Superoxide dismutase partially prevents sympathetic denervation by 6-hydroxydopamine

A. Albino-Teixeira, Isabel Azevedo, Fatima Martel, and Walter Osswald
Department of Pharmacology and Therapeutics, Faculty of Medicine, P-4200 Porto, Portugal

Received January 18, 1991/Accepted March 18, 1991

Summary. The effects of superoxide dismutase (S.O.D.) in two models of chemical denervation induced by 6-hydroxydopamine (6-OHDA) were studied. To evaluate the effects of S.O.D. on in vitro 6-OHDA-induced denervation, fragments of the lateral saphenous veins of mongrel dogs were pre-incubated in oxygenated Krebs-Henseleit solution with or without S.O.D. and then incubated under control conditions, with 6-OHDA or with 6-OHDA + S.O.D. Following the incubation period the fragments were repeatedly washed with Krebs solution and then used for determination of noradrenaline and for morphological study. 6-OHDA produced a profound depletion of noradrenaline. This depletion was significantly reduced although not prevented by S.O.D. The protective effect of S.O.D. was concentration-dependent. The ultrastructural study confirmed the 6-OHDA-induced sympathetic nerve degeneration as well as the protective effect afforded by S.O.D.

In order to evaluate the effects of S.O.D. on in vivo 6-OHDA-induced denervation, male Wistar rats were anaesthetized and the tail vein cannulated. Saline or S.O.D. were intravenously delivered. 6-OHDA was injected five minutes after the beginning of infusions. Fragments of the left ventricle and vasa deferentia were used for determination of noradrenaline and for morphological study. 6-OHDA produced a significant depletion of noradrenaline in the left ventricle and vas deferens (to 8% and 18% of control values respectively). This depletion was reduced, though not prevented by S.O.D. Morphological data confirmed the neurotoxic effect of 6-OHDA and a protective role for S.O.D.

In the concentration shown to afford protection against in vitro 6-OHDA-induced denervation, S.O.D. neither chemically inactivated 6-OHDA, nor did it exert any blocking effect on the neuronal uptake of 3H-noradrenaline. Thus, the protection afforded by S.O.D. against chemical denervation by 6-OHDA appears to be due to the free radical scavenging effect of S.O.D.

Key words: Sympathetic denervation — 6-Hydroxydopamine — Free radicals — Superoxide dismutase

Introduction

We have found that continuous intravenous infusion of noradrenaline (0.1 μg · kg⁻¹ · h⁻¹) causes damage to the sympathetic nerve endings of the infused lateral saphenous vein of the dog (Albino-Teixeira et al. 1989). Concomitant changes in extraneuronal structure and function were observed (hypertrophy of smooth muscle cells, thickening of the vessel wall, impairment of O-methylating capacity). The same study provided evidence for the ability of desipramine (25 μg · kg⁻¹ · h⁻¹) and superoxide dismutase (S.O.D., 5 μg · kg⁻³ · h⁻¹) to prevent the changes induced by denervation. The data suggested that a substance derived from noradrenaline exerted a 6-hydroxydopamine-like effect and was responsible for the neurotoxic effect (Albino-Teixeira et al. 1989). Therefore, in order to further explore this analogy, we decided to investigate the effects of S.O.D. in two models of 6-hydroxydopamine (6-OHDA)-induced denervation.

