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THE EFFECT OF PROSTAGLANDIN E_2 MICROINJECTED INTO THE RAT HYPOTHALAMUS ON URINARY EXCRETION OF WATER AND SODIUM

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1 Effects of prostaglandin E_2 (PGE_2) microinjected into the supraoptic (SON) and the ventromedial nuclei of the hypothalamus (VMH) on urine outflow were studied in rats anaesthetized with ethanol.

2 PGE_2 injected into the SON caused antidiuresis and increased the excretion rate of Na^+. These effects of PGE_2 were prevented by pretreatment with phentolamine but not propranolol, suggesting that they are mediated by α-adrenoceptors in the SON.

3 PGE_2 injected into the VMH caused diuresis and increased the excretion rate of Na^+. These effects were prevented by pretreatment with propranolol but not phentolamine, suggesting that they are mediated through β-adrenoceptors in the VMH.

Introduction

It has been reported that intracerebroventricular (i.c.v.) administration of E type prostaglandins inhibits water diuresis (Anderson & Leksell, 1975) and causes release of antidiuretic hormone (ADH) (Leksell, 1976; Yamamoto, Share & Shade, 1976). On the other hand, Fujimoto & Hisada (1978a, b) using rats anaesthetized with ethanol in which the cerebral ventricles were perfused from the lateral ventricle to the cerebral aqueduct with artificial cerebrospinal fluid, found that prostaglandin E_2 (PGE_2) administered into the lateral ventricle caused diuresis followed by antidiuresis. Since both effects were independently modified by some drugs, it was concluded that the diuretic and the antidiuretic effects could be separated. There was evidence to suggest that the diuretic effect was, at least in part, due to a non-specific action on widespread areas of the central nervous system. The result also suggested that the diuretic and the antidiuretic effects of PGE_2 were mediated by β- and α-adrenoceptors, respectively. An investigation has therefore been carried out into the areas where microinjection of PGE_2 induces diuresis. In the experiments described here, it was found that PGE_2 injected into the supraoptic (SON) and the ventromedial nuclei of the hypothalamus (VMH) produced antidiuresis and diuresis, respectively. The mechanisms underlying both effects were also studied.

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Methods

As described previously (Hisada, Fujimoto, Kamiya, Endo & Tsushima, 1977), male Wistar rats (280 to 300 g) were anaesthetized with 12% ethanol at a volume of 50 ml/kg, orally and anaesthesia and diuresis were maintained by an intravenous infusion of 3% ethanol at a rate of 0.1 ml/min. The urinary bladder was catheterized after suprapubic incision and urine outflow was recorded by a photoelectric drop counter. The animals were placed in a stereotaxic frame and the atlas of König & Klippel (1963) was used as a general guide. Drugs were injected unilaterally into the SON (A 6.28, L 1.3, V 8.5) and the VMH (A 4.90, L 0.25, V 7.9). The outer diameter of the tip of the injector was 0.2 mm and the volume injected was 1.0 μl. After completion of the experiment, the injection sites were verified histologically according to the method of Milton & Paterson (1974). Arterial blood pressure, heart rate and rectal temperature were monitored continuously in all experiments. Sodium (Na^+) in urine collected for 50 min after the administration of PGE_2 was estimated by flame photometry and the excretion rate (mEq/min) was calculated. PGE_2 (Ono Pharmaceutical Co., Ltd., Osaka) was dissolved immediately before use in absolute ethanol and diluted with artificial cerebrospinal fluid (CSF): the ethanol concentration in the PGE_2 solution (5 nmol/μl) was 5%. Phentolamine mesylate (Ciba-Geigy Ltd., Basel) and propranolol hydrochloride (ICI Co., Ltd., Manchester) were dissolved in the CSF and the solutions were adjusted to approximately pH 7.2 and 300 mosmol. Phentolamine and propranolol were injected into

these nuclei at doses of 13 nmol/μl and 8.5 nmol/μl, respectively, 20 to 30 min before microinjection of PGE_2. The ionic composition of the CSF was as follows (mEq/l): Na^+ 150, K^+ 3, Ca^{2+} 2.3, Mg^{2+} 1.6, Cl^- 135, HCO_3^- 21 and HPO_4^{2-} 0.5. The significance of the observations in this study was ascertained by Student's *t* test.

