Continuous servo-controlled replacement of urinary sodium loss in conscious dogs

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ANDERSEN, STIG E., AND PETER BIE. *Continuous servo-controlled replacement of urinary sodium loss in conscious dogs.* Am. J. Physiol. 259 (Regulatory Integrative Comp. Physiol. 28): R313–R316, 1990.—A method for continuous servo-controlled infusion of sodium chloride to dogs is reported. The servo system consists of a sodium-sensitive electrode, a modified commercial flowmeter, a thermistor, a control unit, and a pump. Based on analog inputs from electrode, flowmeter, and thermistor, the control unit generates appropriate numbers of voltage steps, which drive the pump infusing a concentrated solution of sodium chloride. Inputs from the thermistor are necessary to correct for the influence of fluctuations in urine temperature. The sodium servo system has been tested together with a weight servo mechanism in conscious water-diuretic dogs. Furosemide (1 $\mu g \cdot kg^{-1} \cdot min^{-1}$) was infused to change the rate of sodium excretion by a factor of 17 from 3 ± 1 to 50 ± 15 μmol/min. Within this range, the apparatus replaced the sodium losses with great accuracy. From seven experiments the average system response in micromoles per minute (Na_{In}) measured in 10-min periods against mean renal excretion of sodium (Na_{Ex}) was $Na_{In} = 0.98 \cdot Na_{Ex} - 1.37$. The servo system provides a tool for accurate maintenance of body sodium irrespective of large changes in the rate of sodium excretion.

sodium servo system; furosemide

THE REGULATION OF BODY sodium and water in mammals is the result of the combined action of a number of complex mechanisms. To examine the involved mechanisms separately, establishment of well-defined experimental conditions is a necessity. Studies of anesthetized or conscious animals often include some sort of experimental pertubation, e.g., volume expansion. Precise maintenance of new steady-state levels with regard to sodium and water is possible only if the urinary losses of these substances are replaced continuously. Without this artificial replacement the body contents of sodium and water inevitably change during the experiment, affecting the regulating mechanisms unpredictably.

The sodium servo system described here, designed for use in conscious dogs, replaces urinary losses of sodium continuously and precisely. Used together with a weight servo system for replacement of volume (1), the sodium servo makes a valuable feedback system for studies of osmo- and volume regulation.

MATERIALS AND METHODS

A general overview of the sodium and water servo systems is given in Fig. 1.

Equipment

Electrodes. The sodium-sensitive glass electrode (G502Na Sodium Selectrode, Radiometer, Copenhagen, Denmark) has a measuring range of 10^{-5}–1 M NaCl and temperature range of 0–60°C (manufacturers' specification). Approximate selectivity constants for interfering monovalent cations are 0.01 for Li^+, 0.0007 for K^+, 10 for H^+, and 1,000 for Ag^+. The influence of temperature and interfering ions can be estimated from the equation $E = E_0 + RT/F \cdot \ln(a_{Na^+} + K_i a_i^{|1/z|})$, where E_0 is a constant, R is the gas constant (8.3143 $J \cdot K^{-1} \cdot mol^{-1}$), T is the absolute temperature (°K), F is Faraday's constant (96,487 C/g), a_{Na^+} is the sodium ion activity, K_i is the selectivity constant for the interfering ion, a_i is the activity of the interfering ion, and z is the charge of the interfering ion.

Potassium release from the open-tip calomel reference electrode (K102, Radiometer) has been measured to 43 μmol/h at a constant fluid flow of 5 ml/min. This will not affect the measurement of potassium excretion. The sodium-selective electrode and the reference electrode are inserted into a Plexiglas chamber, constructed to minimize dead space. The volume of fluid in the chamber during operation is 2 ml. The electrode chamber is inserted between a modified Foley bladder catheter and the flowmeter.

Flowmeter. The commercial flowmeter (Phasesep Flowmeter, Phase Separations, Queensferry, UK) contains a glass measuring tube and two photoelectric units. The functional volume of the glass tube was measured to 872 μl. When this volume is reached, the magnetic valve of the flowmeter opens, and drainage of the flowmeter chamber takes place by gravitation. Opening time of the magnetic valve averaged 428 ms. The flowmeter has been equipped with a modifying print, allowing the control unit to read the status of the magnetic valve. Operating flow range for the sensing head is ~0.06–65 ml/min (manufacturers' specification). The registration of filling time takes place in the control unit.

