Pharmacology Biochemistry & Behavior, Vol. 7, pp. 121–128.

Inhibition of Water Intake by Ouabain Administration in Sheep[1]

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(Received 7 September 1976)

WEISINGER, R. S., D. A. DENTON AND M. J. MCKINLEY. *Inhibition of water intake by ouabain administration in sheep.* PHARMAC. BIOCHEM. BEHAV. 7(2) 121–128, 1977. – Intracarotid infusion of ouabain (1280 ng/min) over 4½ hr virtually abolished water intake of sheep in response to intracarotid infusion of either angiotensin II (800 ng/min) or 4 M NaCl (1.6 ml/min for 20 min). Ouabain treatment did not affect mean arterial pressure either before or during infusion of angiotensin. Neither ouabain nor angiotensin administration affected plasma [Na] or [K] or CSF [K]. During ouabain, but not during control infusion, angiotensin administration significantly decreased CSF [Na]. Ouabain administration also decreased water intake after 23½ or 48 hr water deprivation. In the 23½ hr deprivation experiments, food was made available immediately prior to water presentation and the ingestion of food appeared to ameliorate the reduction in water intake. Food intake itself, was decreased in some animals, during ouabain treatment. Ouabain infused at 960 ng/min resulted in significant, but smaller, reductions in water intake induced by angiotensin, 4 M NaCl, and 48 hr water deprivation. It was concluded that ouabain treatment affected water intake by influence on Na transport either in the thirst receptors or at some other level in the neural system between receptor and effector.

Ouabain Intracarotid infusion Water intake Inhibition of drinking

IN RATS, it has been proposed that water intake which results from hypovolaemia without increase in body fluid tonicity is caused by increased renal secretion of renin and thus elevated blood antiotensin II levels [19]. Administration of angiotensin II into the blood stream, or directly into the brain elicits drinking in several species [18,20], although it is questionable whether this behaviour is produced by physiological concentrations [1].

The dipsogenic action of angiotensin II injected into the brain ventricles of goats is dependent on the ambient Na concentration of the cerebrospinal fluid. It has been proposed that angiotensin acts by facilitating the entry of sodium into the intracellular fluid of neurons subserving thirst [2,4]. Evidence cited as consistent with this concept of angiotensin II action is the observation that sodium uptake by frog skin [26] or mucosal cells of the jejunum [13] is stimulated by angiotensin II.

If the dipsogenic action of angiotensin II involves stimulation of Na transport into brain cells, the presence of an inhibitor of transmembrane Na movement might affect this dipsogenic action of angiotensin. The aim of our experiments was to test the action of the Na transport inhibitor, ouabain, on water intake caused by intracarotid infusion of angiotensin II. The effects of ouabain administration on plasma and CSF [Na] and [K] and on blood pressure, prior to and after infusion of angiotensin were determined. The effect of ouabain on other stimuli of water drinking, i.e., intracarotid infusion of 4 M NaCl or water deprivation has also been assessed.

METHOD

Eleven merino sheep, ten ewes and one wether, were used in these experiments. In each group of experiments 6–8 of the 11 animals were used. The sheep were 2–5 years old and weighed 30–45 kg. Each animal had bilateral carotid loops [14] and six of the sheep had a permanent indwelling stainless steel cannula over the lateral ventricle [27]. The animals were maintained in individual metabolism cages. Unless otherwise noted, the sheep had free access to water and were fed 0.8 kg oaten-lucerne chaff at 1600 hr each day.

Infusions

All infusions were made into the left or right carotid artery. The infusions were performed as previously described [15]. Briefly, a 22 g needle, attached to polyethylene tubing, was inserted into one artery with the contralateral artery occluded by a pneumatic cuff. This procedure insured that the infused material was bilaterally distributed in the brain [6,7]. The infusion line was connected to a motor driven syringe (Palmer, England).

In all of the experiments described below an intracarotid infusion began at 1000 hr and continued for 4 hr 50 min.

[1] This research was supported by grants-in-aid from The National Health and Medical Research Council of Australia, The Laura Bushell Trust, and the Robert and Helen Kleberg Foundation.

During the initial 4 hr 30 min, the sheep were infused with physiological saline (control condition) or ouabain (ouabain octahydrate, M.W. = 585, Sigma). The ouabain was infused at 1280 ng/min (3200 ng/ml in physiological saline infused at 0.4 ml/min). Later, a second series of control and ouabain experiments were run. This time a lower dose of ouabain, 960 ng/min (2400 ng/ml at 0.4 ml/min) was used.

