INVESTIGATION OF THE PRIMARY STIMULUS AND MECHANISM OF THE AMMONIUM CHLORIDE-INDUCED INCREASE IN THE CONTENT OF POTASSIUM IN CHOROID PLEXUS EPITHELIAL CELLS

by

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SUPERVISORY COMMITTEE APPROVAL

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THE UNIVERSITY OF UTAH GRADUATE SCHOOL

FINAL READING APPROVAL

To the Graduate Council of The University of Utah:

I have read the dissertation of Ronald Eugene Daniel Harbut its final form and have found that (1) its format, citations, and bibliographic style are consistent and acceptable; (2) its illustrative materials including figures, tables, and charts are in place; and (3) the final manuscript is satisfactory to the Supervisory Committee and is ready for submission to the Graduate School.

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ABSTRACT

After young adult male rats were injected intraperitoneally with NH_4Cl, the content of K (hereafter, CP [K]) within the epithelial cells of the choroid plexus increased greatly while that of Na (CP [Na]) decreased. This dissertation has focused on elucidating the primary stimulus and mechanism of the NH_4Cl-induced increase in CP [K], with some investigative focus on the induced decrease in CP [Na]. The primary stimuli under consideration are the NH_4Cl-induced increases in plasma [NH_4], [K], and [H], and decreases in [HCO_3] and [Na]. An acidosis-induced augmentation in the concentration of catecholamines available to CP beta-adrenoceptors has also been considered. Since sympathectomy, adrenalectomy or pretreatment with beta-adrenoceptor blockers did not reduce the effects of NH_4Cl, it was possible to rule out an involvement of catecholamines as the stimulus. A comparison of the time-courses of plasma [ammonia] with CP [K] and [Na] indicated that the primary stimulus was not plasma [NH_4]. A comparison of the effects of nine individual salt treatments on plasma [K] with CP [K] and [Na], revealed that plasma [K] was not the primary stimulus. Significant statistical correlations were drawn between plasma [H] and the induced increase in CP [K] and decrease in CP [Na]. Since an elevation of plasma [H] was most consistently associated with an increase in CP [K], the mechanism

through which [H] operates was investigated. The increase in CP [K] and decrease in CP [Na] must result from either an increase in Na-K exchange (Na-K ATPase), or from a decrease in K efflux and Na influx; or perhaps a combination of both. With a physiological analog of K (i.e., Rb), it was demonstrated that acidosis does not increase the slope of the initial linear uptake of ^{86}Rb *in vitro*; thus no effect of acidosis to increase Na-Rb exchange (i.e., Na-K exchange) was found. Since acidosis augments the steady-state volume of distribution of ^{86}Rb *in vitro*, without an increase in Na-Rb exchange, it would appear that acidosis augments CP Rb by effecting a reduction in Rb efflux and, by analogy, would also reduce the efflux of CP K. In conclusion, NH_4Cl induces an increase in CP [K] by increasing plasma [H] which in turn acts to reduce the efflux of K across the apical and/or basolateral membranes of the CP. An *in vivo* indication that acidosis reduces the efflux of K across the apical membrane was suggested by a decrease in CSF [K].

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CHAPTER 1

INTRODUCTION

Choroid Plexus

Overview. The choroid plexus (CP) is a non-neural epithelial tissue located within the lateral, third and fourth ventricular cavities of the mammalian central nervous system (CNS). The epithelium of the CP acts as a partition between the cerebrospinal fluid (CSF) and the plasma ultrafiltrate of the choroidal capillaries. The blood-CSF barrier, as the above epithelial partition is called, serves to regulate the composition and formation of the CSF (Wright, 1978; Bradbury, 1979; Wright, 1982). The blood-CSF barrier achieves its CSF regulatory role not only by acting as a physical barrier but also by translocating selectively plasma constituents into the CSF and CSF constituents into the plasma. The transport mechanisms involved in the transfer of K and Na, and those factors which modulate these mechanisms, are the subject matter of this dissertation. With a better understanding of CP ion-transport processes, we will more clearly understand the role of the CP in the homeostasis of the CSF and further elucidate its role in the pathology and treatment of hydrocephalus, intracranial hypertension and cerebral edema.

