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OSMORECEPTORS OR SODIUM RECEPTORS: AN INVESTIGATION INTO ADH RELEASE IN THE RHESUS MONKEY

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SUMMARY

1. ADH secretion was studied in trained, preoperated conscious monkeys undergoing water diuresis after administration of isosmolar hypertonic solutions of different substances into any one of the following sites: (i) anterior third ventricle, (ii) the hypothalamus, just anterior to the third ventricle and (iii) common carotid artery.

2. Free water clearance was continuously monitored and the ADH released was measured by bio-assay on the same animals after administering graded doses of standard arginine vasopressin in a comparable manner.

3. Intraventricular infusions of hypertonic solutions of NaCl or Na acetate released significant amounts of ADH while sucrose or mannitol of comparable osmolality were ineffective. Graded increases in the concentration of NaCl infused into the c.s.f. resulted in secretion of ADH proportional to log Na concentration.

4. Infusion of the same hypertonic solutions into the anterior hypothalamus released ADH, though Na salts were more effective than the sugars.

5. Hypertonic solutions of NaCl, Na acetate, sucrose or mannitol were effective in releasing ADH when injected via the carotid artery, but hypertonic solutions of NaCl were significantly more effective than the other solutions.

6. These findings may be explained by the hypothesis that the 'osmoreceptors' of Verney are Na sensitive receptors composed of dendrites innervating the specialized ependyma of the anterior part of the third ventricle.

INTRODUCTION

Osmoreceptors were described originally in the dog as elements located in the anterior hypothalamus and responding to an increase in the osmotic pressure of blood by releasing the antidiuretic hormone (ADH) from the neurohypophysis (Verney, 1947; Jewell & Verney, 1957). Recently, this concept was questioned by Swedish workers who had shown that although intracarotid infusions of hyperosmolar solutions of NaCl, sucrose and fructose caused antidiuresis in the goat (Eriksson, Fernandez & Olsson, 1971), only intraventricular infusions of NaCl were effective (Andersson, Jobin & Olsson, 1967; Andersson, Olsson & Warner, 1967; Olsson, 1969; Eriksson, 1974). According to Andersson (1977), 'the integrated results of these studies imply that ... the osmoreceptors ... are ... juxta-ventricular sodium

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sensitive receptors'. In sheep, McKinley, Denton & Weisinger (1978) found that lateral ventricular infusion of hypertonic sucrose dissolved in artificial c.s.f. was antidiuretic, though less so than a comparable solution of NaCl, while pure hypertonic sucrose by itself was ineffective.

In the rhesus monkey, Verney's osmoreceptor concept has been largely accepted and elaborated upon by Hayward and his coworkers (Hayward, 1977). Intracarotid hypertonic NaCl is more effective than hypertonic D-glucose in triggering the ADH system (Hayward & Jennings, 1973). The available data in primates, however, do not permit one to say whether the signal from the blood or the c.s.f. which acts on the hypothalamus to trigger ADH release is osmolality or NaCl concentration or both. The present work was designed to investigate these questions in healthy conscious monkeys trained for the purpose.

METHODS

1. Female rhesus monkeys weighing between 3 and 4 kg were maintained as described earlier (Kumar & Swaminathan, 1977).

2. *Implantation of cannula in the brain.* Stereotaxic surgery for cannulating the third ventricle has been described in detail earlier (Kumar & Swaminathan, 1977). The following modifications were introduced in this work. The anterior third ventricle was cannulated with a 21 g stainless-steel Luer-Lok hypodermic needle of 30 mm stem length, which served as the outer cannula. An inner cannula was prepared from Hamilton syringe pipette needle of 26 g with outer diameter 0·46 mm and inner diameter 0·25 mm, by mounting it on a male Luer connector with epoxy glue. The inner cannula fitted snugly into the outer cannula and when locked, the tip of the inner cannula was flush with the tip of the outer cannula. Stainless-steel wire (30 g) was threaded through holes made in the bone adjoining four corners of the cranial window before pouring the dental cement. This reinforcement anchored the implant to the bone without any play. The position of the cannula was confirmed in some monkeys by radiography, after injecting 0·1–0·2 ml. Urografin (Schering AG, Berlin/Bergkamen) (Pl. 1) and in all monkeys by injecting bromophenol blue to stain the ventricular ependyma (Kumar & Swaminathan, 1977) under anaesthesia before post mortem examination.

In some monkeys, the cannula was implanted into the anterior hypothalamus just anterior to the third ventricle, 0·5 mm lateral to the mid line.

