Br. J. Pharmacol. (1988), **93**, 151–155

Effect of dopamine antagonists on the urine flow of rats infused with hypotonic saline

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1 The probable involvement of dopamine in the regulation of water excretion was investigated by administering dopamine antagonists intravenously to barbiturate – anaesthetized rats undergoing a water diuresis induced by the infusion of 0.83% glucose with 0.3% NaCl at the rate of 9 ml h^{-1}.

2 Administration of 100 μg of the D_1-/D_2-dopamine antagonist, haloperidol, reduced the enhanced urine flow of rats infused with the hypotonic solution by 69% (from 75.4 ± 13.0 to 23.6 ± 6.0 μl min^{-1}, $P < 0.01$). Similarly, the D_1-receptor antagonist, SCH 23390, reduced urine flow by 58% (from 77.5 ± 9.2 to 32.7 ± 7.2 μl min^{-1}, $P < 0.01$) and the D_2-receptor antagonist, sulpiride, by 47% (from 66.2 ± 8.6 to 35.1 ± 6.8 μl min^{-1}, $P < 0.05$).

3 The injection of SCH 23390 increased the urine osmolality from 189.6 ± 27.5 to 479.8 ± 45.8 mosm kg^{-1} ($P < 0.05$). There was no significant change in sodium and potassium excretion in any of the experiments. Blood pressure (BP) decreased after haloperidol and SCH 23390 injection from control values of 121.7 ± 1.7 and 116.5 ± 7.4 to 113.3 ± 3.3 and 106.0 ± 8.8 mmHg respectively ($P < 0.05$).

4 To study whether the influence of dopamine antagonists on urine flow during water diuresis depends on antidiuretic hormone (ADH), we administered 0.6 μg d(CH2)5-D-Phe-Ile-AVP (an ADH antagonist) shortly after the injection of 100 μg SCH 23390. The preferential V2 ADH-antagonist abolished the antidiuretic effect of SCH 23390 but did not affect its blood pressure reducing effect (from 118.6 ± 5.6 to 103.2 ± 4.6 mmHg, $P < 0.01$).

5 These results suggest that dopamine antagonists blunted the hypotonic saline-induced diuresis by favouring ADH release through an interference with an inhibitory dopaminergic pathway.

Introduction

There is some evidence indicating an involvement of dopamine in the control of sodium excretion and thus, indirectly, in the control of water reabsorption during saline diuresis. It is less clear whether dopamine is more directly involved in the regulation of water excretion.

Central and peripheral interactions between dopamine receptors and the release or action of antidiuretic hormone (ADH) have been reported. The evidence for an involvement of a dopaminergic pathway in the control of ADH release is controversial: exogenous dopamine application into the hypothalamus or into the third ventricle leads to enhanced, unchanged or reduced ADH release (Bridges *et al.*, 1976; Holzbauer *et al.*, 1980; Lightman *et al.*, 1982 Racké *et al.*, 1986). Few data are available on an interaction at the site of action of ADH. At this level dopamine seems to antagonize the hormone effect on water permeability (Arruda *et al.*, 1982; Muto *et al.*, 1985). It appeared, therefore, of interest to investigate whether or not dopamine antagonists would affect the water diuresis following hypotonic saline infusion.

Methods

Male Sprague-Dawley rats (*Mus rattus* GmbH, Brunnthal, Munich, FRG) weighing 230–320 g, which up to the day of the experiment had free access to a commercial rat chow and to tap water, were anaesthetized with 5-ethyl-5(1-methylpropyl)-2-thiobarbituric acid (Inactin, Byk Gulden, Konstanz, FRG) 100 mg kg^{-1} body weight i.v.. Body temperature was kept at 37.5 ± 0.5°C by placing the animals on a heated operation table. After performing a tracheotomy, catheters were placed in the right jugular

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vein (for separate administration of hypotonic saline and drugs), in the right femoral artery for measuring blood pressure by means of an electrical transducer, and in the left ureter for urine collection. A water diuresis was induced by infusing a solution of 0.83% glucose and 0.33% NaCl at the rate of 9 ml h^{-1} throughout the experiment. Urine was collected for two 10 min control periods after an equilibration time of about 120 min. Dopamine antagonists (haloperidol, SCH 23390 and sulpiride) were then administered and the urine collection continued for two 5 min and three 10 min periods. To assess the involvement of ADH on the effect of dopamine antagonists on renal function, we also combined 100 μg SCH 23390 with 0.6 μg ADH-antagonist. The ADH antagonist was injected through a separate catheter shortly after injecting the dopamine antagonist. Urine was collected as described above.

