J. Physiol. (1977), **272**, *pp.* 563–572
With 2 plates and 4 text-figures

SEARCH FOR A NATRIURETIC MECHANISM SENSITIVE TO SODIUM IN THE BRAIN OF THE MONKEY

BY M. ASHWINI KUMAR AND S. SWAMINATHAN
From the Department of Pharmacology, Vallabhbhai Patel Chest Institute, University of Delhi, Delhi-110007, India

(*Received 9 December 1976*)

SUMMARY

1. The effects of hypertonic saline infusion into the third ventricle were investigated in ten monkeys which were pre-operated, trained, and used in the conscious state under controlled conditions.

2. In non-hydrated monkeys, intraventricular infusion of NaCl 1·0 M, 0·01 ml./min for 30 min did not affect urine volume or Na output but produced a small increase in urine osmolality. Comparable infusion of NaCl 0·15 M had no effect on any parameter.

3. In monkeys undergoing water diuresis (with I.V. infusion of 5% dextrose), intraventricular hypertonic saline produced large reciprocal changes in urine volume and osmolality while urine Na showed no significant change. The effects on urine volume and osmolality were greater than those of lysine-vasopressin 30 m-u./kg I.V.

4. The absence of natriuresis after intraventricular hypertonic saline infusion in the monkey was in notable contrast to the results reported in lower species. However, the data suggested that the infusion probably released ADH as in other species.

INTRODUCTION

Smith (1957) postulated that the neuraxis may be the source of a potent antinatriuretic hormone. Subsequently, proponents of a natriuretic hormone seem to have ignored his prefix 'anti' while quoting him (Cort & Lichardus, 1968; de Wardener, 1969, 1973; Levinsky, 1974). The various reports supporting the existence of a natriuretic hormone show little agreement on its chemistry, source and releasing stimulus (de Wardener, 1973; Levinsky, 1974).

The present work was started in the wake of reports that natriuresis is produced by small infusions of hypertonic saline into the third ventricles of conscious goats (Andersson, Dallman & Olsson, 1969), anaesthetized dogs (Dorn, Levine, Kaley & Rothballer, 1969), anaesthetized rats (Dorn &

Porter, 1970) and anaesthetized cats (Chiu & Sawyer, 1974) and by larger infusions into the carotid arteries of conscious goats (Olsson, 1973), anaesthetized dogs (Zucker & Kaley, 1976), anaesthetized cats (Thornborough, Passo & Rothballer, 1973) and conscious sheep (Blaine, Denton, McKinley & Weller, 1975). It has been suggested that a hormone of diencephalic origin may be responsible for these effects (de Wardener, 1973; Levinsky, 1974). Earlier work in this laboratory failed to confirm consistent natriuresis after injection of hypertonic saline into the third ventricles of anaesthetized dogs and monkeys (Kumar, 1972). The object of this study was to test the effects of intraventricular infusion of hypertonic saline in the conscious monkey under controlled conditions.