3. *Cannulation of carotid artery.* The common carotid artery (4 on left side, 3 on right side) was cannulated through the facial artery, using silastic medical grade tubing (Dow–Corning) of outer diameter 1·2 mm and inner diameter 0·64 mm. The other branches of the external carotid artery were tied off. The tubing was brought out subcutaneously over the cranium. It lay coiled inside a small screw-capped polypropylene container implanted into the cranium at the bregma with dental cement reinforced with 30 g stainless-stele wire as described above. When not in use the silastic tubing was filled with heparin solution (1000 i.u./ml.) and sealed. Post mortem, the patency of the internal carotid artery was confirmed by injecting a dye into the cannula which stained the brain areas supplied by the artery. Cerebral angiography also was performed in some monkeys after injecting Urografin via the carotid cannula under anaesthesia.

4. *Experimental protocol.* Experiments on preoperated monkeys were carried out at the same time of the day by the same investigator in a temperature controlled (28–29 °C) sound-proof cubicle. The animals were fully trained to sit on a chair, with light restraint and familiarized with various dummy manipulations before the actual experiment began.

The bladder and a limb vein were catheterized respectively with Foley's FG10, a 3 ml. balloon catheter and appropriate sized polyethylene tubing with sterile precautions. The monkey was put on the chair and after about 1 hr, an I.V. drip of 5% dextrose was started at the rate of 1·5 ml./min to produce a diuresis. In about 40 min, urine output equalled the drip rate and urine collection was started. Collections were for periods of 10 min and after three control collections, artificial c.s.f. or saline, 0·15 M (controls) or one of the test substances

(NaCl, Na acetate, sucrose or mannitol) was administered. Only one test solution was administered on any one day and at least 36 hr were allowed between consecutive experiments. The test solutions and assay doses of standard arginine vasopressin (AVP) were administered in random order. Any given monkey responded in the same way to a particular stimulus over a long period of time. Control free water clearance (FWC) was maintained between 1·0 and 1·4 ml./min in all experiments.

For the monkey with a cannula in the third ventricle or in the hypothalamus, the test substance was dissolved in artificial c.s.f. and infused for a period of 15 min at the rate of 5 μl./min, using Harvard model 902 pump. The volume of the fluid infused was checked by noting the movement of an air bubble in the polyethylene tubing over a calibrated distance. The composition of artificial c.s.f. was as follows: (in m-mole/l.) Na, 150; K, 2·9; Ca, 1·3; Mg, 1·0; Cl, 157 and $NaHCO_3$ to bring the pH up to 7·4. For the carotid artery series, the test substance was dissolved in saline and injected in a volume of 2 ml. over a period of 15 sec. In any given monkey, all the test solutions were of same osmolality.

Between experiments, the monkeys were completely free in their individual cages.

5. *Analytical methods.* Urine volume was measured correct to 0·1 ml. Osmolalities of plasma and urine were measured by the freezing point depression method (Knauer Halbmikro Osmometer). Plasma osmolality values of non-hydrated monkeys were between 300 and 310 m-osmole/kg and after 60–80 ml. water retention, values ranged between 290 and 300 m-osmole/kg; so 300 m-osmole/kg was taken as the plasma osmolality for the calculation of FWC. Plasma Na was estimated by emission spectrometry (Beckman model B, Flame Spectrophotometer). In some animals, blood pressure was recorded via the carotid catheter, connected to a Statham UC-3 strain gauge and Beckman RM Dynograph. The amount of ADH released by each stimulus was determined by bio-assay as described below.

6. *Bio-assay of ADH.* Four or five graded doses of standard AVP (Sigma, synthetic lyophilized powder standardized against international standard posterior pituitary powder, 85·3 i.u./mg) were infused I.V. over a period of 15 min into every monkey of the third ventricle and anterior hypothalamus series or injected I.V. (in 15 sec) into every monkey of the carotid artery series while each monkey was undergoing water diuresis. The order of administration of various doses was randomized. The reduction in free water clearance (ΔFWC) was plotted against each corresponding log dose of AVP. The amount of ADH released by a test stimulus was determined by comparing the ΔFWC against the standard log dose of AVP: ΔFWC curve (Figs. 2 and 7).

7. *Statistical analysis.* In the case of histograms (Figs. 3 and 8) analysis of variance was performed after log transformation of the original data to obtain homogeneity among variances. As this showed the groups to be from different populations, the Student range test was used to detect the significantly different groups (Goldstein, 1964). When there was no homogeneity among variances even after log transformation as in Fig. 1, a confidence interval was calculated for the control points, and the test points considered significantly different from control points if mean + 2 S.D. did not lie within the confidence interval. In the case of Fig. 6 (carotid series) as there was a parallel I.V. control, the significance of the difference between the data obtained for each period after intracarotid and I.V. injections was calculated by paired t test, in terms of the change from final preinfusion control value (Blaine, Denton, McKinley & Weller, 1975; Snedecor & Cochran, 1967). Regression analysis (Figs. 2, 4 and 7) was performed in the usual way.