Experiment 1(a). Effect of Ouabain on Water Intake Elicited by Angiotensin II

After the initial 4 hr 30 min infusion of ouabain (1280 or 960 ng/min), animals in the experimental condition were infused concurrently for 20 min with 800 ng/min angiotensin II (2000 ng/ml infused at 0.4 ml/min). The angiotensin solution was prepared by diluting 0.5 mg Val^5-angiotensin II (Hypertensin, CIBA) in 250 ml of the prevously used ouabain solution. In control experiments, following the initial infusion of physiological saline, animals were infused concurrently with 800 ng/min angiotensin II (2000 ng/ml in physiological saline infused at 0.4 ml/min).

In both experimental and control conditions 4 litres of water was offered at 1000 hr. The volume of water drunk was measured at 1430 and 1450 hr, i.e., prior to and after the infusion of angiotensin.

Experiment 1(b). Effect of Ouabain on Plasma and CSF Na and K Concentrations, and on Blood Presssure Before and During Angiotensin Infusion

The experimental and control conditions were the same as for 1(a), except that venous blood and CSF were sampled at various times, and blood pressure was monitored continuously during the experiment. Only the high dose of ouabain was used.

Venous blood (8 ml/sample) was sampled from a polyethylene cannula inserted into one jugular vein 30–60 min before commencing infusions. CSF was sampled by inserting a stainless steel luer-lock needle into the lateral ventricle guide tube and siphoning off 1–2 ml of fluid. Electrolyte determinations were performed using a Technicon Autoanalyzer.

Blood pressure was monitored by inserting an 18 g needle attached to a blood pressure transducer (micron MP–15) into a carotid artery. Blood pressure was recorded continuously on an Offner Type RS Dynograph.

Experiment 2. Effect of Ouabain on Water Intake Elicited by Hypertonic NaCl

After the initial 4 hr 30 min infusion of ouabain or physiological saline, 4 M NaCl was infused at 1.6 ml/min for 20 min. For the experimental condition, the 4 M NaCl was made up in a ouabain solution one fourth the concentration of that previously used so that the ouabain infusion rate remained constant. Water intake was determined as in Experiment 1(a).

Experiment 3. Effect of Ouabain on Water Intake After 48 hr Water Deprivation

In these experiments, the sheep were fed at the usual time, but had not been allowed access to water for 48 hr. The animals were weighed prior to the onset of deprivation and just before beginning the infusion. The initial infusion (i.e., ouabain or physiological saline) which began at 1000 hr continued for the entire 4 hr 50 min at 0.4 ml/min; water was available from 1430 hr. Water intake was measured at 1450 hr and, in ouabain treated animals, at various times during the next 24 hr.

Experiment 4. Effect of Ouabain on Water Intake During Feeding After 23½ hr Water Deprivation

The purpose of this experiment was to determine the effect of ouabain treatment on water intake during feeding after 23½ hr water deprivation. For two days the animals were fed at 1300 hr. The amount of food consumed was measured at 1430 hr and 0930 hr the next day. At 0930 hr the food was removed. Water was available only from 1430 hr to 1500 hr. On the third day the same procedure was followed except that at 1000 hr the infusion, i.e., physiological saline or 1280 ng/min ouabain began. The infusion ended at 1500 hr.

Statistical Analysis and Design

Each animal served as its own control. The paired *t*-test was used in the statistical analysis of the data. Data are also presented in the text as mean ± SEM.

At least ten days intervened between successive experiments involving ouabain treatment.

RESULTS

Experiment 1(a). Effect of Ouabain on Water Intake Elicited by Angiotensin II

Figure 1 shows the water intake elicited by angiotensin after saline or ouabain infusion. Water intake induced by the infusion of 800 ng/min angiotensin, under control conditions, ranged from 500–1500 ml/20 min. The mean water intake in the two groups of control experiments was 791 ± 114 ml (n = 8) and 821 ± 48 ml (n = 7), respectively. The latency before drinking was usually 2–3 min.

In animals infused with 1280 ng/min ouabain, water intake induced by angiotensin infusion ranged from 0–250 ml with a mean of 66 ± 31 ml ($p<0.001$). Water intake was reduced in all, and virtually abolished in six of the eight animals tested. In animals infused with 960 ng/min ouabain, water intake induced by angiotensin infusion ranged from 50–740 ml with a mean of 377 ± 111 ml ($p<0.05$). Water intake was virtually abolished in two of the seven animals tested. Animals that drank less water during ouabain infusion than during control infusion did repeatedly investigate the water bin.