Results

As controls for the effect of the injection, the CSF or 5% ethanol in CSF at a volume of 1.0 μl was injected into the SON and the VMH. No changes in urine outflow, urinary concentration of Na^+, blood pressure, heart rate or body temperature were observed. None of these parameters was changed by phentolamine or propranolol injected into these nuclei at the doses used.

When PGE_2 was injected into the SON at doses of 0.5 to 5 nmol/μl, urine outflow decreased (Figure 1a). The antidiuretic effect of 0.5 nmol PGE_2 lasted for 40 min and that of 5 nmol PGE_2 for more than 70 min. Urine was collected for 50 min after PGE_2 and the urinary Na^+ concentration determined. The urinary concentration of Na^+ was significantly increased from 6.4 ± 0.9 (s.e.) to 21.6 ± 4.1 mEq/l by 0.5 nmol of PGE_2. PGE_2 at 5 nmol increased the urinary Na^+ concentration from 6.3 ± 0.8 to 28.9 ± 3.0 mEq/l. The excretion rate of Na^+ was approximately doubled by both doses of PGE_2. PGE_2 at a dose of 0.05 nmol had no effects on urine outflow and urinary concentration of Na^+. Phentolamine but not propranolol inhibited significantly both effects of 0.5 nmol PGE_2. An increase in rectal temperature in response to PGE_2 (0.5 nmol) was not altered by phentolamine and propranolol at the doses used.

When PGE_2 was injected into the VMH at doses of 1 and 5 nmol/μl, urine outflow increased significantly (Figure 1b). The effect was dose-dependent and lasted for 30 to 50 min. The Na^+ concentration in urine collected for 50 min after injection of PGE_2 was increased significantly from 5.4 ± 0.3 to 10.3 ± 0.9 mEq/l by 1 nmol PGE_2 and 5.7 ± 0.3 to 23.6 ± 2.0 mEq/l by 5 nmol PGE_2. The excretion rate of Na^+ was increased significantly from 0.52 ± 0.06 to 1.22 ± 0.08 μEq/min in the former and 0.56 ± 0.06 to 3.02 ± 0.21 μEq/min in the latter. PGE_2 at a dose of 0.5 nmol was without effect. Propranolol, 8.5 nmol/μl, injected into the VMH inhibited significantly PGE_2-induced diuresis and natriuresis but phentolamine (13 nmol) was without effect. Rectal tempera-