RESULTS

I. *Third ventricle series*

1. *Control experiments.* In monkeys with the cannula close to the anterior wall of third ventricle, isotonic saline (0·15 M-NaCl) released significant amounts of ADH, as shown by ΔFWC. Therefore it was necessary to use artificial c.s.f. as the solvent for the test substances. Artificial c.s.f. infusion did not release ADH. The ΔFWC (in ml.) observed after isotonic saline and artificial c.s.f. infusions in three monkeys respectively were: $-101{\cdot}14 \pm 27{\cdot}27$ and $-0{\cdot}88 \pm 3{\cdot}07$.

2. *Effect of test infusions.* It was possible to complete the administration of all

test substances and assay doses of AVP in nine monkeys in the third ventricular series. Out of these, four monkeys were sensitive to NaCl, 0·2 M in artificial c.s.f. (total 0·35 M) while in five, NaCl, 0·8 M (total 0·95 M) was needed to produce comparable ADH release. In the latter, the placement of ventricular cannula was found to be slightly posterior compared to the former (Pl. 1). The test substances infused in any given monkey were of identical osmolality. In a monkey sensitive to 0·2 M-NaCl, the following molar concentrations of different substances dissolved in artificial c.s.f. were used: Na acetate, 0·19; sucrose, 0·36 and mannitol, 0·37. When the

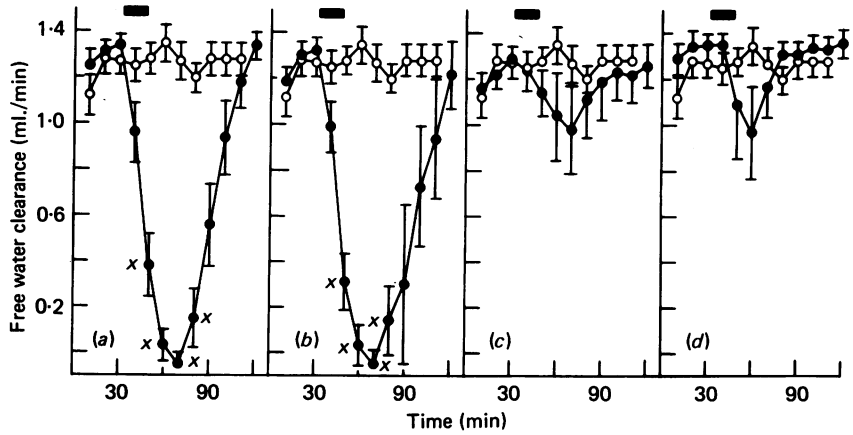

Fig. 1. *FWC* before and after intraventricular infusion (horizontal bar) of artificial c.s.f. (○) and test substances dissolved in artificial c.s.f. (●) in nine monkeys. NaCl, 0·2 M (total 0·35 M) in four monkeys and NaCl, 0·8 M (total 0·95 M) in five monkeys (*a*), isosmolar hypertonic solutions of Na acetate (*b*), sucrose (*c*), and mannitol (*d*). Artificial c.s.f. is shown in all panels for comparison. Statistically significant change from pre-infusion control is denoted by *x* ($P < 0·01$).

monkey was sensitive only to 0·8 M-NaCl, four times these concentrations were used. Artificial c.s.f. did not produce any significant ΔFWC. Of the four substances tested, NaCl and Na acetate produced a sharp, prolonged and significant ΔFWC whereas sucrose and mannitol produced a transient and insignificant ΔFWC (Fig. 1). No behavioural change was noticed after artificial c.s.f., sucrose or mannitol. Hypertonic solutions of NaCl and Na acetate produced tongue and lip smacking, chewing and sometimes defaecation.

3. *Quantification of ADH released.* Five doses of standard AVP 17·5, 70, 280, 1120 and 4480 i. μu./kg.min were tested in all the monkeys and a standard curve of log dose AVP against ΔFWC was plotted (Fig. 2). The ΔFWC produced by each substance in each monkey was individually transformed into standard ADH units using the standard log dose–response curve. The pooled data are shown in the histogram (Fig. 3). NaCl or Na acetate released significantly greater amount of ADH as compared to sucrose, mannitol or artificial c.s.f. ($P < 0·01$). Sucrose and mannitol responses were not significantly different from artificial c.s.f. ($P > 0·05$). There was no significant difference between NaCl and Na acetate ($P > 0·05$).