Experiment 1(b). Effect of Ouabain on Plasma and CSF Na and K Concentrations, and on Blood Pressure Before and During Angiotensin Infusion

Figure 2 shows mean arterial pressure (MAP) = diastolic blood pressure + 1/3 pulse pressure during control and ouabain experiments. Infusion of 1280 ng/min ouabain did not have a significant effect on the MAP prior to angiotensin infusion (n = 5). Ouabain administration did not appear to affect the increased MAP induced by angiotensin infusion. During saline infusion, angiotensin increased MAP from 69.8 ± 5.5 to 110.0 ± 3.0 mm Hg. During ouabain, angiotensin increased MAP from 64.6 ± 3.0 to 109 ± 3.6 mm Hg. Blood pressure increased 2–3 min

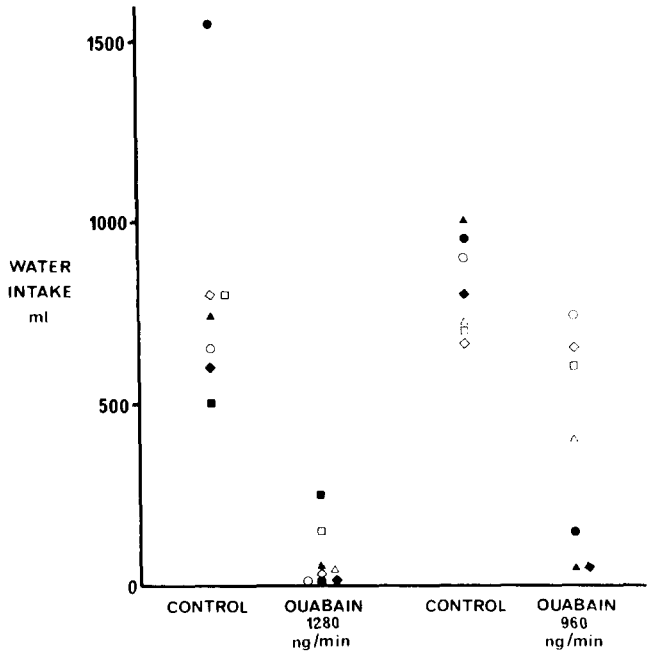

FIG. 1. Water intake (ml) during 20 min intracarotid infusion of 800 ng/min angiotensin II after 4½ hr infusion of either ouabain (1280 or 960 ng/min) or physiological saline (control). Each symbol represents an experiment on an individual animal.

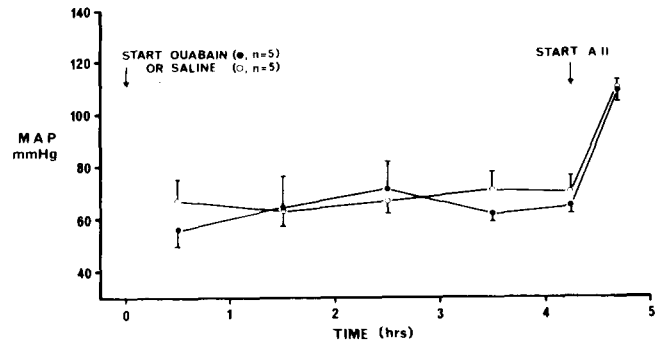

FIG. 2. Mean ± SEM mean arterial pressure (mmHg) during 4½ hr infusion of either ouabain (1280 ng/min) or physiological saline and during 20 min infusion of angiotensin II. Each value represents the average MAP during an interval and is plotted in the middle of that interval. (MAP = diastolic blood pressure + 1/3 pulse pressure).

after the onset of the angiotensin infusion and decreased 2–3 min after its termination.

Figure 3 shows the plasma and CSF Na and K concentrations during control and ouabain experiments. No significant changes in either plasma electrolytes or CSF K concentration were observed. However, angiotensin decreased CSF Na concentration during ouabain treatment from 151 ± 0.58 to 149.2 ± 0.70 mM (n = 6, $p<0.05$).

