EFFECTS OF CENTRALLY AFFECTING DRUGS ON THE DIURETIC AND ANTI迪URETIC ACTIONS OF INTRACEREBROVENTRICULAR PROSTAGLANDIN E\textsubscript{2}

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Abstract—Effects of centrally affecting drugs on the diuretic and antidiuretic actions of intracerebroventricularly (i.c.v.) injected prostaglandin (PG) E\textsubscript{2} in ethanol-anaesthetized rats were studied. PGE\textsubscript{2}, when injected i.c.v. at a dose of 1 nmole, produced diuresis followed by antidiuresis. When morphine (0.1 mM) was perfused i.c.v., urine outflow decreased and neither diuretic nor antidiuretic effects of i.c.v. PGE\textsubscript{2} was apparent. The perfusions with picrotoxin, \(\alpha\)-aminobutyric acid and L-glutamate inhibited either the diuretic or the antidiuretic effect of PGE\textsubscript{2}. On the other hand, when pentobarbital, diazepam, isoniazid and strychnine were perfused i.c.v., the diuretic action of PGE\textsubscript{2} was diminished and antidiuresis in response to PGE\textsubscript{2} remained unchanged. These results suggested that the diuretic and antidiuretic effects of PGE\textsubscript{2} could be separated. The development of the diuretic effect of PGE\textsubscript{2} was completely blocked by amitriptyline and antidiuresis was increased. In rats pretreated i.c.v. with reserpine, the diuretic effect of PGE\textsubscript{2} was prolonged and antidiuresis in response to PGE\textsubscript{2} was not observed. An antidiuretic action of i.c.v. norepinephrine was not varied by reserpine. Mechanisms for both effects of PGE\textsubscript{2} are discussed.

Prostaglandins (PGs) are present in most parts of the brain including the hypothalamus (1, 2), and readily released from the central nervous system (CNS) both spontaneously and by electric or chemical stimulation (3). In addition, since PGs could be detected in effluents of the perfused lateral ventricle (4) and superfusates of the cerebral cortex (5, 6), they may be naturally-occurring components in the cerebrospinal fluid.

Hedge (7) was of the opinion that hypothalamic and pituitary PGs regulated anterior pituitary hormone secretion. On the other hand, regarding with posterior pituitary hormone secretion, PGF\textsubscript{2}α was suggested to inhibit milk ejection by a central block on oxytocin release (8). PGE\textsubscript{1} injected centrally, inhibited water diuresis (9) and increased urinary concentrations of ADH (10). It was shown that central PGE\textsubscript{2} increased plasma ADH contents (11) and caused diuresis followed by antidiuresis (12). PGA\textsubscript{2} injected intracerebroventricularly (i.c.v.), decreased urine outflow in some of ethanol-anaesthetized rats or increased it in others (13). Thus, it is likely that at least E\textsubscript{1}, E\textsubscript{2} and A\textsubscript{2} of the PGs act on the CNS to regulate water metabolism.

Chemicals known to inhibit some effects of the PGs, will be good tools to study mechanisms of the effects of the PGs. For instance, Peng et al. (14) who demonstrated that i.p. morphine inhibited the adrenal ascorbic acid-depleting effect of i.v. PGE\textsubscript{1}, suggested that the action of PGE\textsubscript{1} on ACTH release was at some level in the CNS. Such was later con-
firmed by Hedge and Hanson (15) who reported that ACTH-releasing action of PGE\textsubscript{1} injected into the median eminence was abolished by i.p. morphine.

The febrile response to central PGE\textsubscript{1} was attenuated in rabbits pretreated centrally with 6-hydroxydopamine (16) and the present authors showed that i.c.v. perfusion with picrotoxin reduced the febrile action of i.c.v. PGE\textsubscript{2} in rats (17). Fujimoto (18), furthermore, reported that although the potency of i.v. PGA\textsubscript{2} as a vasodepressor in rats was significantly varied by some anaesthetics, the decrease in blood pressure by i.c.v. PGA\textsubscript{2} was not altered by these drugs.