In three monkeys, four graded doses of hypertonic NaCl (0·1, 0·2, 0·4 and 0·8 M)

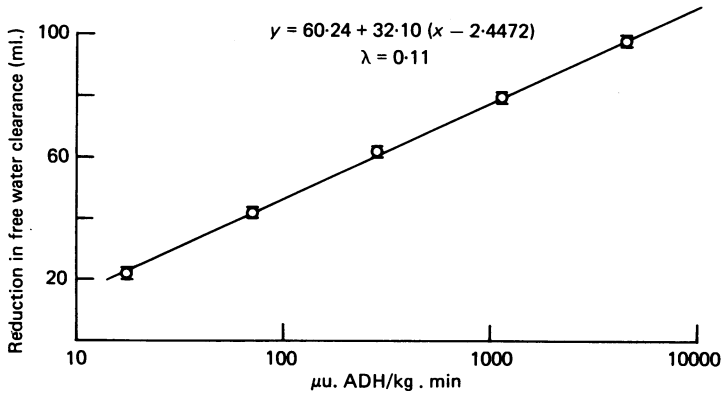

Fig. 2. ΔFWC produced by each of five graded doses of standard ADH (μu./kg.min) infused for a period of 15 min is plotted against the log dose of ADH. The regression of the standard line thus obtained for the nine monkeys of the ventricular series is linear and significant ($P < 0{\cdot}01$).

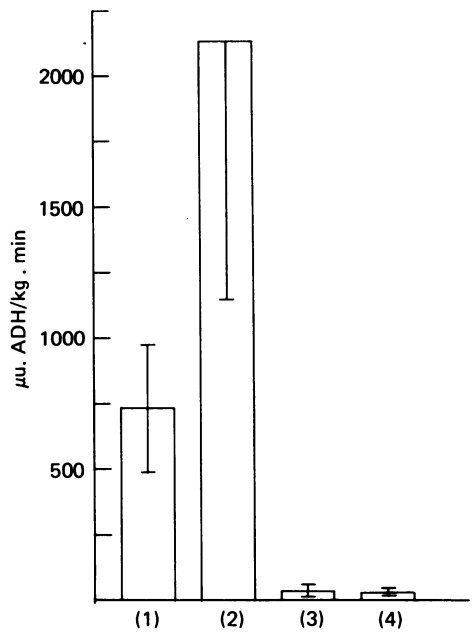

Fig. 3. Histogram showing the amounts of ADH released by different hypertonic solutions in c.s.f. infused into the third ventricle in nine monkeys; NaCl (1), Na acetate (2), sucrose (3) and mannitol (4). Amount of ADH released by artificial c.s.f. ($2{\cdot}8 \pm 0{\cdot}91$ i. μu./kg.min) is not shown in the Fig. NaCl or Na acetate released significantly more ADH than artificial c.s.f., sucrose or mannitol ($P < 0{\cdot}01$).

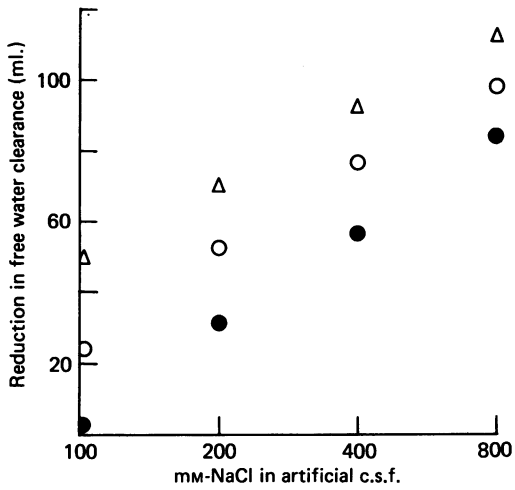

Fig. 4. ΔFWC by four graded concentrations of NaCl in artificial c.s.f. infused into the third ventricle in three monkeys. Each monkey is represented by one symbol. The regression is linear and significant ($P < 0{\cdot}01$).

in artificial c.s.f. were administered and the ΔFWC were plotted against the corresponding log doses (Fig. 4). The regression was linear and significant ($P < 0{\cdot}01$). The same data transformed via Fig. 2 into the amounts of ADH secreted in i. μu./kg.min for the four graded doses respectively were: 54 ± 41, 253 ± 158, 1353 ± 801 and 6167 ± 3159.

II. *Anterior hypothalamus series*

In three monkeys, attempts to infuse test solutions directly into the hypothalamus just anterior to the third ventricle were successful. The protocol was identical to that in the ventricular series. As evidenced by dye staining, post mortem, in one monkey the infused fluid had traversed back along the needle track up to the cranial window. In the other two monkeys, the fluid after travelling a short distance along the needle track had entered the lateral ventricle and thence the third ventricle. The ΔFWC produced by different substances are shown in Fig. 5. The amounts of ADH released in these three monkeys were computed in μu./kg.min by transforming the data from Fig. 5 via the standard ADH log dose–response curve. The respective values for artificial c.s.f., NaCl, Na acetate, sucrose and mannitol were (same symbols as in Fig. 5): ○, 4·0, 475, 42, 195 and 170; ●, 0, 12500, 5150, 24 and 88; △, 0, 280, 1325, 14 and 13.