Experiment 2. Effect of Ouabain on Water Intake Elicited by Hypertonic NaCl

Figure 4 shows the water intake elicited by intracarotid

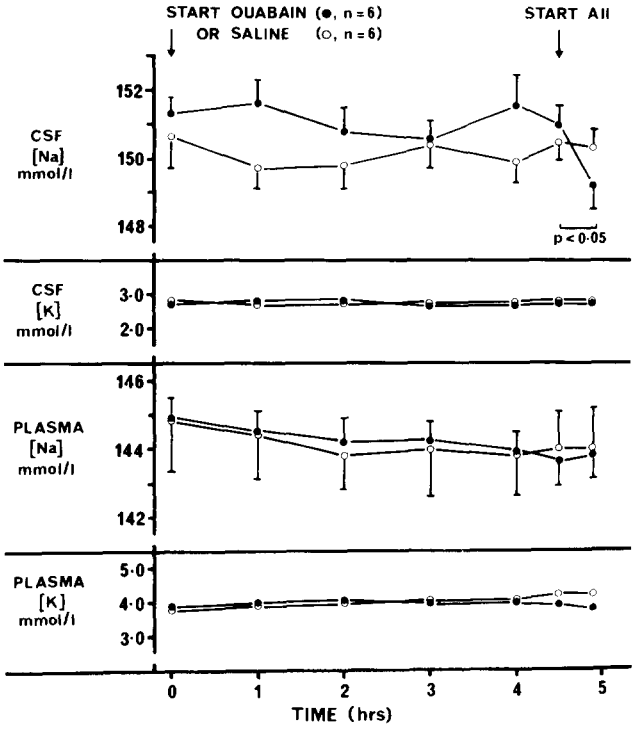

FIG. 3. Mean ± SEM plasma and CSF Na and K concentration (mM) during 4½ hr infusion of either ouabain (1280 ng/min) or physiological saline and during 20 min infusion of angiotensin II.

infusion of 4 M NaCl after physiological saline or ouabain infusion. Water intake induced by the infusion of 4 M NaCl, under control conditions, ranged from 674–1800 ml/20 min. The mean water intake in the two groups of control experiments was 1322 ± 121 ml (n = 6) and 1050 ± 148 ml (n = 7), respectively. The latency to first drink was 2–3 min.

In animals infused with 1280 ng/min ouabain, water intake induced by the infusion of 4 M NaCl ranged from 0–865 ml with a mean of 235 ± 149 ml ($p<0.001$). Water intake was reduced in all, and virtually abolished in four of the six animals tested. In animals infused with 960 ng/min ouabain, water intake induced by 4 M NaCl ranged from 0–850 with a mean of 389 ± 134 ml ($p<0.01$). Water intake was virtually abolished in two of the seven animals tested.

As in Experiment 1(a), although many animals did not drink any water in the presence of ouabain infusion, the animals often investigated the water bin.

Experiment 3. Effect of Ouabain on Water Intake After 48 hr Water Deprivation

Figure 5 shows the results of these experiments. There was no significant difference between weight loss and 20 min water intake in the first or the second series of control experiments. Weight loss was 1625 ± 74 and 1950 ± 371 g while water intake was 1687 ± 194 (n = 8) and 1834 ± 366 ml (n = 8), respectively. Weight loss and water intake were positively correlated (r = .55, $p<0.05$).

Infusion of 1280 ng/min ouabain clearly inhibited water intake. Weight loss was 1750 ± 151 g and the 20 min water

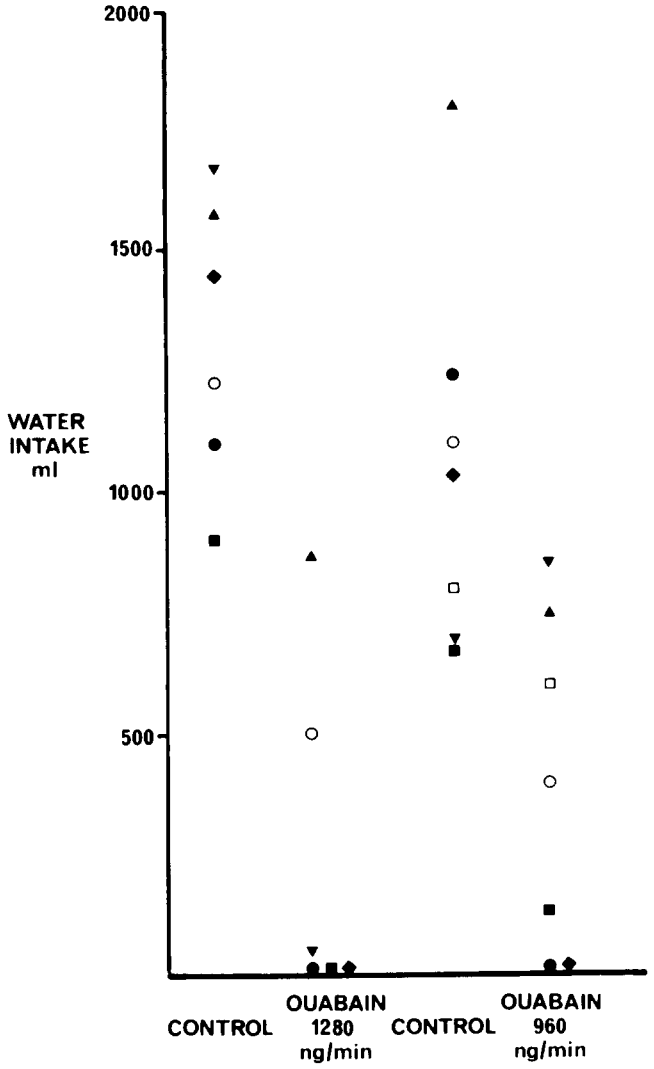

FIG. 4. Water intake (ml) during a 20 min intracarotid infusion of 4 M NaCl (1.6 ml/min) after 4½ hr infusion of either ouabain (1280 or 960 ng/min) or physiological saline (control), each symbol represents an experiment on an individual animal.

intake was 146 ± 92 ml (n = 8, $p<0.001$). Infusion of 960 ng/min ouabain also reduced 20 min water intake. Weight loss was 2250 ± 265 g and water intake was 1369 ± 362 ml (n = 8, $p<0.05$).