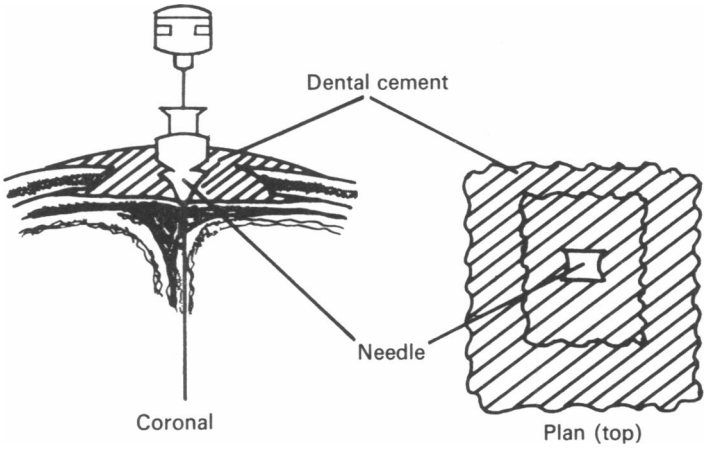
METHODS

Healthy female rhesus monkeys trapped in North Indian forests and weighing 3–4 kg were used after 3 weeks' quarantine. They were housed individually in iron cages 1 m^3 at temperature 25–35 °C. Food was given once daily in the afternoon and consisted of (i) chick peas about 100 g, soaked in water for 24 hr and boiled after adding NaCl 1 g, (ii) dried 'bael' pulp about 25 g, soaked in water ('bael' is the pectin rich fruit of *Aegle marmelos* which prevents diarrhoea in confined animals), (iii) two fruits, e.g. banana, apple, pear, guava, tomato, mango. Water was always available in the cage. The monkeys thrived well on this regimen and often gained weight.

Surgery was performed aseptically under pentobarbitone anaesthesia (40 mg/kg I.P.). All monkeys were episeotomized. The third ventricle was cannulated with a stainless-steel Luerlock hypodermic needle, 23 g with the stem about 32 mm long and blunt, short-bevelled tip. The hub of the needle was indented to aid fixation with dental cement. The cranium was exposed by a mid line incision and a window about 1 cm^2 was made in front of the bregma. The dura was cut along one edge of the sagittal sinus and if the latter bled, gelfoam was applied. The head was fixed in a Horsley-Clarke type of frame with the auditory canals and the inferior orbital margins in the same horizontal plane. In the female rhesus monkey weighing 3–4 kg the co-ordinates for a point in the third ventricle antero-ventral to the intermediate mass were usually (1) 15 mm anterior to the interaural line, (2) about 29 mm vertically below the surface of the cerebrum and (3) 0·5 mm lateral to the midline to avoid the sagittal sinus and the falx. C.s.f. flowed out when the needle attached to a micromanipulator, entered the third ventricle. It was anchored by pouring rapid setting dental cement (AD Stellon Rapid Repair) in the cranial window (Text-fig. 1). The scalp was sutured in layers and skin was shaped around the implant. A stainless-steel wire fixed with epoxy glue in a male luer connector (sawed off an old syringe) served to close and lock the needle (Text-fig. 1). The implant was trouble free for at least 10 weeks. Pl. 1 shows X-ray photographs of the skull taken after 5 ml. air was injected through an implant. After a series of experiments was completed in a monkey, it was anaesthetized with pentobarbitone (30 mg/kv I.V.) and bromophenol blue solution 0·2%, 0·02 ml./min was infused for 20 min through the implanted needle. The brain was thus stained and fixed as described by Feldberg & Fleischhaur (1960). The needles' track and tip could be seen on the cut surface of the brain and if it was correctly placed, the ependyma of the third ventricle was deeply stained with the needle tip antero-ventral to the intermediate mass (Pl. 2). The monkey was included

in the series only if these findings were positive; a total of ten animals were finally included.

After recovery from surgery, the animal was trained to sit on a special restraint chair daily for 6 hr; it got quite used to this within a week. Experiments on trained monkeys were begun in the morning. The bladder and blood vessels were catheterized aseptically (under local anaesthesia if necessary), the former with Foleys' FG-10, 3 ml. balloon catheter and the latter with appropriate sized polyethylene tubes inserted through hypodermic needles. The animal was placed on the chair and given 100 ml. 0·08 M-NaCl through gastric tube. Water diuresis was induced if necessary by giving sterile 5% dextrose solution I.V., 50 ml. load followed by continuous



Text-fig. 1. Diagram showing the intracranial implant. The dental cement interlocked with the needle and the cranial bone. The needle was closed and locked with a home-made stainless stylet.

infusion of 1 ml./min throughout the experiment. Equilibration for 90 min was allowed before urine collections were begun, for successive periods of 20 min. If the urine output was under 0·5 ml./min the bladder was washed out with 4 ml. distilled water after each collection. Injectable pyrogen-free solutions of NaCl (analytical grade) and lysine-vasopressin (Sigma) were prepared or diluted freshly before use. Infusions were given using a Harvard model 902 pump, the infusion volume being corrected for cannula dead space. At least 36 hr were allowed between two consecutive experiments in a monkey.