Embryology. The lateral (LVCP), third (3VCP) and fourth (4VCP) ventricular choroid plexuses (CP's) are derived from two

distinct embryologic origins. The LVCP (telencephalic) is derived from the medial wall of the cerebral hemispheres; the 3VCP (diencephalic) and 4VCP (myelencephalic) both arise from an invagination of the single-layered roof plate (Dohrmann, 1970). The difference in the origins of the CP's may underlie possible functional differences among the LVCP, 3VCP and 4VCP.

Microanatomy and Physiology. The fluid volume of the CP is composed predominantly by the choroidal epithelium (about 50-60% of total wet tissue weight); the vessel luminal and extravascular stromal volumes each constitute about 15% of total wet tissue weight (Quay, 1972; Johanson, Reed and Woodbury, 1976). Since the choroid plexus is primarily composed of epithelial cells, hereafter this cell type will be referred to by CP. The CP may be envisioned as the middle fluid compartment of a three-compartment system in series, where the basolateral (plasma-facing side) and apical (CSF-facing side) membranes of the CP delineate the three compartments. Thus, the three compartments contain the interstitial fluid of the CP (i.e., plasma ultrafiltrate of the choroidal capillaries), the intracellular fluid of CP, and the cerebrospinal fluid. The structure and thus function of each CP membrane differs. Na-K-activated ATPase (Na-K ATPase) is observed predominantly on the apical membrane in rat, rabbit and frog (Quinton, Wright and Tormey, 1973; Smith and Johanson, 1980; Masuzawa, Saito and Sato, 1980; Miwa, Inagaki, Fujiwara and Takaori, 1980; Masuzawa, Saito and Sato, 1981).

Thus, Na-K exchange predominantly occurs across the apical membrane. On the other hand, Na-H exchange (via a Na-H antiporter) has been postulated to occur across the basolateral membrane (Wright, 1977; Murphy, unpublished data). This sidedness with respect to Na-K and Na-H exchange is an important consideration in the elucidation of the mechanisms responsible for establishing and regulating the concentrations of K and Na in the CP.

Potassium Influx. Intracellular K is typically accumulated against a concentration gradient by the activity of Na-K ATPase (Sweadner and Goldin, 1980). Apical Na-K ATPase (Na-K exchange pump) exchanges CP Na for CSF K. The unique apical distribution of Na-K ATPase forms part of the homeostatic mechanism responsible for the clearance of K from the CSF into the plasma (Bradbury and Stulcova, 1970; Johanson, Reed and Woodbury, 1974; Husted and Reed, 1976; Macknight, 1977). Miwa et al. (1980) have shown that the activities of Na-K ATPase in the LVCP, 3VCP and 4VCP of the rabbit are similar.

Potassium Efflux. Our current knowledge of K efflux across the membranes of the CP indicates that K leaves the cells down an electrochemical gradient into the CSF and plasma (presumably through K-selective channels). The rate of this efflux is largely determined by the permeability of the cell membranes to K. Although, Zeuthen and Wright (1981) demonstrated that most of the K efflux from the CP occurs across the apical membrane into the CSF (frog), Wright (1982) also reported that

there is some K efflux across the basolateral membrane into the plasma. Thus, in normokalemic animals a net movement of K from CSF to plasma is achieved by the active uptake of CSF K across the apical membrane (Na-K exchange pump), followed by the efflux of K across the basolateral membrane into the interstitial (stromal) fluid of the CP (and thus into capillary plasma). Although the CP has a role in the removal of K from the CSF, its role to prevent the loss of CSF K in hypokalemia should not be overlooked (Johanson et al., 1974).

Sodium Influx. Moving down an electrochemical gradient, extracellular Na may gain access into the CP by way of facilitated diffusion. Several facilitated diffusion exchangers have been proposed. The exchanger of interest in this dissertation is the aforementioned basolateral Na-H antiporter, which exchanges stromal fluid Na for CP H.

Sodium Efflux. As with K influx, Na efflux is largely a consequence of the Na-K exchanger, i.e., the exchange activity of apically located Na-K ATPase (Johanson et al., 1974).