III. *Carotid artery series*

1. *Control experiments.* Test substances dissolved in 2 ml. isotonic saline were injected over a period of 15 sec. Control experiments were performed in the same way by injecting the test solutions into a vein. All test solutions had the same osmolality, i.e. NaCl, 0·75 M in isotonic saline (total 0·9 M) or equivalent. The molar concentrations of different substances dissolved in isotonic saline were: NaCl, 0·750;

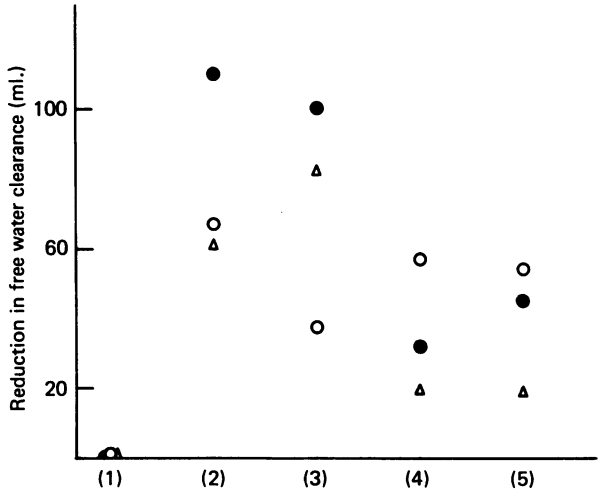

Fig. 5. Hypothalamus series; ΔFWC produced by artificial c.s.f. (1), hypertonic solutions of NaCl (2), Na acetate (3), sucrose (4) and mannitol (5) in artificial c.s.f. Each monkey is represented by one symbol. The monkey represented by (○) was sensitive to NaCl, 0·2 M in c.s.f. and the infused fluid did not enter the ventricular system; (●) was sensitive to NaCl, 0·2 M, in c.s.f. and the infused fluid entered the ventricular system; (△) was sensitive only to NaCl, 0·8 M in c.s.f. and the infused fluid entered the ventricular system; the cannula was located more laterally as compared to the other two monkeys.

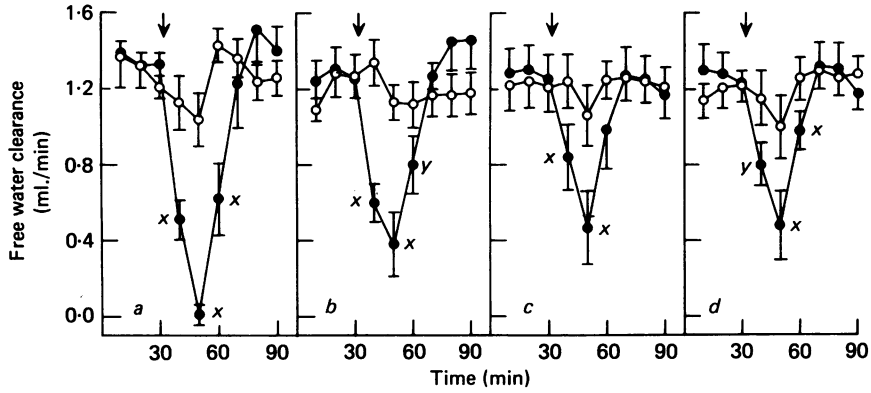

Fig. 6. *FWC* before and after intracarotid (●) and I.V. (○) injections (arrow) of different test substances dissolved in isotonic saline, 2 ml., in seven monkeys. NaCl, 0·75 M (total 0·9 M) (*a*), isosmolar solutions of Na acetate (*b*), sucrose (*c*) and mannitol (*d*). Significance of the difference between the data obtained for each period after intracarotid and I.V. injections was calculated by paired *t* test, in terms of the change from final preinfusion control value. Significantly different periods are represented by *x* ($P < 0·01$) and *y* ($P < 0·05$).

Na acetate, 0·700; sucrose, 1·313 and mannitol, 1·355. Control injection of isotonic saline in the carotid artery did not produce any change in *FWC*.

2. *Effect of test injections.* All the test substances injected into the carotid artery produced significant ΔFWC in some collections when compared with the corresponding collections after I.V. injections (Fig. 6). The integrated ΔFWC produced by

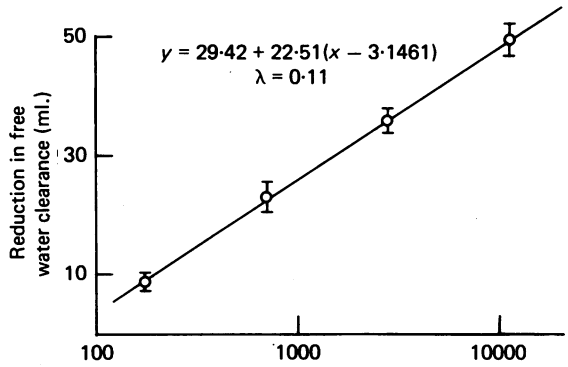

Fig. 7. ΔFWC produced by each of four graded doses of ADH (μu./kg) injections is plotted against log dose of ADH in the seven monkeys of carotid series. Regression of the standard line thus obtained is linear and significant ($P < 0{\cdot}01$).

