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Vasopressin receptor antagonist OPC-31260 prevents cerebral oedema after subarachnoid haemorrhage

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Abstract

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 subarachnoid haemorrhage were studied in rats. Subarachnoid haemorrhage induced significant water retention after water loading, increased the brain content of water and Na^+ and increased plasma vasopressin levels. The water retention and brain water and Na^+ accumulation were prevented by OPC-31260 administration, but the plasma vasopressin levels were further enhanced by OPC-31260. These results demonstrate the important role of vasopressin in the development of antidiuresis and disturbances in brain water and electrolyte balance in response to subarachnoid haemorrhage. The subarachnoid haemorrhage-induced cerebral oedema was significantly reduced following oral OPC-31260 administration. The protective mechanism exerted by OPC-31260 stems from its influence on renal tubular function: it blocks the renal vasopressin V_2 receptors. These observations might suggest a new, effective approach to the treatment of subarachnoid haemorrhage-induced cerebral oedema in humans.

Keywords: Subarachnoid haemorrhage; Cerebral oedema; Vasopressin receptor antagonist; Non-peptide

1. Introduction

The acute phase of subarachnoid haemorrhage is frequently associated with an impaired water metabolism and a disposition to cerebral oedema (Goldberg and Handler, 1960; Joynt et al., 1965; Imbeau and Rock, 1976; Dóczi et al., 1981;). For study of the pathogenesis of brain oedema, an experimental model of subarachnoid haemorrhage was earlier created in the rat (Kamiya et al., 1982; Shigeno et al., 1982; Dóczi et al., 1984). Significant water retention and increases in the brain content of water and Na^+ and in plasma arginine–vasopressin levels have been observed in rats with subarachnoid haemorrhage (László et al., 1995b). The cerebral oedema generated by artificial cerebral bleeding in rats is significantly reduced following the administration of a highly specific antidiuretic (V_2) vasopressin receptor peptide antagonist, [d(CH_2)$_5$D-Ile^2,Ile^4,Ala^9]-vasopressin (László et al., 1993, 1995a). We earlier attained similar results, namely the prevention of hyponatriaemia and cerebral oedema with the vasopressin V_2–V_1 (antidiuretic and pressoric) receptor antagonist d(CH_2)$_5$-Tyr(Et)Val-vasopressin in rats treated with high doses of exogenous vasopressin together with forced water intake (László et al., 1984).

However, the introduction of peptide vasopressin V_2 receptor antagonists into clinical practice seems to be rather problematic: the oral administration of such substances has not been solved, the duration of action of peptide antagonists is quite short and these antagonists threaten to be rather expensive. In this respect, other difficulties must be considered also: several potent peptide vasopressin V_2 receptor antagonists have partial agonist activity (Brooks et al., 1988; Mah and Hofbauer, 1988; Albrightson Winslow et al., 1989), and chronically administered vasopressin V_2 receptor antagonists lose their antidiuretic antagonist effect and exhibit an agonistic effect (Hofbauer et al., 1986; Mah and Hofbauer, 1988; Brooks et al., 1988; Albrightson Winslow et al., 1989). There are also species differences in the antagonistic effects of peptide vasopressin V_2 receptor-blocking compounds (Stassen et al., 1983).

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Yamamura et al. (1992) recently reported on a newly synthetized, 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 in conscious rats. Other authors have described a substantial aquaretic effect of OPC-31260 following intravenous and oral administration to healthy subjects (Ohnishi et al., 1993, 1995; Shimizu, 1995). OPC-31260 appears to be a promising drug from the viewpoint of clinical practice (Yamamura et al., 1993; Okada et al., 1994; Tsuboi et al., 1994a,b; Fujita et al., 1995). We therefore investigated whether the non-peptide vasopressin V_2 receptor antagonist OPC-31260 can prevent the development of cerebral oedema in experimentally induced subarachnoid haemorrhage. A further aim was to study the mode of action of OPC-31260 in subarachnoid haemorrhage.

