Experimental Research

Inhibitory effect of vasopressin receptor antagonist OPC-31260 on experimental brain oedema induced by global cerebral ischaemia

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Summary

The effects of the non-peptide vasopressin V2 receptor antagonist 5-dimethylamino-1-[4-(2-methylbenzoylamino)benzoyl]-2,3,4,5-tetrahydro-1H-benzazepine hydrochloride (OPC-31260) on the cerebral oedema induced by general cerebral hypoxia were studied in rats. The general cerebral hypoxia was produced by bilateral common carotid ligation in Sprague-Dawley rats of the CFY strain. By 6 h after the ligation, half of the rats had died, but the survival rate was significantly higher following OPC-31260 administration. Electron microscopic examinations revealed typical ischaemic changes after the carotid ligation. The carotid ligation increased the brain contents of water and Na+ and enhanced the plasma vasopressin level. The increased brain water and Na+ accumulation was prevented by OPC-31260 administration, but the plasma vasopressin level was further enhanced by OPC-31260. These results demonstrate the important role of vasopressin in the development of the disturbances in brain water and electrolyte balance in response to general cerebral hypoxia. The carotid ligation-induced cerebral oedema was significantly reduced following oral OPC-31260 administration. The protective mechanism exerted by OPC-31260 stems from its influence on the renal vasopressin V2 receptors.

These observations might suggest an effective approach to the treatment of global hypoxia-induced cerebral oedema in humans.

Keywords: Carotid ligation; cerebral oedema; hyponatraemia; SIADH; vasopressin receptor antagonist; non-peptide.

Introduction

Yamamura et al. [43] reported a detailed characterisation of an orally effective, non-peptide vasopressin V2 receptor antagonist, 5-dimethylamino-1-[4-(2-methylbenzoylamino)benzoyl]-2,3,4,5-tetrahydro-1H-benzazepine hydrochloride (OPC-31260), which blocks the binding of vasopressin to renal plasma membranes in vitro, inducing a substantial diuretic effect. The urinary output and osmolality following oral administration of 30 mg/kg OPC-31260 were measured in rats. The duration of the diuretic effect of OPC-31260 proved to be about 6–8 h [26]. Other authors have described a considerable diuretic effect of OPC-31260 following intravenous or oral administration to healthy subjects [28, 29, 35]. OPC-31260 appears to be a promising drug from the viewpoint of clinical practice [8, 30, 38, 39, 44]. We therefore set out to investigate whether the non-peptide vasopressin V2 receptor antagonist OPC-31260 can prevent the development of cerebral oedema following bilateral carotid artery occlusion. A further aim was to study the role of vasopressin in the pathomechanism of...
cerebral oedema observable after cerebral hypoxia, and the mode of action of OPC-31260 in global cerebral ischaemia. We used Sprague-Dawley rats of the CFY strain. Earlier, characteristic symptoms of global cerebral ischaemia were described in this strain following bilateral carotid artery occlusion [19, 37].

**Methods**

**Experimental protocol**

The experiments were performed on 3- to 5-month-old male CFY rats, ranging in weight from 200 to 280 g (bred in our animal house; breeding stock from the Laboratory Animals Producing Institute, Gödöllő, Hungary). The animal care and research protocols were in accordance with the guidelines of our university. The animals were subjected to ether anaesthesia during operations. The rectal temperature was monitored, and cooling was prevented with an electric heating pad. Bilateral ligation of the common carotid arteries was performed for 1, 4 and 6 h under ether anaesthesia. In the control, sham-operated groups, the surgical manipulation was the same, but without carotid ligation. OPC-31260 in a dose of 30 mg/kg, or vehicle only, was administered by gastric tube: OPC-31260 was dissolved in water (15 mg/ml) immediately after the carotid ligation. The dose-response curve for OPC-31260 was reported by Yamamura *et al.* [44]. The antagonist dose and the duration of its effect were described in an earlier publication [26]. At the end of the experiments, the rats were killed by decapitation under ether anaesthesia.

**Blood pressure measurement**

In a separate group of animals (20 rats), the mean arterial blood pressure was measured. Ten rats underwent only bilateral carotid ligation, while the other group (10 rats) was treated orally with OPC-31260 (30 mg/kg) immediately after the ligation. Under ether anaesthesia, a polyethylene tube was inserted into the right carotid artery and diluted heparin was injected as an anticoagulant. Blood gases were monitored. The carotid cannulae were connected to Statham P23D transducers and blood pressure was recorded continuously during 6 h with a Hellige recorder. The method was described in detail earlier [25].

**Brain water and electrolyte contents**

The brain water content was determined by dehydration to weight constancy; 1, 4 or 6 h after the operation, the brain was removed and weighed before and after drying at 200°C for 24 h. This was followed by ashing at 550°C for 20 h, after which the ash was dissolved in 5 ml of 3 mM HNO₃ and the resulting solution was diluted 10-fold with deionised water. With a Perkin-Elmer 306 atomic absorption spectrophotometer (Perkin-Elmer, Norwalk, CT, USA) and use of an air-acetylene flame, the Na⁺ content was determined to be 330.3 nM and the K⁺ content to be 404.4 nM. The slit width was 0.7 and 2 mm, respectively. Tap water (0.5 ml) was administered instead of OPC-31260 to the control animals. The brain Na⁺ and K⁺ determinations were carried out in the Central Research Laboratory, Medical University, Szeged, Hungary. The plasma Na⁺ and K⁺ levels were determined with a flame-photometric micro method, and the osmolality with an Advance osmometer in 10 rats.

**Electron microscopic examination**

Following ether anaesthesia, the animals were killed by decapitation 1, 4 or 6 h after the carotid ligation. The brains were immediately removed and small pieces of the parietal cortex were placed in the fixative solution (1% glutaraldehyde and 1% paraformaldehyde in 0.1 M phosphate buffer, pH 7.4) for 4 h at 4°C. Before the electron microscopic study, light microscopic examination was carried out after staining with methylene blue-Azure II. After overnight washing in 0.1 M phosphate buffer, postfixation was performed in phosphate-buffered (pH 7.4) 1% osmium tetroxide solution for 1 h, and the tissue samples were dehydrated in a graded series of increasing ethanol concentration before embedding in Spurr. During dehydration, they were stained en bloc with uranyl acetate 0.5% (w/v) in 70% ethanol for 15 min. Ultrathin sections were cut on a Reichert-Jung Ultracut E ultramicrotome, contrasted with lead citrate and examined in a Zeiss EM 902 electron microscope. We used 6 rats in each group for light or electron microscopic examination. The evaluation was performed blind.

**Plasma vasopressin determination**

The plasma vasopressin levels were measured by radioimmunoassay (RIA), based on a technique described by Dogterom *et al.* [3] with some modifications, as reported in detail earlier [17, 21]. Synthetic arginine-8-vasopressin (Organon, Oss, The Netherlands; antidiuretic activity 408 IU/mg) was used as reference preparation for antibody production and radiolabelling. Vasopressin antibody was generated against the vasopressin-( ε-aminocaproic