Figure 6 shows water intake as a percentage of weight loss over time. During control infusions, mean water intake within 20 min of water presentation approximated body weight loss (i.e., 108.5 ± 19.1, 102.8 ± 21.9% of weight loss, respectively, for the two groups of control experiments) and no further measurements were made. Twenty min mean water intake during the high dose of ouabain was 7.8 ± 4.7% of weight loss (range = 0–35%) while during the low dose, mean water intake was 61.8 ± 15.3% of body weight loss. The ingestion of water subsequent to the high dose of ouabain was suppressed for at least 4 hr and was 74.1 ± 10.0% of body weight loss by 24 hr. The ingestion of water subsequent to the low dose of ouabain was 82.5 ± 7.7% of body weight loss by 24 hr.

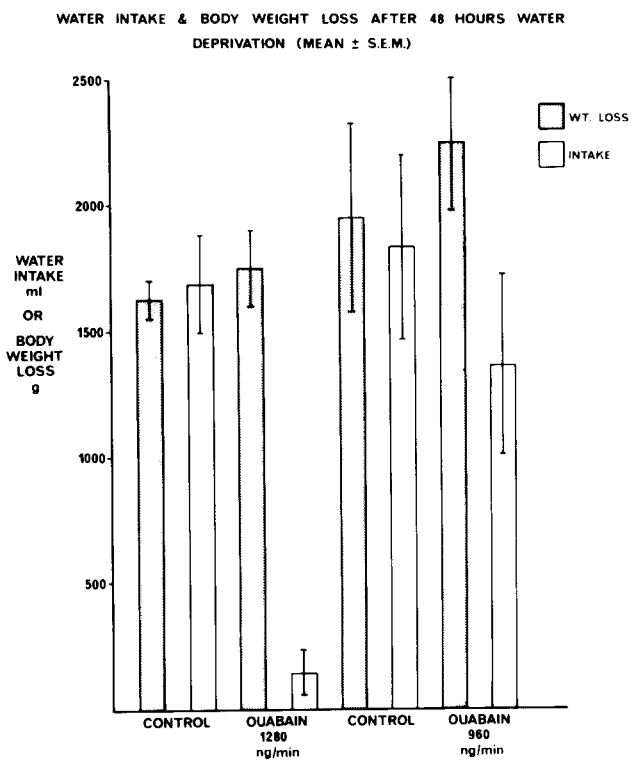

FIG. 5. Mean ± SEM body weight loss (g) and 20 min water intake (ml) during infusion of ouabain (1280 or 960 ng/min) or physiological saline (control) after 48 hr water deprivation.

Experiment 4. Effect of Ouabain on Water Intake During Feeding After 23½ hr Water Deprivation

Table 1 shows the water and food intake for the control and ouabain infusion experiments. The infusion of 1280 ng/min ouabain decreased water intake relative to the average intake on the two days prior to infusion ($p<0.01$). Neither ouabain nor physiological saline infusion had any statistically significant effect on mean food intake over 90 min prior to water presentation. Twenty four hr food intake was not affected by ouabain or physiological saline infusion.

Table 2 shows the percentage change in food and water intake (i.e., [intake during infusion ÷ mean intake of the two baseline days] × 100) for the control and ouabain infusion experiments. Neither ouabain or physiological saline infusion had any significant effect on mean change in 90 min food intake. However, the variance (S^2) of the percentage change in 90 min food intake was markedly increased by ouabain compared to control infusions (i.e., 7276 vs 215 $p<0.001$). That is, 90 min food intake during physiological saline infusion ranged from 92 to 127%, while during ouabain infusion ranged from 10 to 252% of baseline intake. Percentage change in 90 min food intake was significantly correlated with percentage change in water intake during ouabain infusion (r = .90 $p<0.01$) but not during physiological saline infusion (r = .14, $p<0.10$).

