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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 V_2 receptor antagonist 5-dimethylamino-1-[4-(2-methylbenzoylamino)benzoyl]-2,3,4,5-tetrahydro-1*H*-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 V_2 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 V_2 receptor antagonist, 5-dimethylamino-1-[4-(2-methylbenzoylamino)benzoyl]-2,3,4,5-tetrahydro-1*H*-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 aquaretic effect of OPC-31260 proved to be about 6–8 h [26]. Other authors have described a considerable aquaretic 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 V_2 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

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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 HNO_3 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

acid)-thyroglobulin conjugate in sheep. The immunisation procedure and the description of the anti-serum character can be found in an earlier publication [26]. ^{125}I-labelling of vasopressin was performed by the chloramine T method of Hunter and Greenwood [12]. Blood was obtained following decapitation, and 1-ml blood samples were placed in cooled polystyrene tubes [20] containing 1.4 mg of NaEDTA in 30 µl of isotonic NaCl, and centrifuged (1000 $g \times 10$ min) at 4 °C within 10 min. Plasma samples were stored at −20 °C until assaying. RIA was performed within 72 h after sampling. Vasopressin extraction was carried out with an Amprep C8 minicolumn (code RPN 1902 Amersham, Buckinghamshire, UK). The sensitivity of the RIA was 1 pg per assay tube. Vasopressin levels are given in pg/ml plasma.

Statistical analysis

The data are expressed as means ± S.E.M. of the results for the total number of rats per experimental group. Statistical analysis was performed by using the Mann–Whitney non-parallel *U*-test, the Bonferroni multiple comparisons test and the Fisher exact test, where appropriate. *P* values less than 0.05 were considered to be significant.

Results

The survival rate proved to be significantly higher in the OPC-31260-treated rats 4 or 6 h after the carotid ligation (Table 1). During the observation for 1, 4 or 6 h, the plasma osmolality and the plasma Na^+ and K^+ levels did not change significantly following ligation, and these data are therefore not reported here. Immediately after the ligation, the blood pressure significantly increased, but then returned to the control level (basal: 109.0 ± 6.2*, 15 sec: 137.3 ± 9.7, 10 min: 124.5 ± 7.1, 1 h: 124.3 ± 7.4, 4 h: 125.1 ± 9.4, 6 h: 102.3 ± 8.7 Hgmm, $n = 10$, *S.E.M.). The blood pressure did not change following OPC-31260 administration in comparison with the non-treated ligated group. The brain water content and ion concentrations are given in Table 2. These were not changed 1 h after carotid ligation and this was not modified by OPC-31260 administration. Four or 6 h following carotid ligation, the brain water content was significantly increased and the Na^+ concentration was enhanced in parallel, but the K^+level remained normal. The increases in the brain water and Na^+ levels were prevented by the simultaneous administration of OPC-31260.

Table 1. *Higher survival rates were observed in the OPC-31260-treated rats 4 and 6 h after carotid ligation*

Groups	No. of animals	Hours after ligation	No. of surviving animals
1. Control untreated	15	–	15
2. Control + OPC-31260	15	–	15
3. Carotid ligation	15	1	13
4. Carotid ligation + OPC-31260	18	1	18
5. Carotid ligation	18	4	10*
6. Carotid ligation + OPC-31260	20	4	18**
7. Carotid ligation	21	6	8*
8. Carotid ligation + OPC-31260	20	6	15**

The level of statistical significance is (*) $P < 0.05$ as compared with the control group (1), and (**) $P < 0.05$ as compared with the untreated ligated groups (3, 5 and 7)

Table 2. *OPC-31260 was administered by gastric tube in a dose of 30 mg/kg immediately after carotid ligation. OPC-31260 did not alter the brain water content and ion concentrations in the control rats (Group 2). One hour after carotid ligation (Group 3), the brain water content and ion concentrations were unchanged and this was not modified by OPC-31260 administration (Group 4). Four (Group 5) and 6 h (Group 7) following carotid occlusion, the brain water content and Na^+ concentration were significantly increased. The increases in the brain water and Na^+ content were prevented by the simultaneous administration of OPC-31260 (Groups 6 and 8)*