Receptors. Adenylate cyclase has been demonstrated in the CP of mammals (Cramer, Hammer, Maier and Schindler, 1978; Feldman, Epstein and Brusilow, 1979), Nathanson, 1979; Nathanson 1980; Masuzawa et al., 1981). Lindvall (1979) has demonstrated the existence of adrenergic innervation to the CP (see below). Since adenylate cyclase activity and adrenergic innervation are often associated with the presence of beta-adrenoceptors, many authors

have proposed the existence of CP beta-adrenoceptors. Nathanson (1980) has investigated the efficacy of various beta-adrenoceptor agonists to modulate the activity of adenylate cyclase in cat CP; his results indicate that CP adenylate cyclase is modulated by the stimulation of beta-2 adrenoceptors.

Innervation. Adrenergic nerve fibers and terminals have been visualized histochemically near the basolateral surface of the LVCP, 3VCP and 4VCP of many mammalian species, e.g., cat, rabbit and rat (Lindvall and Owman, 1981). Regional variations in the density of innervation follow the order 3VCP > LVCP > 4VCP. Bilateral superior cervical ganglionectomy has been shown to abolish completely the adrenergic innervation to all of the plexuses in the cat and rat. Unilateral sympathectomy produces complete disappearance of adrenergic innervation in the homolateral LVCP of rabbit (Lindvall and Owman, 1981) and rat (Lindvall, Owman and Winbladh, 1981). Lindvall, Owman and Winbladh (1982) demonstrated an adrenergic stimulatory tone on rat CP Na-K ATPase activity; sympathectomy resulted in nearly a 40% decrease in the activity of Na-K ATPase after 6 days. In rabbits, full adrenergic innervation to the entire CP system develops by 3 weeks of postnatal age (Lindvall et al., 1981).

CP Response to Ammonium Chloride

In vivo studies have demonstrated that systemic acidosis induces a unique effect on the contents of K and Na in the CP's of young adult rats (Smith and Johanson, 1980; Pershing and Johanson,

1982). In the above two studies, the authors reported that respiratory or metabolic acidosis, induced by CO_2 inhalation or intraperitoneally injected ammonium chloride (NH_4Cl), augmented the intracellular content of CP K (hereafter, CP [K]) and lowered that of Na (CP [Na]); interestingly, this effect of acidosis on CP [K] and [Na] is opposite to that observed in skeletal muscle (Lade and Brown, 1963; Adler, Roy and Relman, 1965). The acidosis-induced increase in CP [K] and decrease in [Na] will be referred to as the CP response. Systemic acidosis has been shown to raise the titer of plasma catecholamines (Morris and Millar, 1962); a plethora of evidence suggests that catecholamines stimulate the activity of membrane-bound Na-K ATPase in a variety of tissues both in vivo and in vitro (see Discussion); and, the CP is richly endowed with Na-K ATPase on its apical membrane. Therefore, Pershing and Johanson (1982) suggested that the CP response is mediated by the catecholamine-induced stimulation of CP Na-K ATPase. The pathway by which plasma-borne catecholamines could stimulate apical Na-K ATPase has been postulated (Pershing and Johanson, 1982); thus, plasma-borne catecholamines may stimulate beta-adrenoceptors on the basolateral membrane of the CP, which in turn leads to an increase in the production of cyclic-AMP through the coupled stimulation of adenylate cyclase; an increase in the intracellular concentration cyclic-AMP, finally, acts as a second messenger to stimulate Na-K ATPase. Alternately, an increase in the concentration of catecholamines

within the synaptic cleft of the adrenergic nerves innervating the CP may be induced by acidosis (Vanhoutte, Verbeuren and Webb, 1981). Aside from the catecholamine hypothesis, Pershing and Johanson (1982) alluded to the possibility of an acidosis effect on the permeability of the basolateral and apical membranes of the CP to K and Na. Thus, increased CP [K] due to a reduction in K efflux, and decreased CP [Na] to a reduction in Na influx. This alternate hypothesis (effect on membrane permeability) is equally plausible since it can explain the observed changes in CP [K] and [Na] without a need for a stimulated increase in the activity of ATPase.