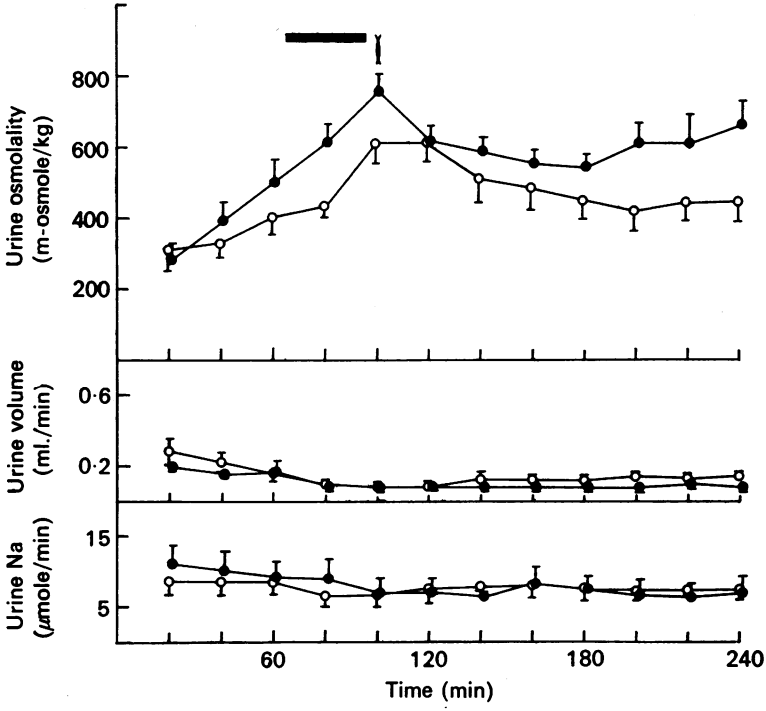
Serum and urine Na were measured by emission spectrophotometry (Beckman model B). Urine osmolality was measured by freezing point depression with appropriate corrections (Knauer Halbmikro-osmometer). In some experiments, radial artery blood pressure was recorded via a fine polyethylene catheter connected to a Statham UC-3 strain gauge and Beckman RM Dynograph.

Statistical analyses of the data included calculation of means and standard errors as well as comparison of individual mean points on a curve with the mean final pre-infusion control value by studentized range test if non homogenity amongst the points in the curve was demonstrable by analysis of variance (Goldstein 1964).

RESULTS

Serum Na in the ten monkeys used in this study was 148·7 ± 0·45 m-mole/l. (total sixteen observations).

Intraventricular infusion of 0·15 M and 1·0 M-NaCl, 10 µl./min for



Text-fig. 2. Urine osmolality, volume and Na output before and after intraventricular infusion (horizontal bar) of 1·0 M-NaCl (●) and 0·15 M-NaCl (○). Urine volume and Na data were obtained in ten monkeys (eleven experiments with 1·0 M-NaCl and thirteen experiments with 0·15 M-NaCl) and osmolality data in only six out of the ten monkeys (seven out of eleven experiments with 1·0 M-NaCl and eight out of thirteen experiments with 0·15 M-NaCl). In this and subsequent Figures statistically significant change from the last pre-infusion control value is denoted by ǀ ($P < 0·05$) or *($P < 0·01$).

