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Effects of Somatostatin and Sandostatin® on Guinea-Pig Urinary Bladder Subjected to Experimental Ischemia and Reperfusion

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Somatostatin and its stable analogue Sandostatin® were tested for their capability to protect the intrinsic nerves in an *in vitro* model of guinea-pig whole urinary bladder subjected to ischemia and reperfusion injury. Sandostatin® (1 to 300 nM) and somatostatin (300 nM) improved significantly the response of the bladder to electrical field stimulation (EFS) during reperfusion as compared to untreated control bladders. Both Sandostatin® and somatostatin exhibited significant antiperoxidant activity with a pIC_{50} M values of 7.0 ± 0.6 and 6.7 ± 0.3, respectively, which could eventually underlie their neuroprotective action during reperfusion.

Key words: somatostatin, sandostatin, bladder, ischemia, reperfusion.

Introduction

It is generally accepted that the partial bladder obstruction represents one of the major pathologies of the urinary bladder that leads to chronic post-void residual urine or acute urinary retention followed by progressive contractile failure. A current hypothesis is that bladder decompensation following partial bladder obstruction is directly related to decreased tissue perfusion, resulting in periods of hypoxia and ischemia [3]. It is important to look for substances which could decrease the ischemia-reperfusion induced neuronal damage in detrusor muscle and in such a way to ameliorate the functional disorders of urinary bladder. Somatostatin-14 and particularly its stable analogue, the cyclic octapeptide Sandostatin®, are known to exert cytoprotective activities in peripheral tissues and in neuronal cells. The somatostatin peptides were also shown to afford protection against neuronal damage caused by experimentally induced cerebral ischemia in rats [8]. However, their effect on detrusor nerves subjected to ischemia-reperfusion, has not been established so far. The aim of this study, therefore, is to examine the efficacy of somatostatin and its stable analogue Sandostatin® to counteract the damage suffered by neurons in an *in vitro* model of whole urinary bladder subjected to ischemia and reperfusion.

Materials and Methods

Male Charles River guinea-pigs (350-500 g) were anesthetized with Ketavet and sacrificed by cervical dislocation. The animals were treated in accordance with the European Committee standards concerning the care and use of laboratory animals. The neuroprotective effects of somatostatin and Sandostatin® were studied in an *in vitro* model of whole urinary bladder subjected to ischemia and reperfusion injury as it has been previously described [7].

Results

The response to electrical field stimulation (EFS) declined rapidly in the combined absence of oxygen and substrate (ischemia-like condition), and was abolished within an hour. After reintroduction of normal conditions, the recovery of the response to electrical field stimulation (neurogenic response) in control bladders was poor, reaching in 2 hours a maximum of about 25 % of the initial response (Fig. 1A and 1B). At this time, however, the response of the muscle to carbachol had fully recovered (data not shown). To see if somatostatin and Sandostatin® could partially reduce the nerve damage described above, the peptides have been perfused during ischemia and the first 30 min of reperfusion, as it is supposed that the major damage to the tissue develops not only during ischemia, but also at the beginning of reperfusion when free radicals are being formed intensively. Sandostatin® at 1, 100 and 300 nM improved significantly the EFS-induced contractile response in reperfusion phase, reaching 67.45 ± 5.10 % at 180 min for a concentration of 300 nM, as compared to 54.15 ± 6.85 % in control bladders ($n = 6$, $P < 0.01$) (Fig. 1B). Somatostatin at concentrations of 1 and 100 nM did not exert any effect (Fig. 1A),

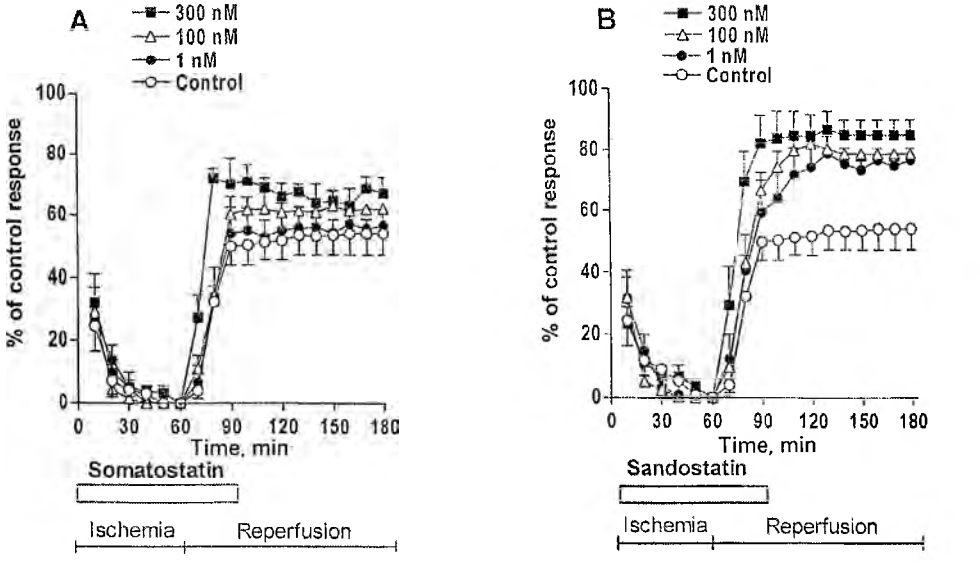

Fig. 1. Electrical field stimulation-induced contractile responses of guinea-pig whole urinary bladder subjected to 60 min of ischemia and subsequent 120 min of reperfusion. Experiments were carried out in the absence or presence of somatostatin (A) or Sandostatin® (B), applied for the first 90 min of the experiment. Results are expressed as mean ± SEM of six experiments in each group