DISCUSSION

The infusion of 1280 ng/min ouabain caused a marked

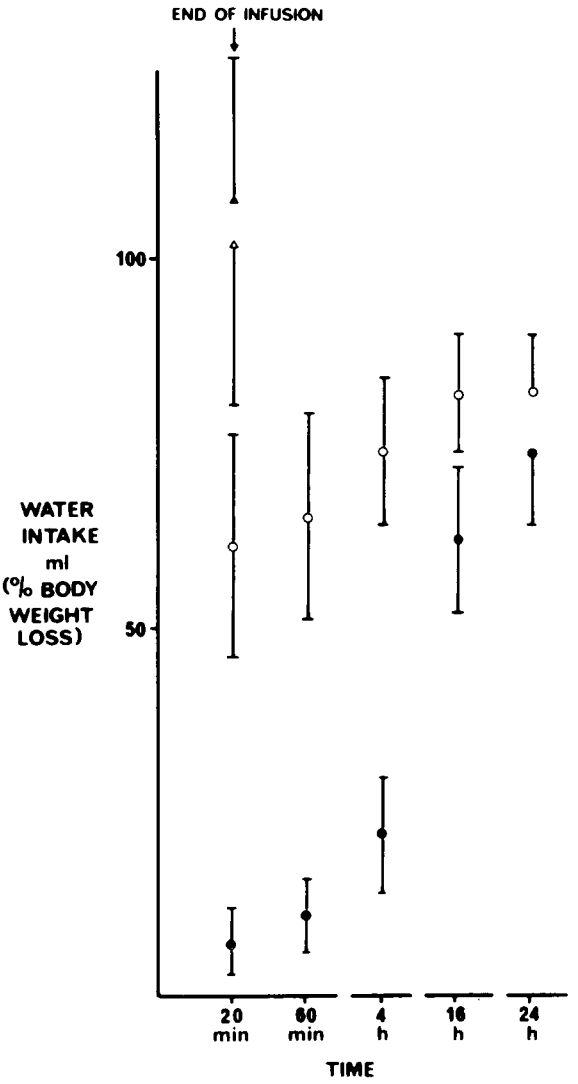

FIG. 6. Water intake, expressed as mean ± SEM percent body weight loss after 48 hr water deprivation, during the 24 hr period subsequent to the infusion of 1280 (•) or 960 (○) ng/min ouabain or during the 20 min period during infusion of physiological saline (▲, △, first and second series of control experiments, respectively).

reduction in water intake induced by intracarotid infusion of 800 ng/min angiotensin II. There was no observable effect of ouabain infusion on either the basal blood pressure or the increased blood pressure during infusion of angiotensin suggesting that the reduction in water intake was not due to the effects of ouabain on blood pressure. Ouabain did not affect either plasma or CSF Na and K concentrations. However, in ouabain treated animals, CSF Na concentration decreased during angiotensin infusion. This effect did not appear to be the explanation for the reduction in intake. That is, there were two animals in which drinking was completely eliminated but in which CSF Na was virtually unchanged (e.g., ± 0.5 mM).

Intake of water subsequent to the intracarotid infusion of 4 M NaCl was also substantially reduced during ouabain treatment. This result is consistent with observations made on rats. Bergmannn *et al.* [9] reported that an intravenous injection of ouabain (0.25 mg/kg) was effective in reducing water intake following subcutaneous injection of hypertonic NaCl. Water intake was decreased at two hours but was normal, relative to control levels, at 24 hr postinjection. Although in the ouabain experiments reported here 24 hr intakes of food and water were not measured subsequent to the 4 M NaCl infusions, it did not appear that ouabain produced long term deficits in these behaviours (e.g., the animals began to eat immediately when food was presented). Further, in experiments where 24 hr food intake was measured (Experiment 4) no deficits in 24 hr intake were observed.

After 48 hr deprivation, water intake in 20 min was similar to body weight loss. During infusion of 1280 ng/min ouabain, however, 20 min water intake was less than 10% of weight loss and remained suppressed for at least 4 hr. These results differ to a degree from those of Denton *et al.* [15]. They studied 48 hr water deprivation in sheep with a parotid fistula. Saliva was returned to the animals at 12 hr intervals by rumen tube and 5–10 gm of $NaHCO_3$ was added to the last collection to cover any loss. The resulting water drinking stimulus was greater than here being mostly in the 3–4 l range. Ouabain infused at 2510 ng/min for 4¼ hr, which is a larger dose than employed here, reduced intake much less – to 72 ± 11% of control. With two other normal nonfistulated animals, deprived of water for 48 hr, infusion at this rate reduced intake from 2200 to 1450 ml in one animal and from 2000 to 300 ml in the other. Depression of intake lasted less than 90 min. By this time both animals had drunk an additional 1500 ml.

The data would suggest there may be a very sensitive relation between intensity of need and dose level in resulting effect. This is supported by the observation in the paper of Denton *et al.* [15] that intracarotid infusion of ouabain at 1803–2510 ng/min for 4¼ hr almost entirely abolished salt appetite of the Na deficient animal. Half the rate of intracarotid infusion had only a small effect, and the same rate of infusion given intravenously had also only a small effect. Thus it appeared that the differential of concentration of intracerebral blood contrived by intracarotid as distinct from intravenous infusion of the same dose was sufficient to produce a marked quantitative result. In the experiments reported here the intracarotid infusion of 960 ng/min ouabain resulted in a smaller reduction in water intake, compared to the higher dose, after infusion of either angiotensin or 4 M NaCl, and after water deprivation. This data is consistent with the previous research indicating a dose related response [15].