Control unit. This custom-built unit contains a microprocessor (8048 CPU, Intel), a number cruncher (MM57409N, National Semiconductor), an analog-to-digital converter (AD574JN, Analog Devices). The control unit receives simultaneous analog inputs from the electrodes, flowmeter, and the thermistor as the flowmeter drains. The inputs of urine sodium concentration, urine flow, and temperature are converted to a number

of control signals for the peristaltic pump. The operation of the unit, which is adjustable to the sodium concentration of the infusate, can be followed by a monitor display.

Output pump. The peristaltic pump (Peristaltic Pump P-1, Pharmacia Fine Chemicals, Uppsala, Sweden) has a maximal pump flow rate of 8.93 ml/min (tubing ID 3.1 mm). The pump delivers 0.74 μl/step. The number of steps is determined by the control unit. The pump is fed from a flask containing a concentrated solution of sodium chloride, e.g., 400 mM.

In Vitro Experiments

The response of the sodium servo system to steps in sodium input was obtained by variations in input pump velocity and concentrations of sodium in input solutions. In each experiment the input and output quantity of sodium per 10-min periods were correlated. Three different experiments were conducted: *1*) concentration of sodium chloride in the input solution constant at 20 mM, flow rates variable, *2*) concentration of sodium chloride in the input solution variable, flow rates approximately constant, and *3*) as *2* but with potassium chloride (50 mM) added to the input solution.

Furosemide Experiments

Animals. Experiments were performed on seven trained, conscious female beagle dogs weighing 10.5–14 kg. They were fed once daily with commercial dog food (Latz Komplett mixed with Latz canned food) usually at 1400 h and had free access to tap water. The intake of sodium and potassium was determined regularly and averaged 3.9 ± 0.3 and 3.1 ± 0.2 mmol·day^{-1}·kg body wt^{-1}, respectively. The dogs were trained to accept percutaneous catheterization, catheterization of the bladder, and to stand quietly for several hours supported by a canvas sling. The common carotid arteries had been placed in skin loops before the experiments by use of sterile procedures and general anesthesia.

Preparation. The dogs were supported by a canvas sling during the experiment. A sterile catheter (Intracath) was placed close to the right atrium via an external jugular vein and another in a saphenous vein. A modified silicone Foley catheter was inserted into the bladder.

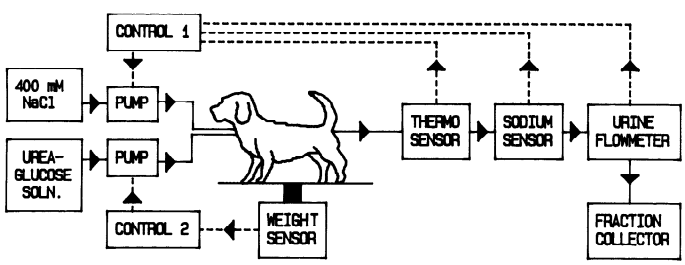

FIG. 1. Sodium and weight servo systems. Sodium servo system consists of control unit (*control 1*), which receives simultaneous analog inputs from thermosensor, sodium-sensitive electrode, and modified commercial flowmeter. Control unit, which drives peristaltic pump connected to flask containing hypertonic solution of NaCl, converts analog inputs to appropriate no. of voltage steps. Weight servo system consists of control unit (*control 2*), which receives analog inputs from weight sensor and converts them to appropriate voltage, controlling Harvard pump infusing hypotonic solution of urea and glucose. Solid line, fluid flow; dashed line, wire connections.

Sodium servo mounting. The sodium selective and reference electrodes were calibrated at room temperature in solutions of sodium chloride, 10 and 90 mM, and mounted in the electrode chamber, which was connected to the flowmeter and the bladder catheter. The thermosensor was likewise mounted in the electrode chamber. The Pharmacia peristaltic pump was connected to a burette (0.1-ml division), containing a 400 mM solution of sodium chloride, and to the jugular catheter. A sterile microfilter unit was inserted between the pump and the jugular catheter. Thus for each flowmeter cycle the quantity of sodium excreted was replaced quantitatively by infusion of 400 mM hypertonic sodium chloride solution.