2. Methods

2.1. Experimental protocol

The experiments were performed on 3- to 5-month-old male Wistar rats, ranging in weight from 200 to 280 g (bred in our animal house; breeding stock from the Laboratory Animals Producing Institute, Gödöllö, 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. Subarachnoid haemorrhage was induced by the administration of autologous blood to the surface of the cerebral cortex. A burr hole was drilled over the left cerebral convexity, 2 mm caudal from the coronal sutures and 2 mm lateral from the sagittal sutures. Care was taken to keep the dura mater intact. The burr hole was sealed with bone wax, and the dura was pierced with a 25-gauge needle. A total of 100 μl of autologous blood was drawn from the tail and injected into the subarachnoid space within 30–60 s. In the sham-operated groups 100 μl of 0.9% saline or cell-free autologous serum was administered instead of total blood. The bone wax and the cyanoacrylate dropped over the needle and the upper surface of the bone prevented leakage back through the burr hole. The position and extent of the blood clot were observed macroscopically in all animals after decapitation. The blood clots were generally confined to the ipsilateral side. The brain ventricles and basal cisterns were free of blood. No extradural or subdural haemorrhages were found. About 10% of the animals died immediately after the injection of blood into the subarachnoid space, and in another 20% of the rats the position of the blood clots was not correct; these cases were excluded from the assessment of the results. The method for induction of experimental subarachnoid haemorrhage was described in detail earlier (Dóczi et al., 1984).

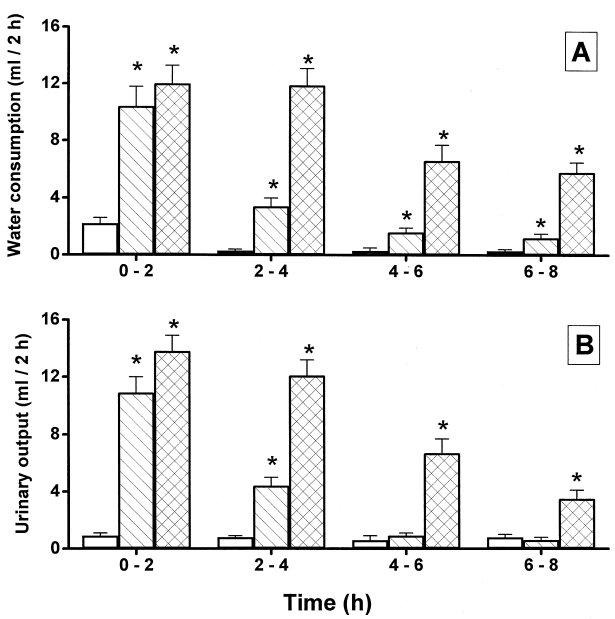

Fig. 1. Effects of oral OPC-31260 (10 or 30 mg/kg) on water consumption (ml/2 h) and urine output (ml/2 h). Spontaneous water consumption (A) was increased to the same level 2 h following administration of both doses of OPC-31260. Two hours after administration of the 10 mg/kg dose of OPC-31260 (hatched columns ▨), the water intake was much smaller than that in the group treated with the 30 mg/kg dose (cross-hatched columns ▩). Similar results were observed for urine output (B), but the lower dose of OPC-31260 (10 mg/kg) did not induce any changes 4–8 h after drug administration. Results are shown as mean values ± S.E.M. for 13–14 animals in each group and where the statistical significance is $^{*}P < 0.05$ compared to the control group (empty columns □).