The ingestion of food immediately preceding the presentation of water after 23½ hr water deprivation attenuated the effects of ouabain administration. After 48 hr water deprivation when food was not available during water presentation, water intake was 7.2% of control levels and was virtually eliminated in 6 of the 8 animals tested. However, after 23½ hr water deprivation when food was available prior to and during water presentation, water intake was 50.1% of baseline levels (Table 2) and was virtually eliminated in 3 of 8 animals tested. This differential effect of 1280 ng/min ouabain on water intake induced by water deprivation or water deprivation and food intake suggests that food intake provides a stimulus to water intake (e.g., dry mouth) that is relatively insensitive to ouabain.

It is also worth noting that during ouabain treatment the variability of 90 min food intake (i.e., percentage change in food intake) was increased and that there was a significant

TABLE 1

TABLE MEAN ± SEM 30 MIN WATER INTAKE (ML) AND 90 MIN FOOD INTAKE (G) ON BASELINE DAYS AND ON DAYS WHEN INFUSION OF 1280 NG/MIN OUABAIN OR PHYSIOLOGICAL SALINE AFTER 23½ HR WATER DEPRIVATION WERE GIVEN. TOTAL DAILY FOOD INTAKE (G) ON BASELINE DAYS AND AFTER INFUSION OF OUABAIN OR PHYSIOLOGICAL SALINE IS ALSO SHOWN. BASELINE INTAKE IS EQUAL TO THE MEAN INTAKE OF THE TWO DAYS PRIOR TO THE INFUSION.(N = 8)

	Baseline Intake	Infusion	Intake during and after infusion
30 min water	1562 ± 151	Physiol. Saline	1459 ± 124
Intake (ml)	1702 ± 178	Ouabain	978 ± 309*
90 min food intake	385 ± 93	Physiol. Saline	398 ± 83
prior to water	262 ± 71	Ouabain	236 ± 76
presentation (g)			
Total daily	775 ± 32	Physiol. Saline	772 ± 35
food intake (g)	722 ± 45	Ouabain	731 ± 55

*$p<0.01$; paired t test; statistical comparison between baseline intake and intake on day of infusion.

TABLE 2

MEAN ± SEM CHANGE IN 90 MIN FOOD INTAKE (%)OR WATER INTAKE (%) FOR PHYSIOLOGICAL SALINE AND 1280 NG/MIN OUABAIN TREATED ANIMALS. THE PERCENTAGE CHANGE = (INTAKE DURING INFUSION − AVERAGE INTAKE DURING THE TWO BASELINE DAYS PRECEDING THE INFUSION) × 100. CORRELATION BETWEEN CHANGE IN FOOD INTAKE AND CHANGE IN WATER INTAKE IS ALSO SHOWN

Infusion	Δ Food intake (%)	Δ Water intake (%)
Physiological Saline	108.2 ± 5.2	98.8 ± 10.9
Ouabain	100.5 ± 30.2	50.1 ± 15.4

Correlation between Δ food intake and Δ water intake:
Control: r = .14, y = 3X + 65.68, $p<0.10$.
Ouabain: r = .90, y = 0.46X + 3.82, $p<0.01$.

correlation between percentage change in 90 min food intake and percentage change in water intake. These data indicate that the effects of ouabain are not restricted to the neural systems subserving water intake. The fact that both food and water intake were severely reduced (i.e., to less than 10% of baseline values) in some animals could suggest that the effect of ouabain on water intake was, in part, due to its toxicity (e.g., the animals decreased intake because of nausea). However, in other animals food intake was unchanged or increased during ouabain treatment. In addition, since none of the animals showed any signs of nausea or distress in so far as they might be visible in sheep (e.g., placing head in bottom of cage) it is conceivable that ouabain interferes with the neural systems controlling both hunger and thirst.

In regard to the neural systems controlling thirst, water intake subsequent to the infusion of hypertonic solutions is often considered to be mediated by the dehydration or shrinking of osmoreceptors located in the hypothalamus [11, 21, 23, 32]. Recently, however, Andersson [2,3] questioned the osmoreceptor theory and instead proposed that drinking in response to infusion of hypertonic solutions is mediated by brain cells, located near the third brain ventricle, which are sensitive either to their immediate environmental Na concentration or to CSF Na concentration. Evidence consistent with this latter proposal is that intraventricular infusion of isotonic or hypertonic saccharides blocks water intake in goats during intracarotid infusion of hypertonic NaCl [28]. In addition, the ingestion of water subsequent to the intraventricular infusion of angiotensin II was also thought to be mediated by brain cells sensitive to Na ions. That is, Andersson suggested that angiotensin II caused thirst by increasing either the entry of Na into these brain cells or the movement of Na from CSF to the brain cells [2, 3, 4, 5].