The drugs used were: haloperidol (Haldol, Janssen Pharmaceutica, Neuss, FRG), R−(+)-8-chloro-2,3,4,5-tetrahydro-3-methyl-5-phenyl-1H-3-benzazepine-7-ol (SCH 23390, Schering-Plough, Bloomfield, USA) and sulpiride (Dogmatil, Schürholz, München, FRG). Doses previously shown to be effective were used (Breese & Mueller, 1985; Racké *et al.*, 1986). The ADH antagonist d(CH2)5-D-Phe-Ile-AVP, a preferential blocker of the antidiuretic effect of vasopressin (Manning & Sawyer, 1985), was kindly provided by Dr M. Manning, Medical College, Toledo, Ohio, USA. All but SCH 23390 were dissolved in 150 mM NaCl. SCH 23390 (1 mg) was dissolved in 100 μl 0.01 M HCl and then diluted to 1 ml with buffered 150 mM NaCl (0.07 M phosphate, pH 7.4).

Sodium and potassium in urine were measured by flame photometry using caesium as internal standard (IL 943, Instrumentation Laboratory, IL-Fisher-Scientific, USA). Urine osmolality was measured by freezing point depression. Glomerular filtration rate was estimated by the clearance of polyfructosan (Inutest, Paesel GmbH & Co., Frankfurt, FRG). This carbohydrate which was infused throughout at 3.0 mg min^{-1}, was measured in urine and in deproteinized plasma by the anthrone method (Führ *et al.*, 1955). The results were given as mean ± standard error of the mean (s.e.mean). The differences in response to drug injection were assessed by the test of multiple comparison of Wilcoxon/Wilcox (Werner, 1984). A P value of < 0.05 was regarded as significant.

Results

The administration of 100 μg haloperidol i.v. induced a 69% reduction in urine flow (from the pre-injection level of 75.4 ± 13.0 to 23.6 ± 6.0 μl min^{-1}, at the 5th–10th min post-injection $P < 0.01$; Figure 1).

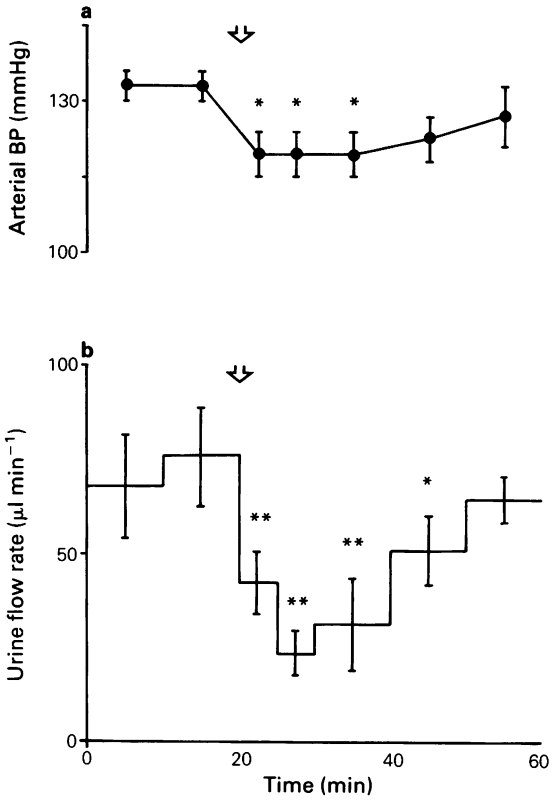

Figure 1 Effect of 100 μg haloperidol i.v. (arrow) on the arterial blood pressure (a) and on the urine flow rate (b) of rats undergoing water diuresis induced by the infusion of 0.83% glucose and 0.33% NaCl i.v. (*$P < 0.05$, **$P < 0.01$; $n = 6$).

Haloperidol injection was associated with a lowering in blood pressure from the control value of 121.7 ± 1.7 to 113.3 ± 3.3 mmHg during the 0–20th min post-injection period ($P < 0.05$). The control urinary sodium excretion (1.0 ± 0.6 μmol min^{-1}) was not altered by the haloperidol injection (1.3 ± 1.0 μmol min^{-1} at the 0–5th min post-injection period. Potassium excretion was 1.30 ± 0.2 μmol min^{-1} prior to and 1.0 ± 0.3 μmol min^{-1} 0–10 min after haloperidol administration (not significant).