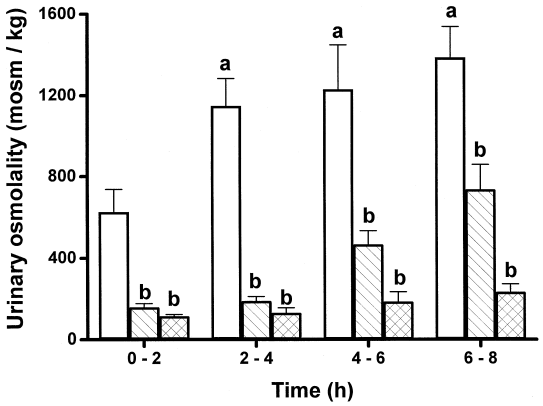

Fig. 2. Effects of oral OPC-31260 (10 or 30 mg/kg) on urine osmolality (mosm/kg). Urine osmolality was increased during the experimental period (2–8 h) in the non-treated control rats (empty column □) and it was decreased following both the lower (10 mg/kg, hatched columns ■) and the higher (30 mg/kg, cross-hatched columns ▩) doses. The higher dose (30 mg/kg) reduced urine osmolality to less than 200 mosm/kg during the 8-h observation period. Results are shown as mean values ± S.E.M. for 13–14 animals in each group and where the statistical significance is (a) $P < 0.05$ compared to the 0–2 h control group, and (b) $P < 0.05$ compared to the control groups for the same observation period (2–4, 4–6, and 6–8 h).

2.2. Water consumption, urine volume and osmolality

The intact non-operated rats were housed individually in metabolic cages at a temperature of 21–23°C. The animals were allowed free access to water and food until 2 h before the start of the experiment and the food was then removed from the cages. OPC-31260 in doses of 10 or 30 mg/kg, or vehicle only, was administered by gastric tube: OPC-31260 was dissolved in water (5 or 15 mg/ml). The antagonist doses (10 or 30 mg/kg) were determined according to earlier published data (Yamamura et al., 1993). Water consumption was measured at 2-h intervals for 8 h. Urine was collected during the same intervals for 8 h to measure the urine volume and osmolality. The latter was determined by freezing-point depression with an Advance osmometer.

2.3. Diuretic reaction following water loading

The diuretic reaction after water loading was examined the next day, following subarachnoid haemorrhage induction. After 12 h of food deprivation, tap water was administered (50 ml/kg) through a gastric tube. From this time, the drinking water was withdrawn. At 1-h intervals for 5 h, the cumulative urine output was determined as a percentage of the water load. Thirty minutes before water loading, OPC-31260 was administered via a gastric tube in doses of 10 or 30 mg/kg (dissolved in water at 5 or 15 mg/ml).

2.4. Brain water and electrolyte contents

The brain water content was determined by dehydration to weight constancy; 3, 6 or 24 h after the operation, the brain hemispheres were 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 mmol/l HNO_3 and the resulting solution was diluted 10-fold with deionized water. The Na^+ content was determined to be 330.3 nm and the K^+ content to be 404.4 nm with a Perkin-Elmer 306 atomic absorption spectrophotometer (Perkin-Elmer, Norwalk, CT, USA) in an air–acetylene flame. The slit width was 0.7 and 2 mm,