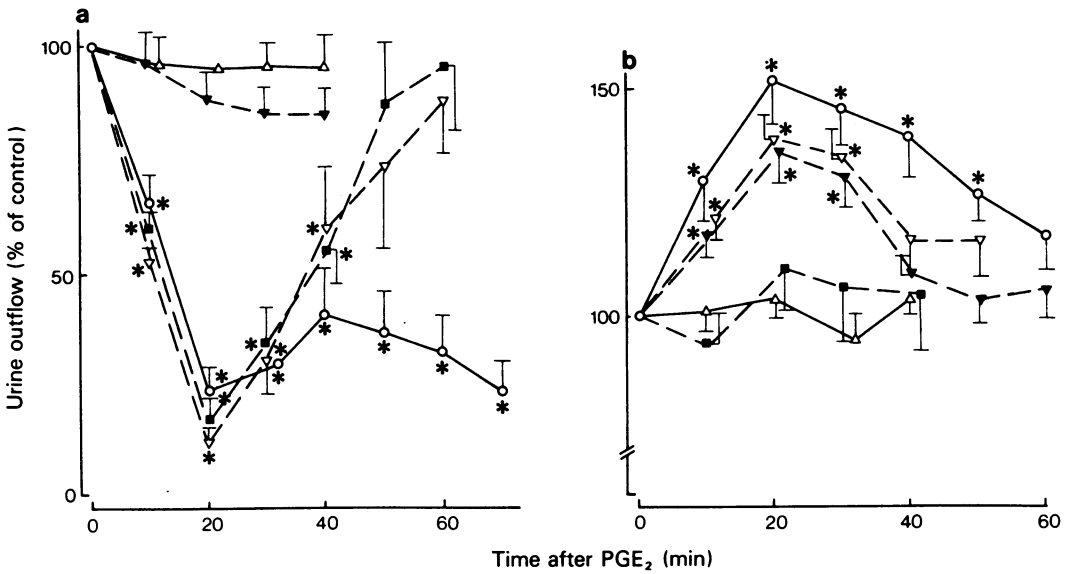

Figure 1 (a) Effect on urine outflow of prostaglandin E_2 (PGE_2) injected into the supraoptic nucleus (SON) at doses of 0.05 (△), 0.5(▽) and 5 (○) nmol/μl. PGE_2 at 0.5 nmol 20 to 30 min after phentolamine 13 nmol (▼) and propranolol 8.5 nmol (■). Ordinate scale; urine outflow, pre-PGE_2 levels (=100%) were as follows (ml/10 min): 1.00 ± 0.08 (○), 1.02 ± 0.04 (▽), 0.99 ± 0.07 (△), 0.98 ± 0.07 (▼) and 1.00 ± 0.08 (■). (b) Effect on urine outflow of PGE_2 injected into the VMH at doses of 0.5 (△), 1 (▽) and 5 (○) nmol/μl. PGE_2 at 1 nmol 20 to 30 min after phentolamine (▼) and propranolol (■). Ordinate scale; urine outflow, pre-PGE_2 levels (=100%) were as follows (ml/10 min): 0.99 ± 0.04 (○), 0.97 ± 0.07 (▽), 1.00 ± 0.07 (△), 0.95 ± 0.04 (▼) and 1.01 ± 0.06 (■). Abscissa scale; time (min) after the microinjection of PGE_2. Vertical lines represent s.e. mean of 6 to 10 injections. *Significant difference from the pre-PGE_2 levels ($P < 0.05$).

ture was increased by PGE_2 (1 nmol) and this effect was not influenced by phentolamine or propranolol at the doses used.

When injected into the SON and the VMH, PGE_2 alone or PGE_2 in combination with phentolamine or propranolol did not change blood pressure and heart rate.

Discussion

It was previously reported that PGE, when injected i.c.v., released ADH from the hypothalamo-neurohypophysial system in the conscious goat (Leksell, 1976) and in the dog, anaesthetized with urethane-chloralose (Yamamoto *et al.*, 1976). As mentioned in the Introduction, Fujimoto & Hisada (1978a, b), demonstrated that in the ethanol-anaesthetized rat, i.c.v. injection of PGE_2 increased urine outflow and then decreased it.

It is well known that the SON is important for the regulation of release of ADH. The microinjection of PGE_2 into the SON resulted in antidiuresis and the excretion rate of Na^+ was increased. These results may suggest that PGE_2 in the SON increases ADH release. The results of the present experiments also suggested that the effects of PGE_2 are mediated through α-adrenoceptors in this area. It was likely that there was α-adrenergic release of ADH in the SON (Milton & Paterson, 1974) and in the hypothalamus (Bhargava, Kulshrestha & Srivastava, 1972). In addition, Hisada *et al.* (1977) found that antidiuresis in response to i.c.v. injected noradrenaline was inhibited by phentolamine but not propranolol. In the present study, the animals were anaesthetized with ethanol. Ethanol reduces the release of ADH and causes diuresis: however, it has been suggested that the effects of ethanol are restricted to ADH released by osmotic stimuli (Wallgren & Barry, 1970). Consequently, even in the rat anaesthetized with ethanol the possibility should not be excluded that PGE_2 injected into the SON evoked ADH release to inhibit diuresis through stimulation of α-adrenoceptors.

The present study showed that PGE_2 injected into the VMH causes diuresis. The urinary Na^+ concentration and then the excretion rate of Na^+ were both very much increased by PGE_2. These increases were prevented by pretreatment centrally with propranolol but not phentolamine. Although the role of the VMH in water metabolism and Na^+ excretion is not clear, this may suggest that PGE_2-induced diuresis and natriuresis are both mediated by β-adrenoceptors. Bhargava *et al.* (1972) suggested that β-adrenoceptors in the hypothalamus are concerned with inhibition of ADH release but it is now questionable whether or not this diuresis resulted from further inhibition of ADH release. Experiments are now underway to elucidate mechanisms for the diuretic and natriuretic effects of PGE_2 in the VMH.

The hyperthermic effect of PGE_2, microinjected into the SON and the VMH, was not affected by phentolamine and propranolol, suggesting that the effects of PGE_2 on urine outflow and rectal temperature are mediated by different mechanisms.

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