In this work, therefore, an attempt was made to determine whether or not centrally affecting drugs could change the diuretic and antidiuretic effects of i.c.v. PGE\textsubscript{2}.

**MATERIALS AND METHODS**

As described previously (19), an artificial cerebrospinal fluid (CSF) was perfused from the lateral ventricle to the cerebral aqueduct of ethanol-anaesthetized rats. The perfusion rate was 10 \mu l/min. Three % ethanol was additionally infused i.v. at a rate of 0.1 ml/min to maintain anaesthesia and diuresis. Urine was collected through a bladder cannula and the rate of urine outflow was recorded by a photoelectric drop counter. Results were expressed as a percentage of change by drugs in urine outflow and control values (expressed as 100\%) of urine outflow ranged from 0.8 ml/10 min to 1.2 ml/10 min.

PGE\textsubscript{2} (Ono Pharmaceutical Co., Ltd., Osaka) was injected at a volume of 10 \mu l through a lateral ventricular cannula. This PG was dissolved immediately before use by ethanol and diluted by the CSF. Ethanol concentration of an original PGE\textsubscript{2} solution (10 nmoles/10 \mu l) was 3 %.

In some experiments, reserpine (Apoplon, Daiichi Seiyaku Co., Ltd., Tokyo) was injected i.c.v. at a dose of 49 nmoles, 24 hr before the i.c.v. perfusion study was carried out.

Morphine sulfate, pentobarbital sodium, diazepam, isoniazid, strychnine nitrate, picrotoxin, amitriptyline hydrochloride, \gamma-aminobutyric acid (GABA), L-sodium glutamate (L-GA) and 1-norepinephrine bitartrate were dissolved by the CSF.

**RESULTS**

The i.c.v. injection of 0.3\% ethanol did not change urine outflow.

When PGE\textsubscript{2} was injected i.c.v. at a single dose of 1 nmoles in ethanol-anaesthetized rats, urine outflow increased by about 50\%, 10 min later. Then, urine outflow decreased gradually and a maximal decrease (to 85\% of pre-PGE\textsubscript{2} level) was observed 40-50 min after the injection (Fig. 1). One tenth nmoles of PGE\textsubscript{2} produced diuresis but antidiuresis was not significantly observed. These diuretic and antidiuretic actions were not produced by intracarotid and i.v. injections of 1 nmoles of PGE\textsubscript{2} (data not shown).

When the ventricular system was perfused at the rate of 10 \mu l/min with CSF containing morphine at a concentration of 0.1 mM, urine outflow decreased (Fig. 2). When this decrease in urine outflow attained a steady-state which was continued for a few hr, PGE\textsubscript{2} was injected i.c.v. at the dose of 1 nmoles. Neither the diuretic nor antidiuretic effects of
PGE\(_2\) were observed in the morphine-treated group. Although the diuretic effect of PGE\(_2\) was slightly but significantly inhibited even by a smaller dose (5 fM) of morphine by which urine outflow was not altered, the antidiuretic effect of PGE\(_2\) remained unchanged (data not shown).

![Fig. 1. Effect of i.c.v. injected PGE\(_2\) at doses of 0.1 (○) and 1 (●) nmoles on urine outflow. Abscissa: time (min) after the injection, ordinate: urine outflow (pre-PGE\(_2\) level: 0.8-1.2 ml/10 min as 100%). Vertical lines represent S.E. of means in 8-12 instances. *P<0.05, significant difference from the controls.]

![Fig. 2. Effects of i.c.v. perfused morphine (0.1 mM) and of PGE\(_2\) (1 nmole) during the morphine-perfusion on urine outflow. The perfusion rate was 10 μl/min. Abscissa: time (min) before and after the injection of PGE\(_2\), ordinate: urine outflow (pre-morphine level=100%). Morphine alone (○), PGE\(_2\) in the presence of morphine (●). Vertical lines represent S.E. of means in 8-12 instances.]