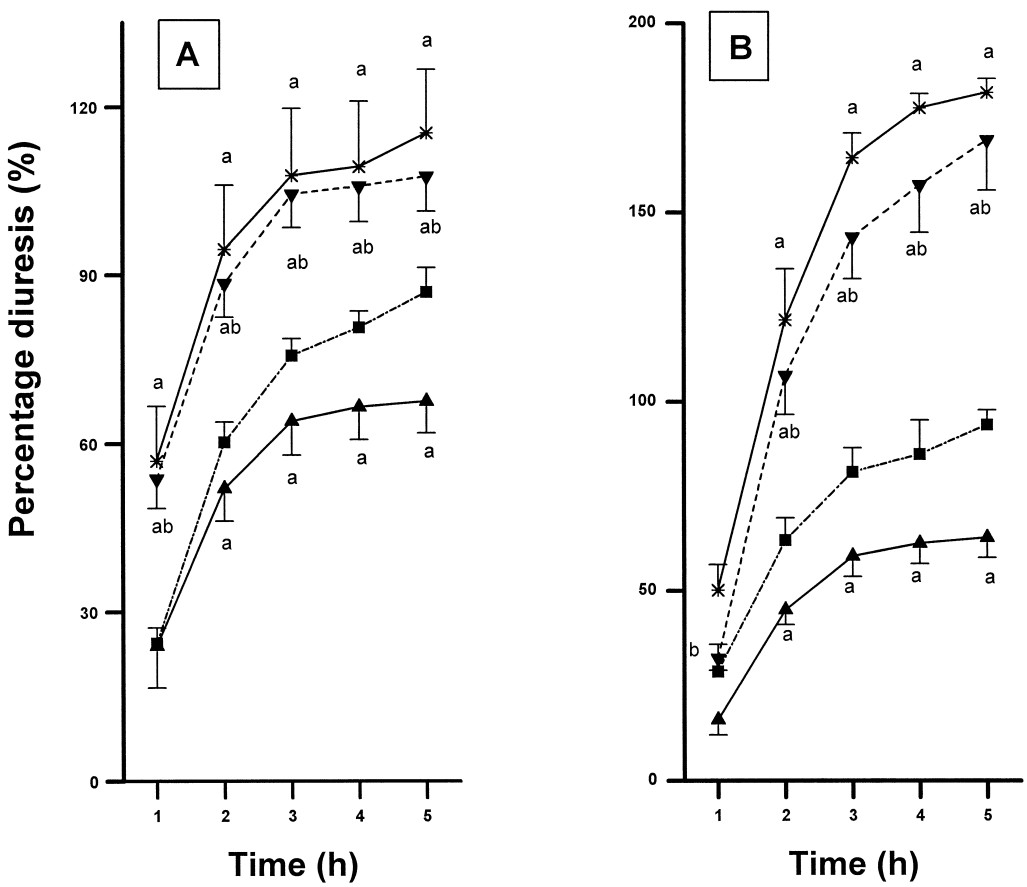

Fig. 3. Effects of subarachnoid haemorrhage and OPC-31260 treatment on the diuretic reaction following water loading (50 ml/kg). The diuretic reaction is expressed as a percentage of the volume of the water load. OPC-31260 was administered 30 min before water loading, in doses of 10 or 30 mg/kg, through a gastric tube. The diuretic curve for the untreated control rats is indicated (■). Significant water retention was detected one day after subarachnoid haemorrhage induction (▲). OPC-31260 in the dose of 10 mg/kg (A) increased diuresis in the control rats (∗) and prevented water retention in rats with subarachnoid haemorrhage (▼). After treatment with 30 mg/kg OPC-31260 (B), the diuresis increased more markedly both in the control rats and in the rats with subarachnoid haemorrhage. Data are shown as means ± S.E.M., where n is at least 8 for each group and where the statistical significance is (a) $P < 0.05$ compared to the control group, and (b) $P < 0.05$ compared to the subarachnoid haemorrhage group.

respectively. Immediately after the surgical intervention, OPC-31260 was administered by gastric tube in doses of 10 or 30 mg/kg (dissolved in water at 5 or 15 mg/ml). The 24-h group was treated with the same doses of OPC-31260 administered every 8 h after subarachnoid haemorrhage induction. Tap water (0.5 ml) was administered to the control animals instead of OPC-31260. The brain Na^+ and K^+ determinations were carried out in the Central Research Laboratory, Medical University, Szeged, Hungary.

2.5. Plasma vasopressin determination

The plasma vasopressin levels were measured by radioimmunoassay (RIA), based on a technique described by Dogterom et al. (Dogterom et al., 1978b) with some modifications, as reported in detail earlier (Jójárt et al., 1987; Laczi et al., 1987).

Synthetic arginine-8-vasopressin (Organon, Oss, 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. For each immunisation, the animals were given about 1 mg (1 ml) of immunogen emulsified in 1 ml of Freund's adjuvant. The emulsion was injected intradermally into as many sites as possible on the back.

The immunisation regimen consisted of injections every 2 weeks for 12 weeks, followed by further boosters monthly. The anti-sera were titrated so as to bind about 50% of iodinated vasopressin. The final antibody dilution used in the assay tube was 1:350 000. The cross-reactions

Table 1
Brain water content and ion concentrations in rats 3, 6 and 24 h following subarachnoid haemorrhage (SAH) and OPC-31260 administration

