EFFECTS OF CENTRALLY AFFECTING DRUGS ON THE DIURETIC AND ANTIDIURETIC ACTIONS OF INTRACEREBROVENTRICULAR PROSTAGLANDIN E₂

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Accepted August 29, 1977

Abstract—Effects of centrally affecting drugs on the diuretic and antidiuretic actions of intracerebroventricularly (i.c.v.) injected prostaglandin (PG) E₂ in ethanol-anaesthetized rats were studied. PGE₂, 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. PGE₂ was apparent. The perfusions with picrotoxin, γ -aminobutyric acid and L-glutamate inhibited either the diuretic or the antidiuretic effect of PGE₂. On the other hand, when pentobarbital, diazepam, isoniazid and strychnine were perfused i.c.v., the diuretic action of PGE₂ was diminished and antidiuresis in response to PGE₂ remained unchanged. These results suggested that the diuretic and antidiuretic effects of PGE₂ could be separated. The development of the diuretic effect of PGE₂ was completely blocked by amitriptyline and antidiuresis was increased. In rats pretreated i.c.v. with reserpine, the diuretic effect of PGE₂ was prolonged and antidiuresis in response to PGE₂ was not observed. An antidiuretic action of i.c.v. norepinephrine was not varied by reserpine. Mechanisms for both effects of PGE₂ 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_{2 α} was suggested to inhibit milk ejection by a central block on oxytocin release (8). PGE₁ injected centrally, inhibited water diuresis (9) and increased urinary concentrations of ADH (10). It was shown that central PGE₂ increased plasma ADH contents (11) and caused diuresis followed by antidiuresis (12). PGA₂ 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 E₁, E₂ and A₂ 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. PGE₁, suggested that the action of PGE₁ 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_1 injected into the median eminence was abolished by i.p. morphine.

The febrile response to central PGE_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_2 in rats (17). Fujimoto (18), furthermore, reported that although the potency of i.v. PGA_2 as a vasodepressor in rats was significantly varied by some anaesthetics, the decrease in blood pressure by i.c.v. PGA_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_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 $\mu\text{l}/\text{min}$. 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_2 (Ono Pharmaceutical Co., Ltd., Osaka) was injected at a volume of 10 μl 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_2 solution (10 nmoles/10 μl) was 3 %.

In some experiments, reserpine (Apoplone, 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, γ -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_2 was injected i.c.v. at a single dose of 1 nmole 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_2 level) was observed 40–50 min after the injection (Fig. 1). One tenth nmoles of PGE_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 nmole of PGE_2 (data not shown).

When the ventricular system was perfused at the rate of 10 $\mu\text{l}/\text{min}$ 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_2 was injected i.c.v. at the dose of 1 nmole. Neither the diuretic nor antidiuretic effects of

FIG. 1. Effect of i.c.v. injected PGE₂ at doses of 0.1 (○) and 1 (●) nmole on urine outflow. Abscissa: time (min) after the injection, ordinate: urine outflow (pre-PGE₂ 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 PGE₂ (1 nmole) during the morphine-perfusion on urine outflow. The perfusion rate was 10 μ l/min. Abscissa: time (min) before and after the injection of PGE₂, ordinate: urine outflow (pre-morphine level=100%). Morphine alone (○), PGE₂ in the presence of morphine (●). Vertical lines represent S.E. of means in 8–12 instances.

PGE₂ were observed in the morphine-treated group. Although the diuretic effect of PGE₂ was slightly but significantly inhibited even by a smaller dose (5 μ M) of morphine by which urine outflow was not altered, the antidiuretic effect of PGE₂ remained unchanged (data not shown).

When PGE₂ (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 μ M and 0.1 mM, respectively, the diuretic effect of PGE₂ was significantly inhibited but antidiuresis in response to PGE₂ remained unchanged. On the other hand, the diuretic effect of PGE₂ was partially inhibited and the antidiuretic effect was completely blocked, when PGE₂ was injected i.c.v. at the dose of 1 nmole 60 min after starting the perfusion with 20 μ M 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 PGE_2 and increased antidiuresis appeared (Fig. 3, bottom panel).

GABA and L-GA were perfused i.c.v. at rates of $0.1 \mu\text{moles/min}$ and $2\text{--}5 \text{ nmoles/min}$, respectively, and 60 min later PGE_2 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 PGE_2 and the PGE_2 -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 PGE_2 was not changed but the diuretic period was extended (Fig. 5). PGE_2 increased urine outflow by 1.6 ± 0.2 (S.E.) ml for 40 min in the reserpine-treated group, while in the control group, PGE_2 increased it by 1.1 ± 0.1 ml for 30 min. The increase in urine outflow in response to PGE_2 in the reserpinized rat was significantly larger as compared with the control. Antidiuresis in response to PGE_2 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 $0.1 \mu\text{moles}$, decreased urine outflow (Fig. 6). This antidiuresis was not varied by the pretreatment with i.c.v. reserpine.