Assuming that the level of ouabain achieved in these experiments inhibited the Na pump (i.e., Na-K dependent ATPase) then there are several possible explanations for the lower water intake observed during infusion of angiotensin II or 4 M NaCl in ouabain treated animals. First, in regard to angiotensin II infusion, if water intake is mediated by increased entry of Na into receptor cells [2], then ouabain administration should have enhanced thirst since ouabain would have, if anything, increased intracellular Na concentration [25]. Since increased water intake did not occur, the data are inconsistent with this type of Na receptor. On the other hand, if water intake is mediated by increased movement of Na from CSF to the receptor cells [2], and this Na movement is dependent on the Na pump [22] then inhibition of the Na pump by ouabain would be expected to decrease water intake. Second, in regard to 4 M NaCl infusions, it is possible that the increased entry of Na into the receptor cells during ouabain administration reduced either the environmental Na of the receptor cells or the amount of cellular shrinkage. Thus, decreased water intake in this situation could be consistent with either the Na receptor or the osmoreceptor theory. Thirdly, in regard to both angiotensin or 4 M NaCl infusions, it should be noted that increased activation of Na-K ATPase, either directly or indirectly (i.e., subsequent to increased intracellular Na), itself has been proposed to induce thirst [10].

The inhibition of Na-K ATPase by ouabain would, therefore, be expected to decrease water intake.

Another possible explanation for the effects of ouabain administration on water intake is that ouabain could interfere with normal neuron functioning by its effects on either Na-K dependent ATPase [12, 16, 17, 29, 30] or neurotransmitter uptake [12,31]. The effects of ouabain on any particular behaviour would be dependent on factors such as the accessibility or affinity of the system with regard to the drug. It has been shown that different areas of the brain have different affinities for ouabain. Donaldson *et al.* [17] injected labelled ouabain into the third brain ventricle of rats and showed that hippocampus and hypothalamus had high affinity relative to cortex, for ouabain. The hypothalamic area is involved in food and water regulation and Bergmann *et al.* [8] demonstrated that placement of ouabain in the hypothalamus, particularly in the region of the supraoptic nucleus, was very effective in reducing both normal and hypertonic NaCl elicited water intake. Further, the decreased effectiveness of ouabain on water intake after 23½ hr water deprivation, which was immediately preceded by food intake (Experiment 4) could be due to the fact that the drinking was, in part, prandial or dry mouth drinking. There is evidence suggesting that prandial drinking is a learned response in both weanling and recovered hypothalamically lesioned rats [24]. Thus, it is possible that the prandial drinking was not disrupted due to its not being predominantly initiated by hypothalamic structures.

Ouabain could exert its inhibitory effects anywhere in the chain of events from the stimulation of the receptor to the end organ responsible for the consummatory behaviour. In this regard several observations are worth noting. First, in some of the ouabain treated animals in which there was marked reduction of water intake in response to angiotensin II or 4 M NaCl infusion, relative to control levels, investigation of the water bin did occur. Similar behaviour was reported by Denton *et al.* [15] in experiments in which ouabain administration eliminated Na intake of Na deficient sheep. These observations could suggest that the receptors have signalled the appropriate information but the inhibition of neural activity beyond the receptor level prevented the consummatory response from occurring. On the other hand, the behaviour is also consistent with the hypothesis that while a majority of the receptors have been blocked there are enough unblocked receptors to initiate a partial response to the stimuli. The fact that ouabain is more effective on some types of thirst than others is consistent with this hypothesis. That is, the observation that ouabain is less effective in inhibiting (1) water deprivation induced thirst when food is available then when food is not available or (2) water deprivation (plus 10 g $NaHCO_3$) induced thirst in sheep with parotid fistula [15] than it is in normal sheep suggests that ouabain is not merely interrupting neurons in a final common pathway of water ingestion behaviour.

In conclusion ouabain infusion into the blood supply to the brain has been shown to inhibit water intake induced by various stimuli. Ouabain inhibited water intake during intracarotid infusion of 4 M NaCl or angiotensin II and after either 23½ or 48 hr water deprivation. The data suggested that the effects of ouabain on water intake after water deprivation were attenuated by having food intake precede the presentation of water. The results could be attributable to the effects of ouabain on Na transport at the receptor level but effects at other levels in the neural pathway between the receptor and the effector organ can not be entirely eliminated.

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