Groups	Number of animals	Hours after SAH	Water content (g/100 g wet brain)	Ion concentrations (mmol/kg dry brain weight)	
				Na^+	K^+
1. Control	15		80.2 ± 0.3	209.3 ± 1.9	405.9 ± 1.8
2. SAH + water		3			
Ipsilateral	15		83.2 ± 0.6[a]	242.2 ± 5.2[a]	410.5 ± 1.8
Contralateral	15		82.5 ± 0.4[a]	242.5 ± 4.3[a]	409.0 ± 2.1
3. SAH + 10 mg/kg OPC		3			
Ipsilateral	10		81.1 ± 0.5[b]	214.2 ± 3.3[b]	407.8 ± 2.7
Contralateral	10		80.3 ± 0.9[b]	217.0 ± 5.6[b]	409.6 ± 2.5
4. SAH + 30 mg/kg OPC		3			
Ipsilateral	10		81.1 ± 0.8[b]	215.4 ± 5.2[b]	407.3 ± 2.7
Contralateral	10		80.6 ± 0.6[b]	213.2 ± 4.3[b]	406.8 ± 2.6
5. SAH + water		6			
Ipsilateral	16		82.9 ± 0.3[a]	250.7 ± 4.9[a]	406.9 ± 1.9
Contralateral	16		82.5 ± 0.4[a]	241.8 ± 5.6[a]	406.6 ± 2.0
6. SAH + 10 mg/kg OPC		6			
Ipsilateral	20		81.0 ± 0.5[b]	225.1 ± 7.4[b]	405.0 ± 1.1
Contralateral	20		80.5 ± 0.3[b]	213.6 ± 3.9[b]	409.2 ± 2.2
7. SAH + 30 mg/kg OPC		6			
Ipsilateral	20		80.6 ± 0.4[b]	213.5 ± 4.2[b]	410.9 ± 1.2
Contralateral	20		79.8 ± 0.4[b]	206.1 ± 4.0[b]	403.9 ± 2.2
8. SAH + water		24			
Ipsilateral	15		83.1 ± 0.6[a]	249.4 ± 7.9[a]	414.1 ± 4.9
Contralateral	15		82.5 ± 0.5[a]	241.5 ± 6.6[a]	409.3 ± 2.4
9. SAH + 10 mg/kg OPC		24			
Ipsilateral	15		80.8 ± 0.6[b]	216.1 ± 6.7[b]	414.4 ± 2.2
Contralateral	15		80.7 ± 0.2[b]	213.1 ± 2.9[b]	409.4 ± 2.7
10. SAH + 30 mg/kg OPC		24			
Ipsilateral	12		80.8 ± 0.5[b]	206.2 ± 2.0[b]	408.3 ± 3.1
Contralateral	12		80.2 ± 0.2[b]	210.2 ± 4.9[b]	414.6 ± 1.7

OPC-31260 was administered by gastric tube in doses of 10 or 30 mg/kg immediately after SAH (3 and 6 h groups) or every 8 h following SAH induction (24 h groups).

Tap water (0.5 ml) was administered to the control rats instead of OPC-31260 (groups 2, 5 and 8).

The brain water content and Na^+ concentration were significantly increased in both the ipsilateral and the contralateral hemispheres, whereas the K^+ level remained normal after 3 (group 2), 6 (group 5) and 24 h (group 8).

The increases in brain water and Na^+ content could be prevented by the administration of OPC-31260 in either 10 or 30 mg/kg doses (groups 3, 4, 6, 7, 9 and 10).

Results are given as mean values ± S.E.M., where the statistical significance is (a) $P < 0.05$ compared to the control group (1), and (b) $P < 0.05$ compared to the non-treated SAH groups (2, 5 and 8).

SAH = subarachnoid haemorrhage.

were 23.3% with [Lys^8]-vasopressin (Organon, Oss, The Netherlands), 0.12% with des-Gly-NH$_2^9$-[Arg^8]-vasopressin (Organon, Oss, the Netherlands), less than 0.01% with oxytocin (Gedeon Richter, Budapest, Hungary), 0.03% with adrenocorticotrophic hormone (1-24) (Organon, Oss, the Netherlands), and 10.7% with 1-deamino-8-arginine-vasopressin (donated by Per Melin, Ferring Research Laboratory, Malmö, Sweden).

^{125}I-labelling of vasopressin was performed by the chloramine T method of Hunter and Greenwood (1962). Reverse-phase chromatography was used for purification of the labelled hormone (Janáky et al., 1982). The specific activity of the [^{125}I]vasopressin in the various experiments was 49.9–61.1 TBq/mmol.