Groups	No. of animals	Hours after ligation	Water content (g/100 g wet brain)	Ion concentration (mmol/kg dry brain weight)	
				Na^+	K^+
1. Control untreated	10		77.52 ± 0.3	281.0 ± 12.3	342.9 ± 12.3
2. Control + OPC-31260	12		77.21 ± 0.2	278.3 ± 11.8	350.5 ± 10.6
3. Carotid ligation	9	1	77.85 ± 0.3	290.5 ± 16.1	340.8 ± 16.1
4. Carotid ligation + OPC-31260	12	1	77.53 ± 0.3	275.7 ± 15.0	337.3 ± 14.9
5. Carotid ligation	7	4	79.51 ± 0.4*	340.9 ± 10.2*	341.7 ± 18.3
6. Carotid ligation + OPC-31260	12	4	77.44 ± 0.2**	276.6 ± 11.8**	339.1 ± 11.8
7. Carotid ligation	6	6	79.75 ± 0.3*	353.1 ± 14.5*	337.5 ± 16.3
8. Carotid ligation + OPC-31260	10	6	77.37 ± 0.3**	283.0 ± 13.6**	341.9 ± 15.7

Results are given as mean values + S.E.M., where the level of statistical significance is (*) $P < 0.05$ as compared with the control group (1), and (**) $P < 0.05$ as compared with the untreated groups (5 and 7).

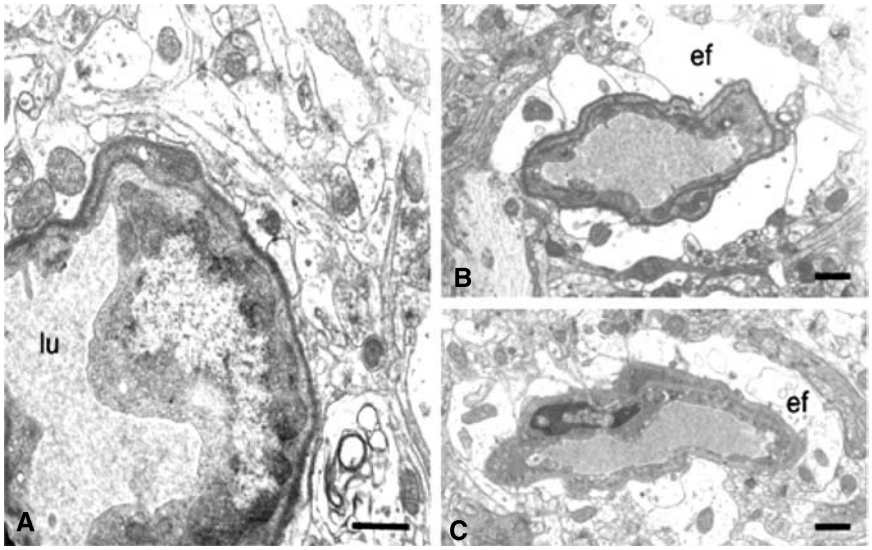

Fig. 1. Electron microscopic changes in cerebral cortical capillaries after carotid occlusion and OPC-31260 treatment. As compared with the control animals (A), 6-h cerebral ischaemia (B) resulted in ultrastructural damage; electron microscopy revealed astrocyte end-foot process oedema (*ef*) and vacuolisation. Vasopressin V_2 receptor antagonist OPC-31260 (C) treatment did not significantly reduce the extent of the blood-brain barrier injury. *lu* Capillary lumen; bar: 1 μm

No changes were detected in the brain structure by light microscopic examination at any time during the whole experimental period (1, 4 and 6 h after the ligation) and electron microscopy did not reveal any morphological alterations in the structure of the capillaries 1 h after the ligation. Similarly as in the control animals, unaltered endothelial cells, astrocytic end-feet and basal lamina were found (Fig. 1A). Six hours after arotid occlusion, however, the morphological signs of the blood-brain barrier damage were obvious; the capillaries were surrounded by swollen astrocytic processes (Fig. 1B). At that time point, moderate swelling of the mitochondria was observed in the neuronal somata and processes, as an indication of the development of hypoxia in the cortical cells. OPC-31260 administration did not result in any significant reduction in the structural damage; the astrocytic swelling was still obvious in animals ligated for 6 h and treated with the drug (Fig. 1C).