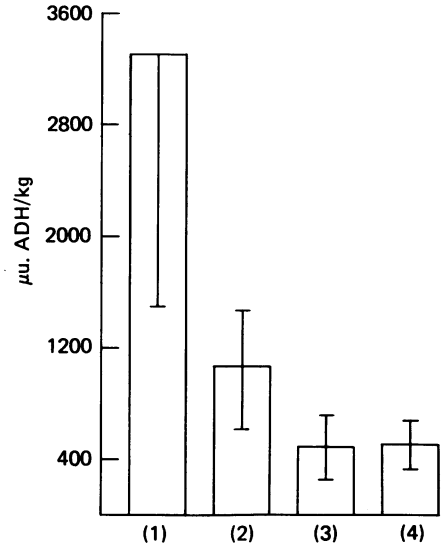

Fig. 8. Histogram showing the amounts of ADH released by hypertonic solutions of different substances injected into the carotid artery of seven monkeys. NaCl (1), Na acetate (2), sucrose (3) and mannitol (4). As intracarotid isotonic saline did not release any ADH, it is not shown in the Fig. All the four test solutions released significant amounts of ADH ($P < 0{\cdot}01$). NaCl released significantly more ADH than isosmolar sodium acetate ($P < 0{\cdot}05$), sucrose or mannitol ($P < 0{\cdot}01$).

the test substances via the carotid artery were significantly higher than the corresponding ΔFWC produced by the venous route ($P < 0{\cdot}01$). In some monkeys, a second injection of the same substance was given into the carotid artery following recovery from the first one and the result was same as that of the first injection.

3. *Quantification of ADH released.* Standard bio-assay curve was obtained with four doses of standard AVP, 175, 700, 2800 and 11200 i. μu./kg injected in 15 sec. I.V. (Fig. 7). The ΔFWC after intracarotid injections were individually transformed into ADH units and a histogram was plotted (Fig. 8). Isotonic saline did not release

any ADH. All the four test solutions released significant amounts of ADH compared to isotonic saline ($P < 0{\cdot}01$). The effect of NaCl was significantly greater than that of Na acetate ($P < 0{\cdot}05$), sucrose or mannitol ($P < 0{\cdot}01$). There was no significant difference between sodium acetate, sucrose and mannitol ($P > 0{\cdot}05$). No behavioural response was observed after intracarotid isotonic saline but all the test solutions elicited mild arousal and transient uneasiness. Intracarotid injections of NaCl, sucrose or I.V. injection of 11·2 i. m-u./kg of standard AVP produced a transient rise of 10–20 mm Hg in mean blood pressure (measured in three monkeys).

DISCUSSION

The above experiments were performed under controlled conditions in the conscious state, the monkey sitting upright on the chair. Trained monkeys remained friendly and cooperative during the experiment without any sign of uneasiness and it was possible to obtain a constant diuresis equal to the rate of the I.V. drip. Between experiments, they were completely free in the cage, where they ate and lived as actively as before surgery. Plasma osmolality and Na were within the normal range throughout the investigation period. The pH of the solutions infused into the brain tissue or c.s.f. was between 7·3 and 7·5 and all the test solutions had the same osmolality for any given monkey.

The aim of the present investigation was to determine whether hypertonicity or an increase in the concentration of NaCl triggers ADH release. When delivered into the c.s.f. of the third ventricle, only Na salts were effective in releasing ADH and non-electrolytes were ineffective. The anterior part of third ventricle was apparently more sensitive to Na than the posterior part, as the monkeys which were sensitive to 0·2 M-NaCl in c.s.f. were found to have the needle closer to the anterior wall (Pl. 1). All these substances when injected through the carotid artery were effective in releasing ADH, though NaCl was significantly more effective than sodium acetate, sucrose or mannitol. When different hypertonic solutions were infused directly into the anterior hypothalamus adjacent to the third ventricle, sucrose and mannitol were also effective in releasing ADH. In one monkey in which the fluid did not enter the ventricular system at all, the sugars were comparatively more effective than in the other two monkeys. In the other two monkeys in which some infused fluid entered the ventricular system via the lateral ventricle, sugars were much less effective than the Na salts.

The above findings suggest that the 'osmoreceptors' of Verney are in fact sensitive to Na because Na salts were more effective than the sugars by all the three routes. The receptors must be located on the neuronal side of the blood-brain barrier because if they were on the haemal side, either intracarotid hypertonic sugar solutions would have released no ADH (assuming that the receptors are sensitive only to sodium) or the hypertonic solutions of sugars and Na salts would have released equal amounts of ADH (assuming that the receptors are sensitive to osmotic pressure). Thus the release of ADH triggered by intracarotid injections of hypertonic solutions must be due to a rise in the c.s.f. sodium concentration, either in the ventricle or in the brain substance, following the withdrawal of water across the blood-brain barrier (Crone, 1963; Fenstermacher & Johnson, 1966).