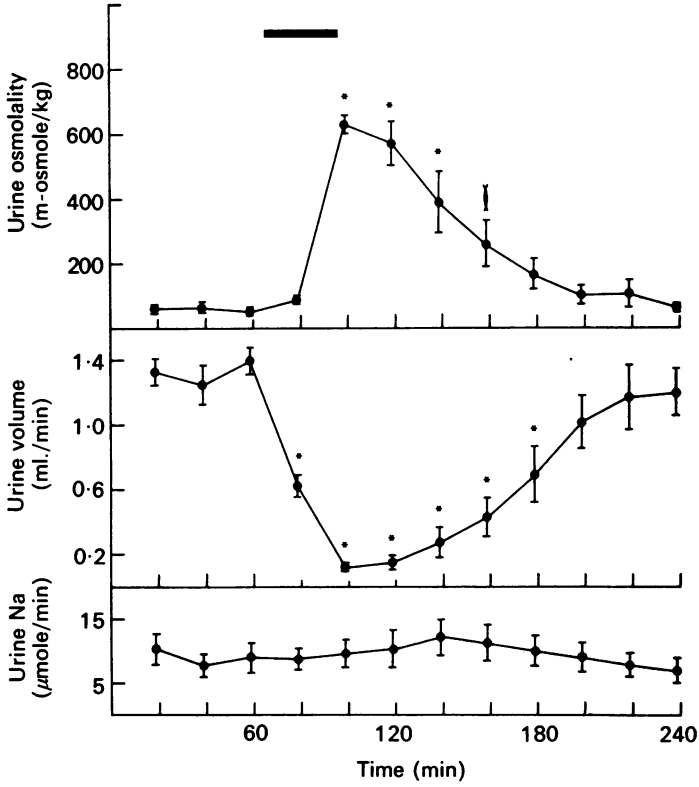
TABLE 1. Radial artery blood pressure (mmHg) before, during and 5 and 90 min after the intraventricular infusion of 1·0 M-NaCl

Monkey	Before infusion	During infusion	5 min postinfusion	90 min postinfusion
1	110/80	105/85	105/90	100/75
2	105/85	125/110	110/95	110/80
3	125/80	135/85	135/85	130/80

TABLE 2. Urine volume and Na output in five monkeys given 1·0 M-NaCl I.V. 10 *µl.*/min for 30 min after the third urine collection. None of the changes are significant

Collection period	1	2	3	4	5	6	7	8	9	10	11	12
Urine volume (ml./min)	0·31 ± 0·069	0·21 ± 0·039	0·19 ± 0·032	0·22 ± 0·096	0·13 ± 0·027	0·09 ± 0·001	0·10 ± 0·014	0·12 ± 0·020	0·14 ± 0·033	0·11 ± 0·022	0·08 ± 0·001	0·08 ± 0·010
Urine Na (*µ*mole/min)	8·6 ± 1·00	11·9 ± 2·92	12·6 ± 2·97	10·8 ± 2·53	10·1 ± 1·97	8·0 ± 1·54	9·1 ± 1·75	8·7 ± 1·26	8·5 ± 2·00	8·4 ± 0·69	8·0 ± 0·41	8·5 ± 1·11

30 min (Text-fig. 2) had little effect on urine Na, urine volume or urine osmolality. However, 1·0 M-NaCl produced a small, significant ($P < 0·05$) rise in urine osmolality in a single sample after the infusion. In the same monkeys, undergoing water diuresis, 1·0 M-NaCl produced a sharp, prolonged decrease in urine volume accompanied by a reciprocal increase in