Protocol. The dogs were hydrated by gastric tube with a load of water equivalent to 2% of body weight heated to body temperature. Thereafter the body weight was kept constant by a servo-mechanism (1) that replaced (within ±0.2% body wt) urinary and evaporative losses with a hypotonic solution of glucose and urea, 40 and 25 mM, respectively (GU-solution). After hydration a 20-ml bolus injection of inulin, 40 g/l, was given, and a continuous infusion was started at a rate of 0.33 ml/min. When steady-state water diuresis was achieved, sampling of urine was started. After a preinfusion period of 20 min, furosemide was infused at 1 μg·kg^{-1}·min^{-1} for 60 min followed by a recovery period lasting 30 min. Urine was sampled at 10-min intervals. Blood samples of 4.5 ml were obtained from the saphenous vein throughout the experiment.

Analyses. Measurements of sodium and potassium were carried out by flame photometry (IL 243 LED flame Photometer). Measurements of inulin were carried out by a method described by Steele (3) with minor modifications.

Statistics. Results are given as means ± SE. Data were subjected to one-way analysis of variance (4). In case of significantly large F values differences were evaluated by Newman-Keuls test (4). The servo-system output was correlated with the servo-system input (2), and the regression of servo-system response on servo-system input was calculated (2). The level of significance was 0.05.

RESULTS

During the furosemide experiments the rate of excretion of sodium rose from 3.0 ± 1.0 to 50 ± 15 μmol/min, and fractional excretion of sodium increased from 0.05 ± 0.01 to 0.84 ± 0.23%, accompanied by an increment in diuresis from a control level of 3.2 ± 0.3 to a maximum of 4.0 ± 0.4 ml/min after 55 min of infusion. Rate of excretion of potassium rose from 10 ± 2 to a maximum of 28 ± 6 μmol/min after 25 min of infusion accompanied by a simultaneous rise in fractional excretion of potassium from 5.6 ± 1.0 to 18 ± 5%. Changes in the clearance of inulin were not observed. Statistically significant changes in plasma concentration of sodium (mean 139.2 ± 0.5 mM) were not found. Plasma potassium decreased from preinfusion value of 3.9 ± 0.1 to a minimum of 3.6 ± 0.1 mM reached 5 min after cessation of furosemide infusion. At the end of the experiment (25 min after cessation of furosemide infusion), plasma potassium, 3.7

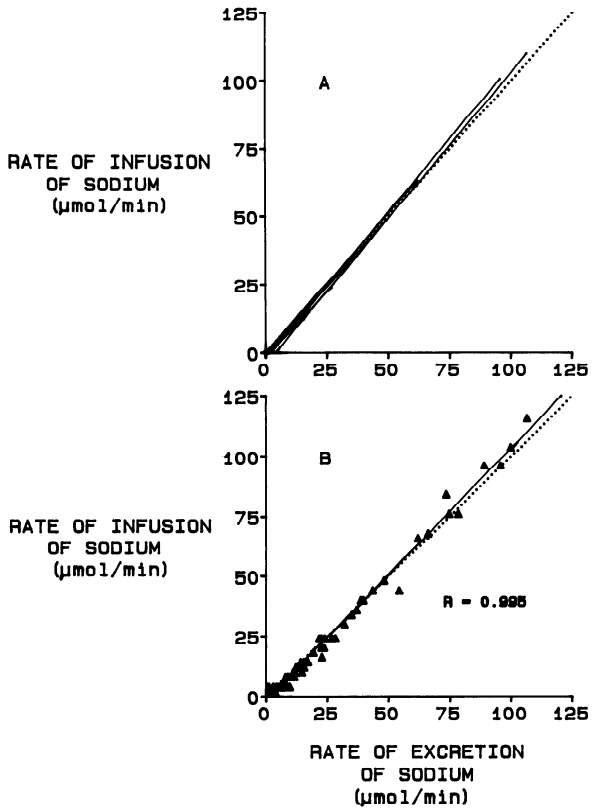

FIG. 2. Responsiveness of sodium servo system. *A*: solid lines, data from experiments in 7 beagle dogs. Variations of urine flow and Na concentrations were obtained by infusing of 1 $\mu g \cdot kg^{-1} \cdot min^{-1}$ furosemide for 1 h after control phase of 20 min. Infusion was followed by recovery phase lasting 30 min. Average regression line of system Na output on system Na input was $Na_{In} = (0.98 \pm 0.04) \cdot Na_{Ex} - (1.37 \pm 0.59)$ where Na_{In} is rate of Na infusion (μmol/min), Na_{Ex} is rate of Na excretion (μmol/min). Dotted line, line of identity. *B*: Na output of servo system plotted against Na input of system for 10-min periods, data from all 7 furosemide experiments. Solid line, line of regression. $Na_{In} = 1.05 \cdot Na_{Ex} - 1.84$, $r = 0.995$.