Methods

In vitro 6-OHDA induced denervation. To evaluate the effects of S.O.D. on in vitro 6-OHDA-induced denervation, mongrel dogs were anaesthetized (sodium pentobarbitone, 30 mg · kg⁻¹ i.v.) and the lateral saphenous veins removed. Fragments weighing about 60 mg were pre-incubated in oxygenated drug-free Krebs-Henseleit solution with the following composition (in mmol · l⁻¹): NaCl 118, KCl 4.7, CaCl₂ 2.4, MgSO₄ 1.2, NaHCO₃ 25, KH₂PO₄ 1.2, glucose 11. Some fragments were incubated in Krebs-Henseleit solution containing S.O.D. (1.25, 2.5, 5 mg · ml⁻¹) for 30 min and then incubated under control conditions or with 6-OHDA (0.1 mg · ml⁻¹) or with 6-OHDA (0.1 mg · ml⁻¹) + S.O.D. (1.25, 2.5, 5 mg · ml⁻¹) for 3 h. All solutions were gassed with 5% CO₂ in O₂ at 37°C throughout the incubation procedure and constantly...
Table 1. Noradrenaline (NA) content of dog saphenous vein strips incubated for 3 h. In S.O.D.-treated preparations S.O.D. was added 30 min before 6-OHDA and maintained thereafter; results expressed as mean ± SEM

<table>
<thead>
<tr>
<th></th>
<th>NA (nmol · g⁻¹)</th>
<th>(% of control)</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>13.95 ± 0.58</td>
<td>100</td>
<td>4</td>
</tr>
<tr>
<td>S.O.D. (5 mg · ml⁻¹)</td>
<td>14.31 ± 0.63</td>
<td>103</td>
<td>4</td>
</tr>
<tr>
<td>6-OHDA (0.1 mg · ml⁻¹)</td>
<td>0.72 ± 0.21*</td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td>S.O.D. (1.25 mg · ml⁻¹) + 6-OHDA</td>
<td>1.38 ± 0.54*</td>
<td>8</td>
<td>4</td>
</tr>
<tr>
<td>S.O.D. (2.5 mg · ml⁻¹) + 6-OHDA</td>
<td>3.17 ± 0.46**</td>
<td>24</td>
<td>4</td>
</tr>
<tr>
<td>S.O.D. (5 mg · ml⁻¹) + 6-OHDA</td>
<td>5.19 ± 0.60**</td>
<td>37</td>
<td>4</td>
</tr>
</tbody>
</table>

* Significantly different from control group; P < 0.05
** Significantly different from 6-OHDA group; P < 0.05

Noradrenaline content

In vitro incubation of dog saphenous vein strips with 6-OHDA (0.1 mg · ml⁻¹) for 3 h resulted in a marked depletion of noradrenaline, to 5% of controls (Table 1). S.O.D. alone did not change the noradrenaline content of the venous tissue and significantly reduced, in a concentration-dependent way, the effect of 6-OHDA (Table 1).

In vivo administration of 6-OHDA (100 mg · kg⁻¹) to rats resulted in a significant depletion of noradrenaline both in the left ventricle and in the vas deferens (to 8% and 18% of control values, respectively) (Table 2). S.O.D. alone did not change the noradrenaline content of the left ventricle or vas deferens. When administered together with 6-OHDA to rats, S.O.D. significantly reduced the effect of 6-OHDA, in a dose-dependent way (Table 2).

Statistics

Results are presented as arithmetic means ± S.E.M. Student's t-test for unpaired results, and Tukey-Kramer's test (when multiple comparisons were carried out) were used for statistical analysis (Sokal and Rohlf 1981). The level of significance was set at P < 0.05.

Drugs used

Superoxide dismutase (Peroxinorm, Chemie Gruenenthal GmbH, Stolberg, FRG); sodium pentobarbitone (Abbott, Lisbon, Portugal); 6-OHDA and pargyline hydrochloride (Sigma Chemical Company, St. Louis, MO, USA); U-0521 or 3'-4'-dihydroxy-2-methylpropionophenone (Upjohn Co., Kalamazoo, MI, USA); (-)-7-3H-noradrenaline 13.0 Ci/nmol (NEN, Dreieich, FRG).

Results

Morphological results

Saphenous vein strips incubated with 6-OHDA had no clearly identifiable adrenergic varicosities. Schwann cells exhibited very dense bodies probably corresponding to degenerated adrenergic nerves (Fig. 1a). No other changes were observed. When vein strips were pretreated