The blood was obtained following decapitation, and 1-ml blood samples were put in cooled polystyrene tubes (Laczi et al., 1983) containing 1.4 mg of Na_2 EDTA 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 standard curves covered the range 1.0–128 pg per assay tube. Each dilution of the reference preparation was extracted from 1 ml of vasopressin-free plasma from homozygous diabetes insipidus rats (CPB-TNO, Zeist, the Netherlands). The extraction was carried out in duplicate. The dry residue was redissolved in 125 μl of assay buffer and 50-μl aliquots were used for the RIA in duplicate. The RIA procedure was the same as that of Dogterom et al. (1978b). The sensitivity of the RIA was 1 pg per assay tube. Vasopressin levels are given in pg/ml plasma.

OPC-31260 was administered by gastric tube immediately after induction of subarachnoid haemorrhage in a dose of 30 mg/kg (dissolved in water at 15 mg/ml). The 24-h group was treated with the same dose of OPC-31260 administered every 8 h after induction of subarachnoid haemorrhage.

2.6. 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 between two values was performed by Fisher's lowest significance of difference test following one-way analysis of variance. *P* values less than 0.05 were considered significantly different.

3. Results

The spontaneous water consumption and urine output are shown in Fig. 1. Orally administered OPC-31260 (10 and 30 mg/kg) dose dependently increased water consumption. It was found that the duration of the effect of OPC-31260 exceeded 8 h. Similar results were observed for urine output, but the lower dose of OPC-31260 (10 mg/kg) did not induce any changes 4-8 h after drug administration. Urine osmolality was increased during the experimental period (2–8 h) in the non-treated control rats (Fig. 2) and it was significantly decreased following both doses of OPC-31260 (10–30 mg/kg). The higher dose (30 mg/kg) reduced urine osmolality to less than 200 mosm/kg during the 8-h observation period, as demonstrated in Fig. 2.

Significant water retention was detected in rats one day after subarachnoid haemorrhage induction (Fig. 3). OPC-31260 administered in a dose of 10 mg/kg increased diuresis in the control rats and prevented water retention in rats with subarachnoid haemorrhage (Fig. 3). After treatment with the higher dose of OPC-31260 (30 mg/kg), the percentage diuresis increased more markedly both in the control rats and in the rats with subarachnoid haemorrhage than after administration of the lower dose of OPC-31260 (10 mg/kg), as shown in Fig. 3. No significant changes were detected in the diuretic reaction of the sham-operated group (13 rats). These data are not shown in Fig. 3.