PGE\(_2\) were observed in the morphine-treated group. Although the diuretic effect of PGE\(_2\) was slightly but significantly inhibited even by a smaller dose (5 μM) of morphine by which urine outflow was not altered, the antidiuretic effect of PGE\(_2\) remained unchanged (data not shown).

When PGE\(_2\) (1 nmole) was injected i.c.v. 60 min after starting the i.c.v. perfusions with pentobarbital (Fig. 3, top panel), diazepam (Fig. 3, top panel), isoniazid (Fig. 3, middle panel) and strychnine (Fig. 3, middle panel) at doses of 0.1 mM, 0.5 mM, 20 μM and 0.1 mM, respectively, the diuretic effect of PGE\(_2\) was significantly inhibited but antidiuresis in response to PGE\(_2\) remained unchanged. On the other hand, the diuretic effect of PGE\(_2\) was partially inhibited and the antidiuretic effect was completely blocked, when PGE\(_2\) was injected i.c.v. at the dose of 1 nmole 60 min after starting the perfusion with 20 μM of picrotoxin (Fig. 3, bottom panel). The perfusion with amitriptyline at a concentration of 0.1 mM in the
same manner prevented completely the diuretic action of PGE₂ and increased antidiuresis appeared (Fig. 3, bottom panel).

GABA and L-GA were perfused i.c.v. at rates of 0.1 μmoles/min and 2-5 nmole/min, respectively, and 60 min later PGE₂ was injected i.c.v. at the dose of 1 nmole. As shown in Fig. 4, these drugs inhibited partially the development of diuresis in response to PGE₂ and the PGE₂-induced antidiuresis did not occur during the experiments.

Control perfusions with pentobarbital, diazepam, picrotoxin, amitriptyline, GABA and L-GA at doses used did not alter the rate of urine outflow. Isoniazid alone at the dose used increased significantly urine outflow. The increase was maintained during the perfusion with the drug. Strychnine by itself at the dose used temporarily increased urine outflow.

In rats which had been treated with i.c.v. reserpine (49 nmoles) 24 hr before the perfusion study was carried out, the maximal diuretic response to PGE₂ was not changed but the diuretic period was extended (Fig. 5). PGE₂ increased urine outflow by 1.6±0.2 (S.E.) ml for 40 min in the reserpine-treated group, while in the control group, PGE₂ increased it by 1.1±0.1 ml for 30 min. The increase in urine outflow in response to PGE₂ in the reserpinized rat was significantly larger as compared with the control. Antidiuresis in response to PGE₂ was not observed in the reserpinized group (Fig. 5).

As reported in a previous paper (19), 1-norepinephrine, when injected i.c.v. at doses ranging from 1 nmole to 0.1 μmoles, decreased urine outflow (Fig. 6). This antidiuresis was not varied by the pretreatment with i.c.v. reserpine.
**DISCUSSION**

The present result that the diuretic and antidiuretic effects of i.c.v. PGE2 were independently affected by some of drugs used, strikingly suggested that these effects were dissociated each other. The effect of PGE2 is, therefore, a summation of both effects. The fact that injections of PGE2 at the dose of 1 n mole by i.v. and intracarotid routes did not change urine outflow in the ethanol-anesthetized rat, suggested that i.c.v. PGE2 at this dose could produce these effects by acting on the CNS. The hypothalamus has been proposed...
to be an action site for PGE1-induced inhibition of water diuresis (9) and PGA2-induced change in urine outflow (13). However, a more distant area of the CNS and pituitary sites cannot be ruled out. In fact, it was reported that PGE2 released ADH from isolated and incubated neural lobes (20-22).