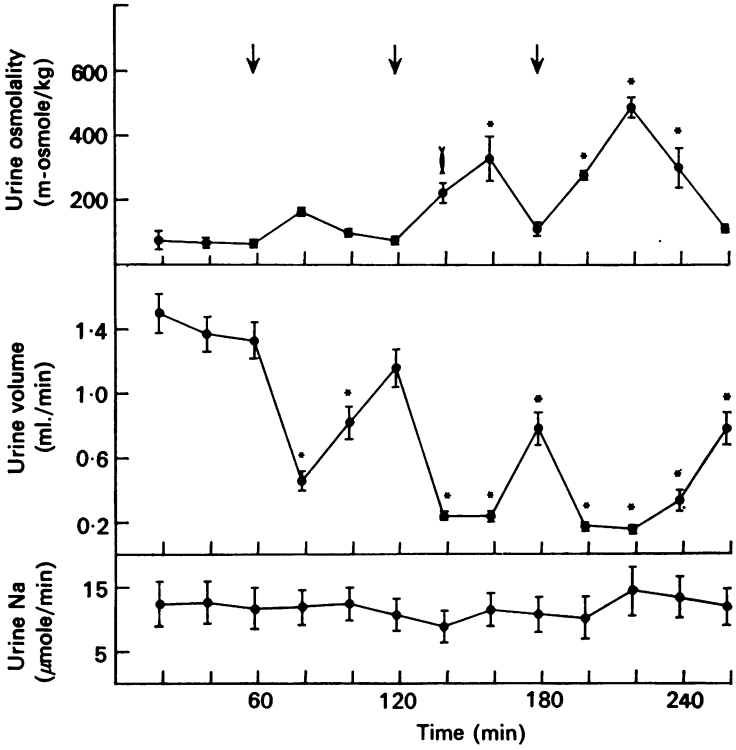
Text-fig. 3. Urine volume and Na output (twelve experiments in ten monkeys) and osmolality (eight out of the twelve experiments in six out of the ten monkeys) with continuous I.V. infusion of 5% dextrose (1 ml./min), before and after intraventricular infusion (horizontal bar) of 1·0 M-NaCl.

urine osmolality but no change in urine Na output (Text-fig. 3). Intraventricular infusion of hypertonic saline also produced slight excitement during the first few minutes in some monkeys and defaecation during the infusion in all monkeys. Radial artery blood pressures before, during and 5 min and 90 min after the infusion in three monkeys are shown in Table 1. There was some increase in blood pressure in two out of three monkeys as a result of the infusion.

In five monkeys, 1·0 M-NaCl was infused I.V., rate and total volume

being similar to the intraventricular infusion. The urine volume and Na data (Table 2) showed no significant changes.

In order to obtain an estimate of the quantity of ADH presumably released by intraventricular hypertonic saline during water diuresis all monkeys in the series were given three doses of lysine-vasopressin during water diuresis. Text-fig. 4 shows that the effect of the highest dose (30 mu./kg) of lysine-vasopressin on urine volume and osmolality (Na was not affected) was less than that of intra-ventricular hypertonic saline (Text-fig. 3).



Text-fig. 4. Urine volume and Na output (eleven experiments in ten monkeys) and osmolality (six out of the eleven experiments in six out of the ten monkeys) with I.V. dextrose as in Fig. 5; lysine vasopressin injected I.V. 3, 10 and 30 mu./kg at 60, 120 and 180 min respectively.

DISCUSSION

The main object of this work was to determine whether a high concentration of Na in the region of the third ventricle can trigger natriuresis. All monkeys were kept healthy under constant conditions and were trained for experiments in the conscious state sitting upright in the restraint chair. The head was always completely free. The trained monkeys showed no

signs of distress (e.g. vocalization, struggling) during experiments. They were completely free in the cages between experiments. All experimental animals ate heartily and remained active, friendly and cooperative up to the end of the investigation. The third ventricle implant was judged finally successful by post-mortem localization. Any transient salt or water deficit was ruled out by oral administration of 100 ml. 0·08 M-NaCl followed by an equilibration period. Serum Na was normal in all monkeys and lay within a range narrower than other reported levels (McClure, 1975).