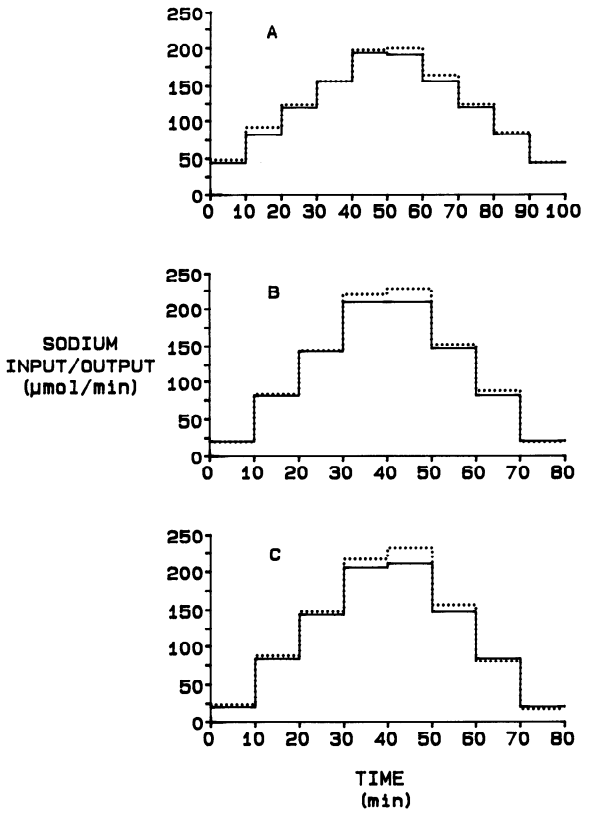

FIG. 3. Response of Na servo system to steps in Na input obtained by variations in input pump velocity and concentrations of Na in input solutions. In each experiment, quantities of Na delivered per 10-min periods by Sage pump (system input) and peristaltic pump (system output) were correlated. Solid line, Na input; dotted line, Na output. *A*: concentration of NaCl in input solution constant at 20 mM; flow rates, 2.2, 4.1, 6.0, 7.8, 9,7, 9.6, 7.8, 6.0, 4.1, 2.2 ml/min. Maximal system output was 200 μmol/min. System output of NaCl during flow step-up experiment exceeded system input by 4.1 ± 1.2%. Regression of system output on system input was $OP = 1.02 \cdot IP + 2.49$, $r = 0.999$, where OP is system output (μmol/min), IP is system input (μmol/min). *B*: concentrations of NaCl of input solutions 5.0, 20.0, 35.0, 50.0 mM; flow rate approximately constant (4.2 ± 0.2 ml/min). Maximal system output was 228 μmol/min. System output during Na step-up experiment lasting 80 min exceeded system input by 2.5 ± 1.6%. Regression of system output on system input was $OP = 1.05 \cdot IP - 1.84$, $r = 0.999$. *C*: as *B* but with KCl (50 mM) added to input solution. Maximal system output was 232 μmol/min. System output during Na step-up experiment lasting 80 min exceeded system input by 3.5 ± 3.4%. Regression of system output on system input was $OP = 1.09 \cdot IP - 4.41$, $r = 0.997$ ($C_{K^+} = 50$ mM).

± 0.1 mM, was still lower than control. Diuresis and excretion of sodium and potassium had returned to control levels. The regression lines of the servo-system response (rate of infusion) against renal sodium excretion for each experiment are shown in Fig. 2. The average regression line was $Na_{In} = (0.98 \pm 0.04) \cdot Na_{Ex} - (1.37 \pm 0.59)$ where Na_{In} is the infusion of sodium (μmol/min), Na_{Ex} is the renal excretion of sodium (μmol/min). Slope and intersection are given as means ± SE ($n = 7$).

DISCUSSION

We have constructed an apparatus that provides continuous and precise replacement of the urinary sodium loss in the laboratory dog. It was designed to become a useful tool in the investigation of the sodium regulation in animal models by ensuring well-defined steady-state levels of body contents of sodium and water.