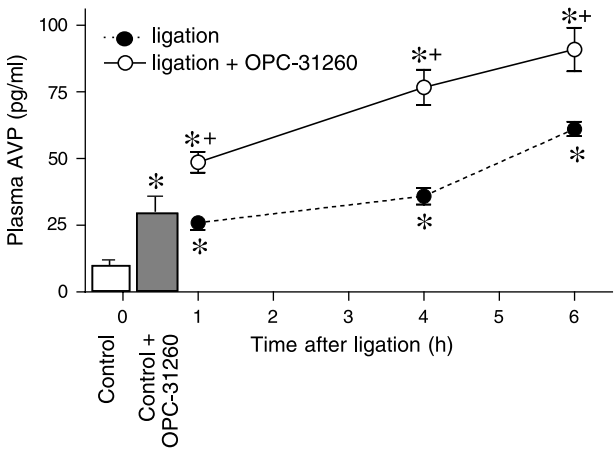

Fig. 2. Plasma vasopressin level following bilateral carotid artery ligation and treatment with OPC-31260. The vasopressin levels were determined 1, 4 and 6 h after the ligation. OPC-31260 was administered orally immediately after the carotid ligation (30 mg/kg). The plasma vasopressin level was increased 1 h after ligation. The plasma vasopressin concentration enhancements were more pronounced 4 and 6 h following ligation. OPC-31260 significantly increased the vasopressin level in the sham-operated control rats. After the common carotid ligation, OPC-31260 administration elicited a further increase in plasma vasopressin level, depending on the time that had elapsed after the ligation. Data are shown as means ± S.E.M. for 10 rats in each group, where the level of statistical significance is (*) $P < 0.05$ as compared with the control group, and (+) $P < 0.05$ as compared with the OPC-31260-treated group

The plasma vasopressin level was increased 1 h after carotid ligation (Fig. 2). The enhancement of the plasma vasopressin concentration was more pronounced 4 or 6 h following ligation. OPC-31260 significantly increased the vasopressin level in the sham-operated control rats. After the common carotid ligation, OPC-31260 administration elicited a further increase in plasma vasopressin level, depending on the time that had elapsed after the ligation.

Discussion

We earlier reported that the vasopressin levels in the plasma and cerebrospinal fluid were elevated after induced subarachnoid haemorrhage [23–25]. It was concluded that vasopressin plays an important role in the development of antidiuresis following water loading and in the disturbance of the brain water and electrolyte balances after subarachnoid haemorrhage induction. Our results revealed that vasopressin elicits cerebral oedema in a complex manner: it increases the water permeability of the brain capillary system (a central effect) and it in-

duces water retention, natriuresis and hypervolaemia by (a peripheral effect) influencing the renal tubular function [24, 25].

The present results demonstrate that a high level of vasopressin and a significant degree of cerebral oedema develops following bilateral carotid ligation. The increase in the vasopressin may originate as a result of hypoxic stress, and the elevated vasopressin concentration may play a significant role in the development of cerebral oedema, influencing the brain capillary permeability and the water metabolism.