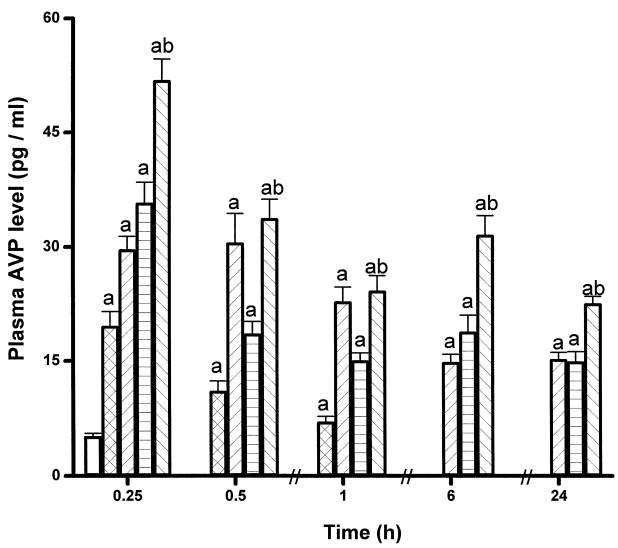

Fig. 4. Effects of subarachnoid haemorrhage and OPC-31260 administration on the plasma vasopressin level (pg/ml) in rats. The vasopressin levels were determined 0.25, 0.5, 1, 6 and 24 h after subarachnoid haemorrhage. OPC-31260 was administered orally immediately after subarachnoid haemorrhage induction (30 mg/kg). The 24-h group was treated every 8 h after subarachnoid haemorrhage. The plasma vasopressin level was increased for a short time (0.25–1.0 h) in the sham-operated rats (cross-hatched columns ▨). Enhanced vasopressin levels were detected throughout the experimental period following subarachnoid haemorrhage (parallel diagonal columns ▤). OPC-31260 significantly increased the vasopressin level in the non-operated controls (hatched column ▨). Following subarachnoid haemorrhage, OPC-31260 administration (hatched column ▧) elicited higher vasopressin levels than those in rats with subarachnoid haemorrhage but without OPC-31260 treatment. Data are shown as means ± S.E.M. for at least 10 rats in each group, where the statistical significance is (a) $P < 0.05$ compared to the control group (empty column □), and (b) $p < 0.05$ compared to the subarachnoid haemorrhage group.

The brain water content was significantly increased and the Na^+ concentration was enhanced in parallel, but the K^+ level remained normal 3, 6 and 24 h after induction of subarachnoid haemorrhage (Table 1). The increases in the brain content of water and Na^+ were prevented by the simultaneous administration of OPC-31260 (10–30 mg/kg p.o.), as shown in Table 1. The water content of the control rats was 80.2 ± 0.3 g/100 g wet brain; the Na^+ concentration was 209.3 ± 1.9 mmol/kg dry brain weight. No significant alterations in brain water content (79.9 ± 0.3-80.7 ± 0.6 g/100 g wet brain) or Na^+ concentration (207 ± 4.1-215 ± 6.3 mmol/kg dry brain weight) were found in the sham-operated groups (12 rats in each groups) or in the control rats treated with OPC-31260 (10 or 30 mg/kg) 3,6 and 24 h (n: 8–17) following surgical intervention. These data are not given in Table 1.

The plasma vasopressin level increased during the first hour in the sham-operated rats (Fig. 4). Enhanced vasopressin levels were detected throughout the whole experimental period (24 h) following induction of subarachnoid haemorrhage (Fig. 4). OPC-31260 significantly increased the vasopressin level in the non-operated controls. Following subarachnoid haemorrhage induction, OPC-31260 administration elicited a further increase in plasma vasopressin level (Fig. 4).

4. Discussion

The present results demonstrate that significant water retention and increased levels of water and Na^+ in the brain develop in rats with subarachnoid haemorrhage. We earlier reported that the vasopressin levels in the plasma and cerebrospinal fluid were also elevated after subarachnoid haemorrhage induction (László et al., 1993, 1995a,b). 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 balance after subarachnoid haemorrhage induction. Indeed, in Brattleboro homozygous rats, which are unable to synthesize vasopressin, the early brain water accumulation was found to be delayed. This reduction in brain oedema was suspected to be a consequence of the reduced increase in capillary permeability because of the lack of central vasopressin release after subarachnoid haemorrhage induction (Dóczi et al., 1984).

The literature dealing with the pathological mechanism of brain oedema indicates that the increase in the permeability of the brain capillary system is the most important point of attack (Joó and Klatzo, 1989). It is known that the brain vasculature, including the capillary network, is not freely permeable to water. It exhibits a permeability similar to that of membranes known to regulate water permeability (Peachey and Rasmussen, 1961; Raichle et al., 1977). All such membranes are under the influence of circulating vasopressin (Peachey and Rasmussen, 1961). Vasopressin has also been detected in significant concentrations in the cerebrospinal fluid (Heller et al., 1968; Jenkins et al., 1980; Wood, 1982). However, a number of studies have revealed that vasopressin enters the cerebrospinal fluid directly from the brain and not from the systemic circulation, and thus blood vasopressin levels may not actually reflect central vasopressin activity (Zaidi and Heller, 1974; Dogterom et al., 1978a). Raichle and Grubb reported that centrally released vasopressin increased the permeability of the brain capillaries to water (Raichle and Grubb, 1978). It was found in the rat that intracerebroventricular administration of vasopressin enhanced brain capillary permeability (Crone, 1963; Dóczi et al., 1982), whereas intravenous injection of vasopressin had no such effect (Raichle and Grubb, 1978). In our earlier studies, an increased vasopressin level in the cerebrospinal fluid was found after induction of subarachnoid haemorrhage, which supports the significance of centrally released vasopressin in this respect (László et al., 1995b).