Proper maintenance of the sodium content of an experimental animal can be obtained by manual sodium replacement, which may be suitable in a number of situations even though it requires rapid and repeated measurements of the sodium concentration in the urine, calculation of the excreted quantity, and an injection of the calculated mass of sodium, e.g., as hypertonic saline. With the described apparatus this procedure is carried out automatically with great precision.

The continuous infusion of negligible volumes of hypertonic sodium chloride solution in the right atrial area ensures a rapid dilution in the blood. The infusion of hypertonic fluid might otherwise affect the vasculature unspecifically in a less predictable manner.

During experiments rapid changes in urine flow and rates of excretion of sodium and potassium may occur. The pump experiments demonstrated a satisfactory responsiveness of the servo system to steps in the sodium input in the order of 60 μmol/min (Fig. 3). It was further shown that the electrodes were unaffected by urine flows between 2.2 and 9.7 ml/min. Likewise potassium at a concentration of 50 mM did not influence the function of the sodium servo system.

The volumes of the lower urinary tract, tubing, the electrode chamber, and the flowmeter (~5 ml total) are not insignificant, the drainage can be delayed several minutes, depending on the urine flow rate. If infusions lag behind an acute increase in renal sodium excretion of, e.g., 100 μmol/min by 4 min the consequence will be a deficit of infusion of 0.4 mmol NaCl. A 12-kg beagle dog (extracellular volume 2.4 l, plasma sodium 145 mmol/l) has an extracellular mass of sodium of ~350 mmol. The sodium excreted during the 4-min lag period thus represents ~0.1% of the extracellular sodium mass. This is most likely a functionally insignificant deficit.

The maximal output capacity of the system is dependent on the concentration of sodium of the infusate. The system is thus easily adjusted to a higher maximal capacity by an increase in this concentration. In the furosemide experiments where the infusate was a 400 mM NaCl solution, system responses were between 0 and 232 μmol/min, an interval useful in most investigations using physiological stimuli in small conscious dogs.

We measured the release of potassium from the open tip calomel reference electrode average <1 μmol/min, so this has no detectable influence on the function of the sodium servo. In theory it introduces an error in the measurements of potassium concentrations of the urine; however, this is negligible. Alternatively, a sealed reference electrode may be used.

The flowmeter operates at flows >0.087 ml/min, resulting in lack of replacement of sodium losses accompanied by urine flow <0.087 ml/min. This flow requirement and the dead space of the measuring cell reduce the usefulness of the system to larger animals or conditions associated with a large diuresis.

The sodium servo system has been tested in short-term experiments in which high urine flow rates were obtained. The weight servo system is accurate over periods of hours, and the sodium servo system could be used in long-term experiments provided that intermittent recalibrations of the electrodes are performed.

Despite great efforts over many years to improve our knowledge of the mechanisms of sodium homeostasis many questions remain unanswered. The described system allows the experimenter to sustain body sodium contents at a fixed level and thus to obtain precise steady-state relations between body contents of sodium and rate of excretion of sodium.

Constant conditions or precise, predictable, and repeatable changes are especially important in experiments including a massive stimulation of the sodium excretion (administration of potent natriuretic substances, volume expansion, etc.). The effect of a infused natriuretic substance theoretically could be obscured by sodium- and water-conserving mechanisms. Another experimental condition, where important information of sodium and water homeostasis could be obtained, is in the situation of blockade of sodium-conserving control systems with, i.e., converting-enzyme inhibitors and aldosterone-receptor blockers. The usefulness of such pharmacological blockade is to a certain extent reduced by the associated natriuresis that prevents acquisition of stable conditions in terms of body sodium. The experimental protocol need not to be very complex before difficulties in sustaining steady-state conditions of body weight and body content of sodium develop. Almost innumerable experiments in numerous animal models have included volume expansion by saline loading. The obtained natriuresis gradually eliminates the stimulus, thus rendering the comparison of results obtained at different times of the experiment very difficult. As far as we know investigations including individualized maintenance of the volume expansion have not been published. The infusion is often set to a fixed rate regardless of the actual rates of excretion of sodium and water. The described sodium and water servo systems allow the experimenter to maintain a volume expansion by saline loading for many hours by ensuring rates of infusion of sodium and water very similar to the rates of excretion. Less rapid effects of volume expansion, e.g., secretion of hormones could thereby be examined. In three conscious dogs, preliminary data obtained after gastric load of isotonic sodium chloride equivalent to 3% of body weight indicate significant differences between the renal responses with and without the use of the described servo mechanism.

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