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Effect of Somatostatin Analogs on Chemically Induced Ischemia in Rat Retina

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S u m m a r y. The retina is subject to ischemic damage resulting from different causes such as occlusion of blood flow to the retina. Ischemia underlies the pathophysiology of many retinal diseases such as diabetic retinopathy. Somatostatin and the novel neuropeptide cortistatin have been shown to act as neuroprotectants in different experimental paradigms. The purpose of the present study was to examine the putative neuroprotective effect of somatostatin, cortistatin and sst_2 selective agonists in retinal explants that were subjected to chemical ischemia.

INTRODUCTION

In recent years, research has been directed towards the search of agents that ameliorate neuronal damage and protect from neurotoxicity and ischemia in CNS areas, including the retina. Different animal models such as permanent middle cerebral artery occlusion, and glutamate-mediated excitotoxicity have been employed to induce ischemic insults. The peptide somatostatin has been shown to have a neuroprotective effect against NMDA-induced neurotoxicity in cortical cultures (1), while somatostatin and its analog octreotide reduced the infarct size when administered (icv) 5 min after middle cerebral artery occlusion (2). The recently discovered novel neuropeptide cortistatin (3) which resembles somatostatin structurally and binds to somatostatin receptors, was also shown to have protective effects against kainate-induced neurotoxicity (4) as well as on ischemic neuronal damage following middle cerebral artery occlusion (2). The reported neuroprotective effect of somatostatin and analogs may be due to their ability to inhibit voltage-dependent Ca^{++} currents (5) and reduce intracellular calcium levels. Activation of excitatory NMDA receptors and subsequent increases in Ca^{++} levels appear to be instrumental in the development of neurotoxicity (6). In the retina, ischemia-induced retinal neovascularization is one of the major causes of diseases such as diabetic retinopathy, and age-related macular degeneration that result in visual loss. Somatostatin is one of many neuroactive substances that influence retinal physiology by activating specific receptors (sst_1-sst_5) found in retinal neurons and the retinal pigment epithelium (7). The purpose of the present study was to investigate the effect of somatostatin, selective sst analogs, and cortistatin on chemically induced ischemia in rat retina. We employed a pharmacological model of chemical ischemia first employed in hippocampal slices (8). This model involves the blockade of oxidative phosphorylation and glycolysis and is believed to be useful in the understanding of the early events underlying the pathophysiology of ischemia.

METHODS

Female Sprague-Dawley rats (250-300g) were employed in accordance with Greek National Laws (Animal Act, P.D. 160/91). Euthanasia was performed with ether inhalations. To induce ischemia, eyecups were immersed in PBS buffer containing iodoacetic acid (IAA; 0.5, 5, 50,100 mM) and sodium cyanate (NaCN; 2.5, 25, 250, 500mM)[chemical ischemia mixture] for 15, 30, 45, 60, 120 min, and subsequently fixed and sectioned for immunohistochemistry studies. To ascertain the protective properties of somatostatin, cortistatin, BIM23014 and MK678, the eyecup was incubated with a) somatostatin (1, 10, 100 μM) which was added prior to, together or after the chemical ischemia mixture using different time regimens, and b) cortistatin, BIM23014 or

MK678 (1 μM) added together with the chemical ischemia mixture for 60 min, with subsequent additions (every 30 min, for up to 120 min), after the removal of the ischemia mixture. Chemical ischemia was ascertained initially by Choline Acetyl Transferase (ChAT), and subsequently by Tyrosine Hydroxylase (TH), and Protein Kinase C (PKC) immunohistochemistry.

RESULTS

Treatment of the eyecups with IAA (5, 50, 100 mM)/NaCN (25, 250, 500mM) for 60 and 120 min abolished ChAT. Therefore a mixture of 5mM IAA/25 mM NaCN was used to induce chemical ischemia in subsequent experiments. TH immunoreactivity was abolished, while PKC immunoreactivity was reduced. Cortistatin and the sst_2 agonists reversed the chemical ischemia as ascertained by ChAT, TH and PKC immunoreactivity. Somatostatin had no effect due to its degradation under the experimental conditions [$1x10^{-6}$M somatostatin was reduced to 306nM in the ischemia mixture during the 30 min treatment].

CONCLUSIONS

These results demonstrate the usefulness of somatostatin analogs as neuroprotectants in retinal ischemia.

REFERENCES

1. Forloni G., Lucca E., Angeretti N., Chiesa R., Vezzani A.: Neuroprotective effect of somatostatin on nonapoptotic NMDA-induced neuronal death: role of cyclic GMP. *J. Neurochem. 68*: 319-327 (1997)
2. Rauca C., Schafer K., Hollt V.: Effects of somatostatin, octreotide and cortistatin on ishemic neuronal damage following permanent middle cerebral artery occlusion in the rat. *Naunyn-Schmied Arch. Pharmacol. 360*: 633-638 (1999)
3. de Lecea L., Criado J.R., Prospero-Garcia O., Gautvik K., Schweitzer P., Danielson P.E., Dunlop C.L.M., Siggins G.R., Henriksen S.J., Sutcliffe J.G.: A cortical neuropeptide with neuronal depressant and sleep-modulating properties. *Nature 38*: 24-245 (1996)
4. Braun H., Schulz S., Becker A., Schroder H., Hollt V.: Protective effects of cortistatin (CST-14) against kainae-induced neurotoxicity in rat brain. *Brain Res. 803*: 54-60 (1998)
5. Wang H.L., Bogen C., Reisine T., Dichter M.: Somatostatin-14 and somatostatin-28 induce opposite effects on potassium currents in rat neocortical neurons. *Proc. Natl. Acad. Sci. USA 86*: 9616-9620 (1989)
6. Choi D.W.: Glutamate neurotoxicity and diseases of the nervous system. *Neuron 11*: 623-634 (1988)
7. Thermos K.: Functional mapping of somatostatin receptors in the retina: a review. *Vision Res. 43*: 1805-1815 (2003)
8. Reiner P.B., Laycock A.G., Doll C.J.: A pharmacological model of ischemia in the hippocampal slice. *Neurosci. Lett. 119*: 175-178 (1999)