Besides its central effect on brain capillary permeability, vasopressin may also act at peripheral sites, i.e., the kidney, which is the most important target organ for the antidiuretic effect of vasopressin. Vasopressin V_2 receptors have been observed in the renal tubules (Imbert et al., 1975; Morel et al., 1987) and may mediate water retention, hypoosmolar hypervolaemia and the development of cerebral oedema (Stassen et al., 1982, 1985; Kim and Schrier, 1985; Thibonnier, 1988). The importance of the kidney was highlighted in our earlier experiments, i.e., the water retention and cerebral oedema with hyponatriaemia induced by the administration of high doses of vasopressin and a forced water intake were prevented by a peptide vasopressin V_2 receptor antagonist (László et al., 1984, 1991). This peptide vasopressin V_2 receptor antagonist increased the diuresis, the plasma Na^+ content and osmolality, and decreased the hypervolaemia by acting on renal function (Kinter et al., 1984; Schrier and Kim, 1984; Tang and Ho, 1988; Thibonnier, 1988). Our earlier experimental results showed that vasopressin elicits cerebral oedema in a complex manner: it increases the water permeability of the brain capillary system (central effect) and it induces water retention, natriuresis and hypervolaemia by influencing renal tubular function (peripheral effect) (László et al., 1995a,b).

As regards the protective mechanism of OPC-31260, at least three possibilities have to be considered: (1) A block of the increased vasopressin release following subarachnoid haemorrhage induction. (2) A decrease in brain capillary permeability (direct effect). (3) An effect on renal tubule function (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 vasopressin levels both in controls and in rats with subarachnoid haemorrhage. It should be mentioned here that the mechanism of the plasma vasopressin enhancement following OPC-31260 administration is un-

known. Further experiments are needed to clarify this observation. We have no direct evidence relating to the effect of OPC-31260 on cerebral capillary permeability and to the possible role of a vascular vasopressin V_1 receptor antagonist effect of OPC-31260 (Yamamura et al., 1993). Most of the findings support the third possibility; namely, 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 antagonizes the binding of vasopressin to vasopressin V_2 receptors in rat kidney plasma membranes in vitro (Ishikawa et al., 1992; Yamamura et al., 1992, 1993), inducing a long-lasting and significant diuresis (Yamamura et al., 1992, 1993) by blocking the antidiuretic action of both endogenous and exogenous vasopressin in conscious rats (Tsuboi et al., 1994a). 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 urine Na^+ excretion, whereas OPC-31260 selectively increases water excretion rather than Na^+ excretion (Yamamura et al., 1992, 1993). Grove et al. (1995) recently reported that infusion of the V_2 receptor blocker OPC-31260 increased 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 hyponatriaemic cerebral oedema, including the syndrome of inappropriate secretion of antidiuretic hormone (Fujisawa et al., 1993). The aquaporin of the collecting ducts is involved in the pathogenesis of water retention in rat models of the syndrome of inappropriate secretion of antidiuretic hormone and liver cirrhosis. The increased expression of aquaporin mRNA is abolished after the administration of OPC-31260 (Fujita et al., 1995). It is well known that the water metabolism disturbance seen after induction of subarachnoid haemorrhage is a feature of the syndrome of inappropriate secretion of antidiuretic hormone, but a longer period is needed for the development of significant hyponatriaemia after subarachnoid haemorrhage induction. Indeed, during our experimental period (24 h), we did not observe any significant changes in the plasma Na^+ level (László et al., 1995a). OPC-31260 has proved to be an effective diuretic agent in the treatment of different experimental oedematous states involving high plasma vasopressin levels, such as liver cirrhosis (Tsuboi et al., 1994b) and progressive renal failure (Okada et al., 1994).

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 subarachnoid haemorrhage induction. Our observations might suggest a new and effective approach to the treatment of cerebral oedema following subarachnoid haemorrhage in humans. This suggestion is strongly supported by a recent publication (Saito et al., 1997) which described a good therapeutic effect following the intravenous administration of OPC-31260 to hyponatriaemic patients with the syndrome of inappropriate secretion of antidiuretic hormone.

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