Similar results were observed after the injection of 100 μg SCH 23390. This D_1-dopamine antagonist induced a 58% reduction in urine flow (from 77.5 ± 9.2 μl min^{-1} to 32.7 ± 7.2 μl min^{-1}, $P < 0.01$; Figure 2). This result was associated with a change in blood pressure from the control value of 116.5 ± 7.4 mmHg to 106.0 ± 8.8 mmHg 0–10 min after injection ($P < 0.05$). The osmolality in-

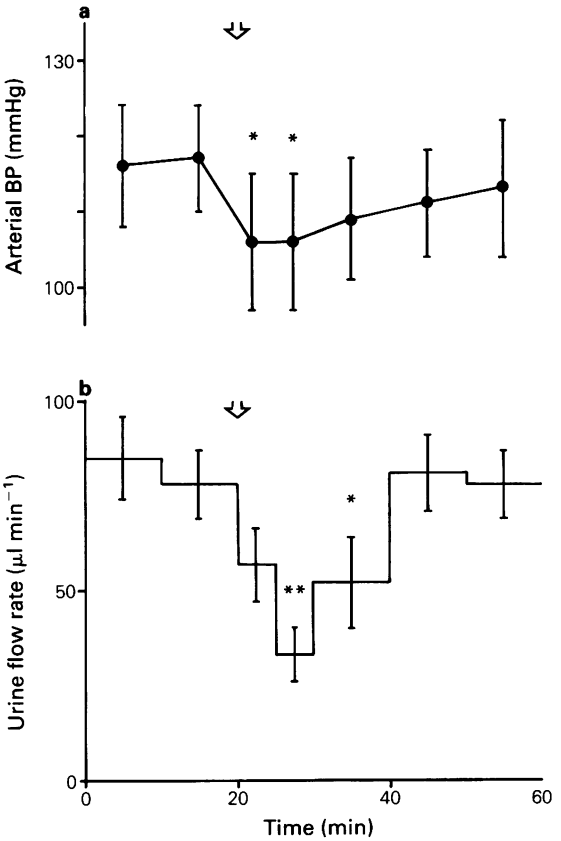

Figure 2 Effect of 100 μg SCH 23390 i.v. (arrow) on the arterial blood pressure (a) and on the urine flow rate (b) of rats undergoing water diuresis induced by the infusion of 0.83% glucose and 0.33% NaCl i.v. (*$P < 0.05$, **$P < 0.01$; $n = 4$).

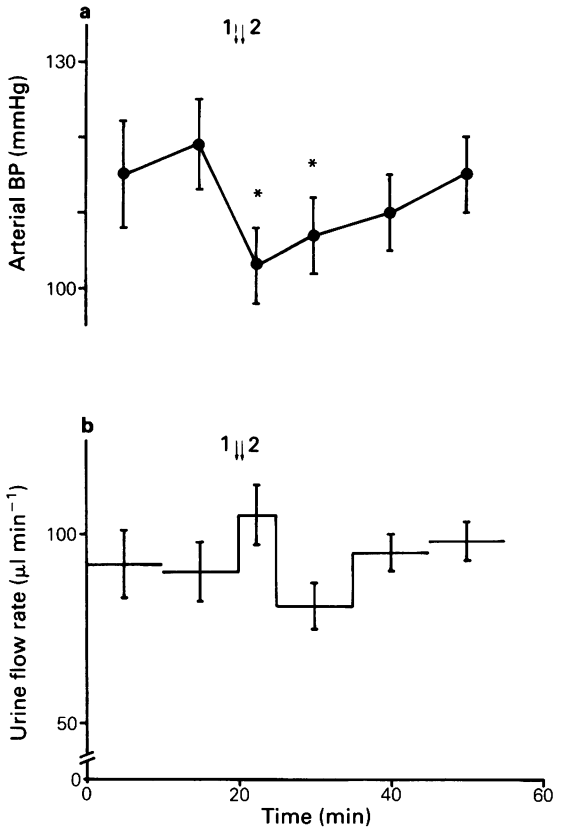

Figure 3 Influence of 0.6 μg d(CH2)5-D-Phe-Ile-AVP i.v. (arrow 2) on the effect of 100 μg SCH 23390 (arrow 1) upon the arterial blood pressure (a) and the urine flow rate (b) of rats undergoing a water diuresis induced by the infusion of 0.83% glucose and 0.33% NaCl i.v. ($n = 4$).

creased gradually from 189.6 ± 27.5 mosm kg^{-1} to 479.8 ± 45.8 mosm kg^{-1} by the 5–10th min after injection returning to control thereafter (50th–60th min = 123 ± 20 mosm kg^{-1}). The glomerular filtration rate (1.2 ± 0.2 ml min^{-1}), as well as the urinary excretions of sodium (2.6 ± 0.6 μmol min^{-1}) and potassium (1.5 ± 0.2 μmol min^{-1}) did not change significantly after SCH 23390.