The negligible effect of intraventricular hypertonic sugar solutions and the somewhat greater effect noted after the administration of the same solutions into the hypothalamus suggest that these Na sensitive receptors may be situated on the ependymal surface of the anterior third ventricle exposed to the c.s.f. Intrahypothalamic infusions of hypertonic solutions of different substances, close to the ependyma, could have caused ADH release by drawing out water across the ependyma and thereby raising the c.s.f. Na concentration (Heisey, Held & Pappenheimer, 1962; Fenstermacher, Patlak, Levin & Rall, 1969). In one of the three monkeys with cannulae in the hypothalamus, infused fluid did not enter the ventricles at all. In this monkey, hypertonic sugar solutions produced a greater antidiuretic response than in the other two. The other two monkeys in which some infused fluid backtracked into the ventricles, showed much greater antidiuretic response after hypertonic sodium salts than after hypertonic sugars, because of an additive effect through the c.s.f. In the monkey which was sensitive only to 0·8 M-NaCl in artificial c.s.f., the needle was more laterally placed in the hypothalamus than in the other two. If the Na sensitive receptors were located in the brain substance, it would be difficult to explain the antidiuretic effects of intrahypothalamic sugar infusions. Thus the evidence obtained in monkeys supports the hypothesis of Na sensitive receptors of the anterior third ventricle (Andersson, 1977).

The results of intracarotid injections in monkeys are different from those in sheep (McKinley *et al.* 1978), goat (Eriksson *et al.* 1971) and dog (Verney, 1947), as the sugars are significantly less effective than NaCl in releasing ADH. In sheep (McKinley *et al.* 1978), intracarotid hypertonic sucrose caused a greater increase in c.s.f. Na concentration and proportionately a greater decrease in renal FWC than isosmolar NaCl. They questioned the validity of the Na sensitive receptor hypothesis on the basis of the finding that intracarotid infusion of 4·6 M-urea produced a greater increase in c.s.f. Na concentration, but a smaller ΔFWC than 2 M-sucrose or 1 M-NaCl. On the other hand, they do not offer any convincing explanation as to why urea was not equally effective in triggering the osmoreceptors. Further, if intracarotid hypertonic urea, in sheep, produced an increase in c.s.f. Na concentration by withdrawal of water across the blood-brain barrier, a corresponding fall in jugular vein plasma Na concentration should have occurred. Surprisingly, this was not found to be the case for urea, while such a fall was observed for sucrose. In sheep, intraventricular infusions of hypertonic sugar solutions in artificial c.s.f. were fairly effective in eliciting antidiuresis, whereas in the monkey they are ineffective. The positive finding in sheep could be explained if the Na sensitive receptors were presumed to be on the neuronal side of the ependyma in this species. The relative lack of effect of intraventricular infusion of pure non-saline hypertonic sucrose in goat (Andersson, 1977) and sheep (McKinley *et al.* 1978) may be due to the changes in the c.s.f. concentration of other important ions like Ca and K.

A unitary explanation of the above findings in the monkey is that the osmoreceptors of Verney are probably the specialized ependymal cells innervated by dendrites, which are known to occur in the anterior third ventricle (Bleier, 1971; Weindl & Joynt, 1972; Dellmann & Simpson, 1975; McKenna & Rosenbluth, 1975; Millhouse, 1975). Scanning electron microscopic observations have shown that the surface of the choroid plexus and distinct areas known as the circumventricular

organs are free of cilia, while major parts of the ventricular ependyma are covered by a dense layer of cilia in rabbit, cat and squirrel monkey (Weindl & Joynt, 1972). Many workers have attributed a sensory function to the processes of magnocellular neurosecretory cells ending freely in the ependyma of the third ventricle of fishes and amphibia (Dierickx, 1962; Smoller, 1965; Hayward, 1974). On the basis of the present data as well as the considerable histological evidence (see above), it is proposed that the osmoreceptors of Verney are sodium sensitive elements composed of the ependymal dendrites alone or specialized ependymal cells plus the dendrites in the anterior third ventricle.