Since i.c.v. injected PGE2 may affect less specifically some sites of the CNS, influence of centrally affecting drugs on the diuretic and antidiuretic effects of PGE2 was analyzed.

It was shown that i.p. morphine inhibited ACTH secretion evoked by PGE1 injected i.v. (14) and directly into the median eminence (15) of the pentobarbital-anaesthetized rat, suggesting that PGE1 acted on the level of the CNS, probably the median eminence. Morphine is a drug which induces ADH secretion by acting on the CNS. In the present work, it was demonstrated that i.c.v. perfusion with morphine (0.1 mM) caused sustained antidiuresis and inhibited both the diuretic and antidiuretic effects of PGE2 in the ethanol-anaesthetized rat. Even when the smaller dose (5 \( \mu \)M) of morphine which did not reduce urine outflow was perfused i.c.v., the diuretic but not antidiuretic effect of PGE2 was inhibited. Picrotoxin at the dose of 20 \( \mu \)M was also shown to inhibit both the diuretic and antidiuretic effects of PGE2. It has been reported that the thermogenic effect of i.c.v. PGE2 at doses ranging from 10 pmoles to 0.1 pmoles was inhibited by picrotoxin at doses of 20 \( \mu \)M-0.4 mM in rats (17).

Pentobarbital, diazepam, isoniazid and strychnine were all potent inhibitors of the diuretic effect of PGE2. However, antidiuresis in response to PGE2 remained unchanged in the presence of these drugs. Since pentobarbital does not inhibit PGE2-induced ACTH secretion (23), it is likely that mechanisms for the diuretic action of PGE2 differ from those for the ACTH-releasing effect of PGE2. Although possible interaction between PGE1 and strychnine (24) was confirmed, a further study to explain in detail interactions between PGE2 and the centrally acting drugs was not reported.

The effect of PGE2 on urine outflow in the presence of the CNS depressants was similar to that in the presence of the convulsants. Hence, since GABA and L-GA appeared to be inhibitory and excitatory transmitters in the CNS, respectively (25, 26), we attempted to determine whether or not the effects of PGE2 were influenced by these drugs. We found that those compounds behaved similarly towards the diuretic and antidiuretic effects of PGE2. Thus, at least the diuretic effect of PGE2 was less specifically inhibited by various drugs. Over all, the diuretic effect of i.c.v. PGE2 might be, at least in part, due to a non-specific action on wide-spread areas of the CNS.

Although isoniazid and strychnine at the doses used produced sustained and temporal diuresis, respectively and morphine decreased urine outflow, a further study was not done to elucidate the mechanisms of these effects. The doses of other drugs perfused would be maximal within doses which did not change urine outflow. When all of the drugs, including isoniazid, strychnine and morphine, were perfused at doses 6-10 times as large as those used, urine outflow decreased irreversibly, and 40-80 min later, the outflow of urine stopped completely. On the other hand, when perfused at one tenth of the dose used, none of these drugs altered either the diuretic or the antidiuretic effects of PGE2.
The antidiuretic effect of PGE₂ might be mediated by adrenergic mechanisms, since amitriptyline prevented completely the development of diuresis in response to PGE₂ and increased antidiuresis, and since centrally injected norepinephrine acted as an antidiuretic (19, 27, 28) and released ADH (28, 29). The pretreatment with reserpine did not vary the antidiuretic effect of exogenous norepinephrine (Fig. 6), suggesting that the sensitivity of adrenoceptors to norepinephrine was not changed by this treatment. Reserpine increased the diuretic effect of PGE₂, but the antidiuretic effect of PGE₂ was not observed in these rats. These observations suggest that amounts of norepinephrine mediating the antidiuretic effect of PGE₂ were decreased by pretreatment with reserpine. Although ample experimental evidence indicates that E series of the PGs might control the adrenergic neuroeffector transmission by inhibiting norepinephrine release from the peripheral nervous system (30–32), such may not be the case in the CNS.

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