FIG. 3. Top panel; Effects of PGE_2 in the presence of pentobarbital (0.1 mM , ●) and diazepam (0.5 mM , ▲) on urine outflow. Pentobarbital alone (○), diazepam alone (△). Middle panel; The effect of PGE_2 in the presence of isoniazid ($20 \mu\text{M}$, ●) and strychnine (0.1 mM , ▲) on urine outflow. Isoniazid alone (○), strychnine alone (△). Bottom panel; The effect of PGE_2 during the perfusion with picrotoxin ($20 \mu\text{M}$, ○) and amitriptyline (0.1 mM , ▲) on urine outflow. Picrotoxin alone (○), amitriptyline alone (△). PGE_2 at the dose of 1 nmole was injected at zero min (in abscissa) 60 min after starting the perfusion with the centrally acting drugs. The same legends as Fig. 1. Significant differences when compared with the diuretic (a, $P < 0.05$) and the maximally antidiuretic effects (b, $P < 0.05$) of PGE_2 (1 nmole) alone and significant differences from the centrally acting drugs by themselves (c, $P < 0.05$) and from the non-treated group (d, $P < 0.05$).

FIG. 4. Effects of PGE₂ in the presence of GABA (left) and L-GA (right) on urine outflow (●). GABA and L-GA were perfused i.c.v. at the rates of 0.1 μ moles/min and 2-5 nmol/min, respectively. GABA and L-GA by themselves (○). See Fig. 3 for detailed explanations. * $P < 0.05$, significant difference from the diuretic effect of PGE₂ alone.

FIG. 5. Prolongation by reserpine pre-treatment of the diuretic effect of 1 nmole of PGE₂. Reserpine at the dose of 49 nmol was injected i.c.v. and 24 hr later PGE₂ was injected i.c.v. (●). PGE₂ in the control group (○). The same legends as in Fig. 1. * $P < 0.05$, significant difference from urine outflow 30 min after the PGE₂-injection in the control group. ** $P < 0.05$, significant difference when compared with the pre-PGE₂ level.

FIG. 6. The effect of reserpine on the 1-norepinephrine-induced decrease in urine outflow. Abscissa: doses (moles) of NE ($-\log$ scale), ordinate: % of urine outflow to the control (=100%). NE alone (○), NE in the reserpine treated rat (●). Vertical lines indicate S.E. of the mean in 8-12 instances. The dose response curve for NE which was represented in the previous paper (19) was again quoted here.

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 nmole by i.v. and intracarotid routes did not change urine outflow in the ethanol-anaesthetized 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 PGE_1 -induced inhibition of water diuresis (9) and PGA_2 -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 PGE_2 released ADH from isolated and incubated neural lobes (20–22).

Since i.c.v. injected PGE_2 may affect less specifically some sites of the CNS, influence of centrally affecting drugs on the diuretic and antidiuretic effects of PGE_2 was analyzed.

It was shown that i.p. morphine inhibited ACTH secretion evoked by PGE_1 injected i.v. (14) and directly into the median eminence (15) of the pentobarbital-anaesthetized rat, suggesting that PGE_1 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 PGE_2 in the ethanol-anaesthetized rat. Even when the smaller dose (5 μM) of morphine which did not reduce urine outflow was perfused i.c.v., the diuretic but not antidiuretic effect of PGE_2 was inhibited. Picrotoxin at the dose of 20 μM was also shown to inhibit both the diuretic and antidiuretic effects of PGE_2 . It has been reported that the thermogenic effect of i.c.v. PGE_2 at doses ranging from 10 pmoles to 0.1 μmoles was inhibited by picrotoxin at doses of 20 μM –0.4 mM in rats (17).

Pentobarbital, diazepam, isoniazid and strychnine were all potent inhibitors of the diuretic effect of PGE_2 . However, antidiuresis in response to PGE_2 remained unchanged in the presence of these drugs. Since pentobarbital does not inhibit PGE_2 -induced ACTH secretion (23), it is likely that mechanisms for the diuretic action of PGE_2 differ from those for the ACTH-releasing effect of PGE_2 . Although possible interaction between PGE_1 and strychnine (24) was confirmed, a further study to explain in detail interactions between PGE_2 and the centrally acting drugs was not reported.

The effect of PGE_2 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 PGE_2 were influenced by these drugs. We found that those compounds behaved similarly towards the diuretic and antidiuretic effects of PGE_2 . Thus, at least the diuretic effect of PGE_2 was less specifically inhibited by various drugs. Over all, the diuretic effect of i.c.v. PGE_2 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 PGE_2 .

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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