses in striatal slices. Striatal LTD generation was found to be regulated by intracellular free calcium ion concentration (1).

Oxidative stress is thought to be responsible for neurotoxicity through the generation of oxygen free radicals (7,8). Free radicals have been correlated with neurodegenerative diseases such as Parkinson's, Huntington's and Alzheimer's diseases (9,10). The aim of the current study was to determine the generation of superoxide radicals under depolarization conditions in rat striatal slices. We also investigated the effect of intracellular and extracellular calcium ion concentrations on superoxide radical formation.

MATERIALS AND METHODS

The study was approved by the Animal Care and Ethics Committee of Marmara University School of
Forty rats were divided into control and experimental groups; n_c=10 and n_e=30, respectively. Each study day two Sprague-Dawley rats (3 weeks old) were decapitated and their brains were quickly removed. The hemispheres were separated and a 2 mm block was removed from the anterior and a 3 mm block was removed from the posterior part of each hemisphere. Then, the hemisphere containing striatum was glued to a vibroslicer stage and slices of 400 mm were obtained using a vibroslicer (Campden Instruments Ltd, UK). Striatum was isolated and striatal slices were kept for 45 minutes in artificial cerebrospinal fluid (ACSF) under carbogen (95% O_2+5% CO_2) aeration for functional recovery. Number of striatal slices were 12-14. ACSF had the following composition (mM): 125 NaCl, 3.75 KCl, 1.2 NaH_2PO_4, 2 CaCl_2, 1.3 MgCl_2, 10 glucose, 26 NaHCO_3. After equilibration, striatal slices were incubated in ACSF (as control) and in depolarizing ACSF (dACSF) for 45 minutes. Composition of the dACSF was as follows (mM): 79 NaCl, 50 KCl, 1.2 NaH_2PO_4, 2 CaCl_2, 1.3 MgCl_2, 10 glucose, 26 NaHCO_3. Calcium free dACSF and ethylene glycol-bis (ß-aminoethyl ether) N,N,N',N'-tetraacetic acid (EGTA, 500 μM) were used to block calcium ion mediated events. Composition of the calcium free dACSF was as follows (mM): 81 NaCl, 50 KCl, 1.2 NaH_2PO_4, 1.3 MgCl_2, 10 glucose, 26 NaHCO_3. Ryanodine (4 and 12μM) was used to block intracellular calcium release. The formation of superoxide radicals was detected using a luminometer (EG&G Berthold, Mini Lumat LB 6506, Germany) (11). Chemiluminescence was recorded at intervals of 15 seconds for ten minutes after the addition of lucigenin into tubes; each tube containing one striatal slice in Hank's buffer. The formation of superoxide radicals was recorded at intervals of 15 seconds for ten minutes after the addition of lucigenin into tubes; each tube containing one striatal slice in Hank's buffer. The significance of differences between the experimental groups was estimated by one-way analysis of variance (ANOVA) using Tukey-Kramer multiple comparison post-test. The differences were considered significant when the p value was smaller than 0.05 (p<0.05).

RESULTS

Chemiluminescence detection of superoxide radical generation was performed in ACSF and dACSF incubated striatal slices. Superoxide radical generation was significantly increased (p<0.001) in dACSF incubated striatal slices (Fig. 1). LDH activity was used as a parameter to detect neuronal damage and was measured in the incubation medium of slices (n=7). LDH activity of control slices (ACSF) was 37±5 IU/L. It was significantly increased (p<0.001) to 157±50 IU/L under depolarization conditions (dACSF).

![Fig. 1.](image)

Effect of depolarization conditions on superoxide radical generation. Under depolarization (dACSF) conditions superoxide formation was significantly increased compared to that generated under control conditions (ACSF).
Superoxide radical generation in rat striatal slices

Figure 2 shows the effect of intracellular and extracellular calcium concentrations on superoxide radical formation. Superoxide radical generation in dACSF incubated striatal slices was compared with that of slices incubated in Ca²⁺-free dACSF+EGTA. Our results indicated that conditions of extracellular calcium ion deficiency enhanced superoxide radical formation significantly (p<0.001). In order to further provoke superoxide radical generation, calcium chelator EGTA was added to calcium-free dACSF and striatal slices were incubated in this solution under carbogen aeration. The addition of intracellular calcium release blocker ryanodine at two different concentrations (4 and 12 μM) into the incubation medium significantly increased (p<0.001) superoxide radical formation. The effect of ryanodine at two different concentrations was also significantly (p<0.001) different in each case.

DISCUSSION

In this study we have shown that depolarization conditions stimulate superoxide radical generation in striatal slices. Furthermore, generation of superoxide radical was enhanced under intracellular and extracellular calcium ion deficiency conditions. In a previous study increased external potassium concentration was reported to induce spontaneous depolarizing potentials in striatal neurons (12). In addition, depolarization with 50 mM K⁺ generated a calcium load in the dorsal root ganglion neuron in vitro (13).

Neuroplasticity is the adaptation or modification of the synaptic response. It is activity dependent and repetitive activation of the major excitatory input to striatum; i.e. corticostratal glutamatergic fibers, have been shown to evoke LTD of the excitatory synaptic transmission (14). LTD is thought to be important in striatum for motor activity control. Intracellular application of calcium chelators EGTA and BAPTA were shown to block striatal LTD (1). Striatal LTD generation was also shown to involve calcium mobilization and phosphatidylinositol turnover and it was found to be sensitive to glutamate metabotropic receptor antagonists (15,16).

Ryanodine sensitive intracellular calcium channels have been reported to cease their activity upon ryanodine application (17). In this study, we observed that ryanodine at two different concentrations increased superoxide formation in striatal slices. Aggravation of superoxide radical generation was dependent on ryanodine concentration of the medium; i.e. 12 μM was more effective in inducing superoxide generation. Ryanodine may lead to increased superoxide radical generation by blocking intracellular calcium channels and LTD generation in striatal slices. High frequency stimulation of corticostratal afferents is a requirement for LTD generation; besides free calcium ion is essential for its maintenance. In cultured Purkinje neurons, long-duration depolarizing pulses have been reported to elicit more prominent calcium-induced calcium release that may play an important role in LTD generation (18). Thus, LTD maintenance is correlated with calcium homeostasis and the activity suppression should be critical in striatum because of its high iron content.

Mice fed on a high iron diet were reported to have increased oxidized glutathione and hydroxyl radical...
levels in the striatum (19). Under these conditions superoxide can rapidly be converted to highly reactive hydroxyl radical which has been implicated as the primary toxic mediator of damage to proteins, carbohydrates, DNA and lipids. We have shown that both calcium free depolarization conditions and the presence of EGTA (calcium free dACSF+EGTA) exacerbate superoxide radical generation in comparison to that generated under depolarization conditions (dACSF). In rat neostriatal slices depolarizing postsynaptic potentials was shown to be followed by afterhyperpolarization; calcium influx may lead to potassium permeability increase (20). Therefore, calcium ion deficiency may block potassium ion permeability of the membrane that is responsible for repolarization and that might be another reason for increased superoxide radical generation in striatal slices.

It is apparent that in striatal slices, disturbance of calcium ion homeostasis under depolarization conditions can increase superoxide generation. We suggest that suppression of oxygen free radical generation from different sources in the neuron will help to develop new therapeutic strategies which may be important in the control of neurodegeneration. However, clinical application of therapeutic agents causing changes in calcium homeostasis should be carefully considered.

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