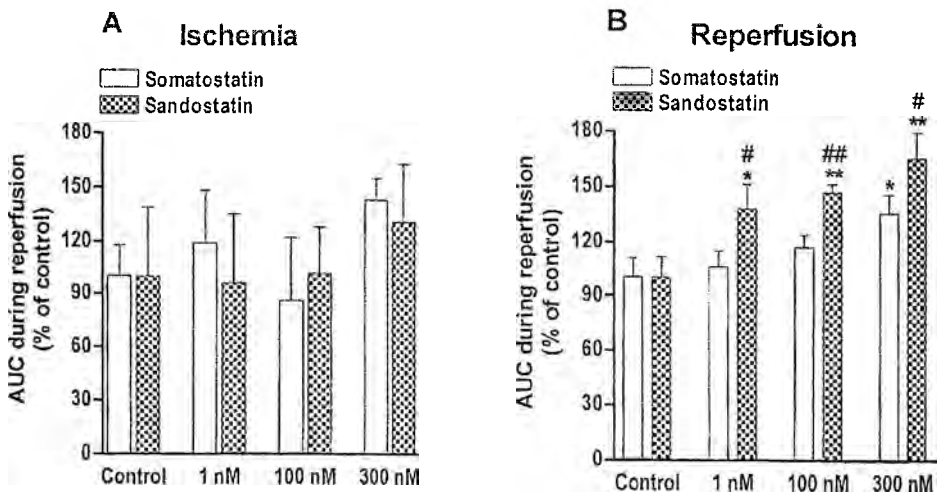

Fig. 2. Electrical field stimulation-induced contractile responses of guinea-pig whole urinary bladder subjected to 60 min of ischemia (A) and subsequent 120 min of reperfusion (B). Experiments were carried out in the absence or presence of somatostatin or Sandostatin®. Results are expressed as mean of area under curve (AUC) ± SEM of six experiments in each group. Differences among groups were evaluated by one-way ANOVA followed by Dunnett's *post hoc* comparison test ($^{*}P < 0.05$ and $^{**}P < 0.01$ versus controls; $^{\#}P < 0.05$ and $^{\#\#}P < 0.01$ versus somatostatin-treated group)

while at 300 nM it increased the recovery of contractile response during reperfusion (Fig. 2B). The antioxidant activity of the peptides was assessed for their capability to prevent linoleic acid peroxidation. Both somatostatin and Sandostatin® exhibited remarkable antiperoxidant activity with a pIC_{50} M values of 6.7 ± 0.3 and 7.0 ± 0.6, respectively (Table 1).

Discussion

Bladder outlet obstruction generally due to prostatic hyperplasia, a common problem in men over 60 years of age, is a major urologic problem that has been the subject of many clinical and experimental studies. The hyperplasia of prostate leads to obstructed micturition, during which occurs periodic bladder ischemia. The latter has been suggested to result in the partial denervation of the detrusor smooth muscle through ischemia and reperfusion injury to the post-ganglionic parasympathetic neurons within the bladder wall [1]. Previous investigations showed that *in vitro* ischemia-like condi-

T a b l e 1. Inhibition of lipid peroxidation

ANTIOXIDANT	IC_{50} (mM) ± SEM	pIC_{50} (mM) ± SEM
DTBHA	0.088 ± 0.006	7.1 ± 0.5
Sandostatin®	0.097 ± 0.011	7.0 ± 0.6
Somatostatin	0.164 ± 0.019	6.7 ± 0.3
Vasoactive intestinal peptide	0.380 ± 0.008	6.4 ± 0.1
BHA	0.428 ± 0.005	6.3 ± 0.4
β-TAG	1.040 ± 0.330	6.0 ± 0.4
Propofol	3.100 ± 0.380	5.5 ± 0.3
β-GLU	9.910 ± 2.480	5.0 ± 0.4

tions were more damaging to the nervous tissue than to the detrusor muscle [6]. Somatostatin-14 and its stable analogue Sandostatin® were shown to ameliorate pancreatic microcirculatory injury and enzyme release after ischemia-reperfusion of the pancreas [4] and to reduce liver and intestinal injury induced by hypoxia/ischemia in rats [5]. In this study we showed for the first time that Sandostatin® counteracts neuronal damage of the whole urinary bladder during reperfusion. The mechanisms by which Sandostatin® protects bladder nerves from reperfusion injury is only a matter of speculation, though a reduction in the amount of somatostatin among loss of other sensory neuropeptides in the obstructed human bladder has been previously described [2]. In the present study, a remarkable antioxidant activity of Sandostatin® has been found, which could underlie its neuroprotective action during reperfusion, when a significant amount of free radicals has been formed. In summary, the pharmacological action of drug Sandostatin® (Novartis), outlined in the present study, may represent a new therapeutic option for the control of functional disorders of the urinary bladder.

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