The results showed that infusions of 1·0 M-NaCl (10 μl./min for 30 min) into the third ventricles of monkeys had no natriuretic effect irrespective of their state of hydration (Text-figs. 2, 3). The following ancillary effects of hypertonic saline infusion into the third ventricle are noteworthy: (i) large reciprocal changes in urine volume and osmolality in hydrated monkeys undergoing water diuresis (Text-fig. 3); (ii) a small but significant increase in urine osmolality in non-hydrated monkeys (Text-fig. 2); (iii) defaecation during the infusion in all monkeys; (iv) a small rise in blood pressure during and after the infusion (Table 1). Taken together, these effects suggest but do not prove the release of ADH, though the absence of isotonic saline infusion controls in Text-fig. 3 makes the changes in urine volume and osmolality less meaningful. Judging by the effect of lysine-vasopressin I.V. in the same monkeys (Text-fig. 4), the quantity of ADH probably released was greater than 30 m-u./kg.

The total quantity of Na infused (300 μM) was under 0·4 % of the total extracellular Na, assuming the e.c.f. to be 200 ml./kg (Bourne, 1975) and was too small to have any direct effect on renal Na excretion (Nizet, Godon & Mahieu, 1968*a*, *b*; Levinsky, 1974), as borne out by Table 2.

The natriuresis reported to occur in other species after comparable intraventricular (or intracarotid) infusion of hypertonic saline has not been satisfactorily explained so far. Intracranial or systemic administration of hypertonic saline releases ADH in the dog (Verney, 1947), sheep (Blaine *et al.* 1975; Scott & Morton, 1976), goat (Andersson, Olsson & Warner, 1967; Lishajko & Andersson, 1975) and rat (George, 1976). It has been established also that ADH is natriuretic in the dog (Humphreys, Friedler & Earley, 1970; Kurtzman, Rogers, Boonjarern & Arruda, 1975), sheep (Scott & Morton, 1976), goat (Andersson, 1955) and rat (Gershkovich, 1975). It is possible that the natriuretic effect of hypertonic saline in all these species is brought about by the ADH released as shown in sheep by Scott & Morton (1976). In the present work up to 30 mu./kg of lysine vasopressin was not natriuretic in the monkey. The renal action of ADH differs somewhat in the monkey perhaps because ADH has a smaller effect on the distal tubule and/or the monkey has a poorly developed inner medulla with few long loops of Henle (Tisher, Schrier & McNeil, 1972). Species dif-

ferences in the effect of ADH on natriuresis are not surprising. Fluid-electrolyte regulation in ruminants evidently differs from that in other mammals; as for the dog, it can remain in Na balance with normal plasma Na even after oral intake of 4 g NaCl per kg body weight i.e. over 10 times the maximum normal human intake, for 6 days (Smith, 1957). The natriuretic effect of ADH in lower mammals may be physiologically relevant for it would tend to correct the Na error signal which triggers ADH release. The mechanism of ADH natriuresis in lower mammals remains unknown. It is not due to inhibition of the renin-angiotensin-aldosterone system or changes in arterial pressure or GFR (Humphreys, Friedler & Earley, 1970). In the dog, ADH has been claimed to release a natriuretic substance (Buckalew & Dimond, 1976).

To conclude, the infusion of hypertonic saline into the third ventricle has no natriuretic effect in the monkey. Although this work does not rule out the existence of a central natriuretic or antinatriuretic system sensitive to Na in primates, it does suggest that such a system, if it exists, is scarcely the overriding omnipotent 'monkey-wrench' postulated by Smith (1957).

Valuable advice and help were received from Professor A. S. Paintal, Dr T. Desi Raju and Dr I. S. Dusaj. Partial financial support was obtained from Grant AFOSR-71-2149, U.S. Air Force Office of Scientific Research (European Office of Aerospace Research and Development).

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EXPLANATION OF PLATES

PLATE 1

Pneumoencephalogram with the third ventricular needle implant.

PLATE 2

Cut surface of a brain showing a successfully implanted needle; the third ventricle ependyma was stained blue. Scale in mm.