The injection of 100 μg sulpiride reduced the urine flow rate about 47% from 66.2 ± 8.6 μl min^{-1} to 35.1 ± 6.8 μl min^{-1} during the 5 min post injection period ($P < 0.05$). This agent did not affect blood pressure, glomerular filtration rate or electrolyte excretion (data not shown).

When given in combination with an ADH-antagonist, SCH 23390 changed neither urine flow rate (Figure 3) nor the excretions of sodium (2.9 ± 0.5 μmol min^{-1}) or potassium (1.2 ± 0.1 μmol min^{-1}). The glomerular filtration rate (0.9 ± 0.1 ml min^{-1}) and the urine osmolality (172 ± 33 mosm kg^{-1}) remained unaffected. The blood pressure decreased after drug administration from 118.6 ± 5.6 mmHg to 103.2 ± 4.6 (0–5 min) and 107.1 ± 4.6 mmHg (5–15 min; $P < 0.05$).

Discussion

The administration of dopamine antagonists reduced the diuretic response to hypotonic saline infusion without changing the urinary sodium excretion. Thus, the change observed in our experiment cannot be explained by an antagonism of a dopamine-dependent natriuretic pathway. The rise in urine osmolality after SCH 23390 suggests that the plasma ADH concentration was enhanced by the injection of this D_1-selective dopamine antagonist. This drug could act centrally to

enhance the ADH release and thus indirectly lead to a reduced urine flow. Nerve terminals of the central dopaminergic system have already been described in the hypothalamus and neurohypophysis. Although controversial, there is some evidence for an inhibitory effect of dopamine on ADH secretion from the neurohypophysis (Lightman *et al.*, 1982). Variable effects of exogenously administered dopamine on ADH release have been reported (Forsling *et al.*, 1979; Holzbauer *et al.*, 1980; Racké *et al.*, 1986). Similarly, conflicting results on dopamine content in hypothalamus and hypophysis after water deprivation or salt loading in rats have been reported (Holzbauer *et al.*, 1978).

An alternative possibility to explain our results could be that the dopamine antagonists interact with dopamine receptors on collecting duct cells. It has been shown that dopamine inhibits the ADH-stimulated water flow at this level (Muto *et al.*, 1985). Since this effect was inhibited by metoclopramide it appears to depend on an interaction of dopamine with its D_2-receptors. In our experiment the unselective (D_1/D_2) and the D_1-selective antagonist had a somewhat more prolonged and more marked effect suggesting that the D_1-receptors are involved in the blunting of the diuresis due to hypotonic saline infusion.

The effect of dopamine antagonists on urine flow may also depend on changes in renal blood flow. By counteracting a dopamine-dependent vasodilatation, and consequently increasing the cortico-medullary osmotic gradient, dopamine antagonists could favour collecting tubule water reabsorption (Chapman *et al.*, 1980). This would mimic the effect of enhanced plasma ADH concentration and lead to reduced urine flow and increased urine osmolality. Our finding that an ADH-antagonist with selectivity for the V_2-receptors reversed the 'antidiuretic' effect of dopamine antagonists supports the hypothesis that in the present experiments these agents acted by favouring ADH-release. We cannot totally exclude a participation of the blood pressure change in a release of ADH, but it is unlikely that the moderate reduction observed in our study was primarily responsible (Robertson, 1976).

A lack of change in sodium excretion in the present study is at variance with the previous observation that dopamine antagonists reduce the rise in sodium excretion which follows isotonic saline loading (Krishna *et al.*, 1985; McClanahan *et al.*, 1985; Marin-Grez *et al.*, 1987). The activation of a dopamine-dependent 'natriuretic pathway' during isotonic expansion of the extracellular fluid volume but not during hypotonic loading may explain this discrepancy. Our previous finding that dopamine antagonists counteract the natriuretic response to the administration of exogenous ANP suggests that this peptide may represent the above mentioned 'natriuretic pathway' (Marin-Grez *et al.*, 1985).

This work was supported by grants from the Deutsche Forschungsgemeinschaft and the Bayer AG, Leverkusen, FRG. The authors are indebted to the Schering-Plough Corp. (Brommfield, USA), the Schürholz GmbH (Munich, FRG) and to Dr M. Manning (Toledo, U.S.A.) for supplying the drugs used in this investigation. Dr P. Angchanpen was a fellow of the DAAD.

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(Received May 30, 1987.
Revised August 30, 1987.
Accepted September 4, 1987.)