It appeared very important to study the different modes of prevention of cerebral oedema. In experimental global cerebral hypoxia, significant protection was found following treatment with a TRH analogue (YM 14673) [42], dexamethasone [18], dopamine [4], or kainic acid [5, 31]. The most effective protection was observed after treatment with the antidiuretic V_2 receptor antagonist in hyponatraemic cerebral oedema (the syndrome of inappropriate secretion of antidiuretic hormone, SIADH). Several V_2 antagonists have been used for the treatment of SIADH: the peptide V_{1-2} antagonist afforded protection in experimental SIADH [22] and in cerebral oedema induced by subarachnoid haemorrhage [24]; the non-peptide V_2 antagonist OPC-31260 proved to be an effective protective agent in spontaneous SIADH in dog [6], in rats after subarachnoid haemorrhage [26], and in cirrhotic rats [7, 38, 39]. A favourable effect was described in SIADH following treatment with other non-peptide V_2 antagonists: Conivaptan (YM 087) [1, 32], VPA-985 [11, 29, 33], or SR121463A [16, 34]. In humans, OPC-31260 was the first proved effective diuretic agent [13, 29, 35, 36, 43] in the treatment of different oedematous states involving high plasma vasopressin levels, hyponatraemia and cerebral oedema, such as liver cirrhosis [14], congestive cardiac failure [40], or neoplastic disease with SIADH [9].

As regards the protective mechanism of OPC-31260, at least four possibilities have to be considered: (1) Blockade of the increased vasopressin release following subarachnoid haemorrhage induction. (2) A decrease of the ischaemia following carotid ligation. (3) A decrease in brain capillary permeability (a direct effect). (4) An effect on the renal tubule function (an indirect diuretic effect). The first of these hypotheses is unacceptable, since the present study revealed that OPC-31260 did not inhibit vasopressin release: it increased the vasopressin levels both in the controls and in the rats which underwent carotid ligation. Similar observations were made in earlier experiments: The V_2 receptor antagonists increase the plasma vasopressin concentration [2, 11, 26, 41]. It should be mentioned here that the mechanism of plasma vasopressin enhancement following OPC-31260 administration is unknown. The longer biological half-life of vasopressin after OPC-31260 administration may play a role in the elevation of the plasma vasopressin level [27]. The second possibility can also be excluded. Electron microscopic examination demonstrated that there is no significant difference in the ischaemic signs between the non-treated and OPC-31260-treated rats after carotid ligation. We have no direct evidence relating to the effect of OPC-31260 on the cerebral capillary permeability or to a possible role of a vascular vasopressin V_1 receptor antagonist effect of OPC-31260 [44]. Most of the findings support the fourth possibility, i.e. that the renal tubular effect of OPC-31260 is the most important action in the prevention of the cerebral oedema induced by subarachnoid haemorrhage. Many data show that OPC-31260 antagonises the binding of vasopressin to vasopressin V_2 receptors in rat kidney plasma membranes *in vitro* [15, 43, 44], inducing a long-lasting and significant diuresis *in vivo* [43, 44] by blocking the antidiuretic action of both endogenous and exogenous vasopressin in conscious rats [38]. However, the diuretic effect of OPC-31260 is quite different from the actions of other traditional diuretic agents, such as furosemide, hydrochlorothiazide and spironolactone. The diuretic effects of these traditional diuretic drugs are closely associated with the urine Na^+ excretion, whereas OPC-31260 selectively increases the water excretion rather than that of Na^+ excretion [43, 44]. Grove *et al.* [10] reported that infusion of the V_2 receptor blocker OPC-31260 increased the diuresis 15-fold and tended to halve the Na^+ excretion. This selective aquaretic effect of OPC-31260 is rather advantageous with a view to the treatment of hyponatraemic cerebral oedema, including SIADH [7]. It is well known that the water metabolism disturbance seen after general cerebral ischaemia is a feature of SIADH, but a longer period is needed for the development of significant hyponatraemia after bilateral carotid ligation. Indeed, during our experimental period (6 h), we did not observe any significant changes in the plasma osmolality and Na^+ level.

Our findings and the above-mentioned experimental data lead us to conclude that the renal tubule-selective diuretic effect of OPC-31260 is the most important factor in the reduction of cerebral oedema following bilateral carotid ligation. Our observations might suggest a new and effective approach to the treatment of cerebral oedema following general cerebral hypoxia in humans.

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