Several other observations can be reconciled easily if the osmoreceptors are identified with a sodium sensitive innervated complex in the ependyma of the anterior third ventricle: (i) the difference in responses to hyperosmolar solutions of NaCl and sugars injected into the carotid artery: while NaCl and the sugars are both only slightly permeable through the blood-brain barrier (Crone, 1963; Fenstermacher & Johnson, 1966; Milhorat, Hammock, Fenstermacher, Rall & Levin, 1971), the small amount of sodium which does permeate into the c.s.f. may continue to activate the ADH system, the sugars being ineffective. Further, hypertonic solutions when perfused via the carotid artery open the blood-brain barrier reversibly in monkeys (Rapoport, 1976). In the present series, osmolality was elevated only for brief periods and the elevation was uniform for all the substances. Apparently, there was no damage to the barrier as a second injection following recovery from the first one (about 1 hr later) produced an identical response. Thus intracarotid injection of hypertonic NaCl released significantly more ADH than comparable solutions of mannitol or sucrose. (ii) The difference in the responses to sodium acetate administered into the third ventricle and into the carotid artery: the third ventricular infusions of Na acetate or NaCl were more effective than the sugars suggesting that the Na concentration provided the relevant signal. The difference between sodium acetate and NaCl in this series was not significant ($P > 0{\cdot}05$). However, the results of Na acetate infusion show a large scatter which was traceable to the large amounts of ADH released by three monkeys in which the cannulae were located posteriorly compared to the others, and required 0·8 M infusion. Nevertheless, it is beyond doubt that Na and not chloride is the triggering ion. On the other hand, Na acetate infused through the carotid artery was less effective than NaCl, perhaps because acetate is a powerful vasodilator even in low concentrations (Frohlich, 1965; Holbert, Pearson & Gonzalez, 1976). The vasodilation could be expected to dilute the signal reaching the osmoreceptors. (iii) The precisely graded response to intraventricular NaCl: the linear relationship between log Na concentration infused into the c.s.f. and ADH release (Fig. 4) strongly suggests a physiologically relevant mechanism. (iv) In physiological and even pathological conditions, changes in plasma osmolality coincide with corresponding changes in plasma Na concentration (the most abundant plasma cation) and therefore it should be hardly surprising if osmoreceptors are in fact Na sensitive receptors.

The ependymal Na sensitive receptors of the anterior third ventricle appear to be osmoreceptors by virtue of their anatomical relations. The determination of their true nature does not detract much from Verney's original concept and in any case does not warrant a change in terminology. Whether the dendrites in the osmoreceptor complex belong to the neurosecretory cell itself or to a 'sensory' neurone

and whether they are functional units by themselves or in conjunction with specialized ependymal cells are questions which need further investigation.

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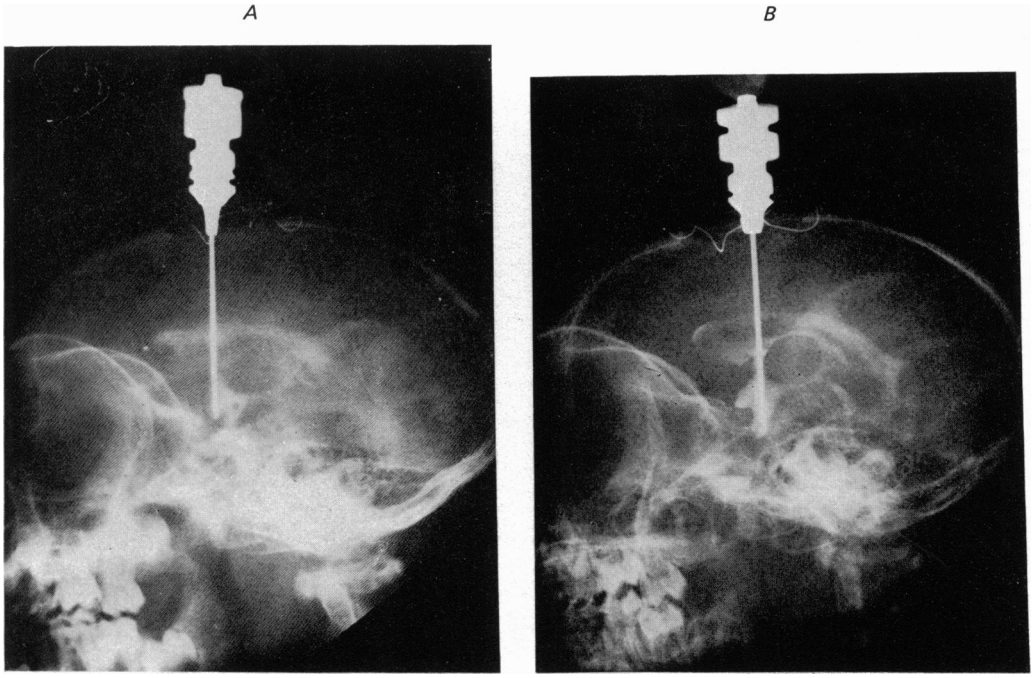
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EXPLANATION OF PLATE

Ventriculogram after 0·1–0·2 ml. Urografin. Note that in the monkey marked (*A*) the cannula was closer to the anterior wall of the third ventricle and was sensitive to NaCl, 0·2 M in artificial c.s.f. whereas monkey (*B*) with the needle slightly posterior was sensitive only to NaCl, 0·8 M in artificial c.s.f. The 30 g wire threaded through the four holes in the skull for better anchoring is also seen.