Focusing my attention on the NH_4Cl-induced CP response, I have attempted to answer the following two questions:

(1) What is the primary stimulus to the response? Is it an NH_4Cl-induced increase in plasma [NH_4], [catecholamines], [K] or [H], or decrease in plasma [HCO_3] or [Na]?

(2) What invoked mechanism(s) generates (generate) the response? Is it a stimulus-induced increase in apical Na-K exchange pump activity or decrease in the permeability of the apical and basolateral membranes to K and Na?

CHAPTER 2

DOSE-RESPONSE AND SIXTY-MINUTE TIME-COURSE ANALYSES OF THE EFFECTS OF NaCl AND NH_4Cl ON [K] AND [Na] IN CHOROID PLEXUS: METHODS

Experimental Protocols

Sixty-eight 6-8 week-old male Sprague-Dawley rats were used in the studies described below. Prior to the experiments, the animals were housed in cages wherein they had free access to food and water, and were regularly exposed to alternating 12-hour periods of darkness or overhead-fluorescent lighting. During the experiments, rats had continued free access to food and water in cages receiving room-light. Five minutes before fluid sampling and tissue removal were initiated, each animal was injected intraperitoneally with 80 mg/kg ketamine hydrochloride. This anesthetic dose of ketamine, which did not change the animal's rate of respiration, required 5 minutes to effect sedation. Each rat was killed by exsanguination after the blood sample was taken. In the experiments described below, the following were measured: plasma [H], [HCO_3], [K] and [Na], blood pCO_2 and pO_2, and choroid plexus [K] and [Na]. In some experiments, [K] and [Na] in the cerebrospinal fluid were also measured.

Dose-Responses. Sixteen rats (180-250 grams) were divided equally into four groups in this first experiment. The animals in each respective group were injected intraperitoneally

with one of four doses of NH_4Cl, i.e., 0.00 (control), 2.33, 4.67 or 7.00 mmol/kg; NaCl was formulated into each of these four solutions in order to maintain a constant concentration of Cl (Table 2-1). Sixty minutes after NH_4Cl was administered, a sample of blood and of cerebrospinal fluid were taken, and the fourth and lateral ventricular choroid plexuses were removed from each animal (all in the order described).

Time-Responses. Thirty-six rats (140-250 grams) were separated into two equal groups in this second experiment. The animals in each respective group were injected intraperitoneally: controls received NaCl (see Table 2-1); treated animals received NH_4Cl (4.67 mmol/kg, Table 2-1). Five, 15, 30 or 60 minutes after injection, a sample of blood was taken, and the fourth (4VCP), third (3VCP) and lateral (LVCP) ventricular choroid plexuses were removed from each animal (all in the order described).

Regional Analyses of Choroid Plexus [K] and [Na]. Four rats (330-380 grams), representing eight lateral ventricular choroid plexuses (2 LVCP's per rat), were used in this third experiment. Following the ketamine anesthetic, a sample of blood was taken, and the 4VCP, 3VCP and LVCP's were removed from each animal. The removed LVCP samples (approximately 10 mm in length) were sectioned into anterior and posterior halves. Thus, four 3VCP's and 4VCP's, and eight anterior LVCP's (A/LVCP) and posterior LVCP's (P/LVCP), were available for a comparative analysis.

Table 2-1. COMPONENTS OF INJECTION SOLUTIONS

SOLUTIONS:	I	II	III	IV
NH_4Cl	0.00	2.33	4.67	7.00
NaCl	7.00	4.67	2.33	0.00
TOTAL Cl	7.00	7.00	7.00	7.00

The amounts of NaCl, NH_4Cl and Cl given with solutions I-IV are shown. In the dose-response experiment, the injections referred to as 0.00 (control), 2.33, 4.67 and 7.00 mmol/kg NH_4Cl, respectively refer to solutions I-IV. In the time-response experiment, 4.67 mmol/kg NH_4Cl refers to solution III; control refers to solution I. All solutions were injected intraperitoneally (0.020 ml/kg).

Extracellular Fluid Volumes of Choroid Plexuses. Twelve rats (200-250 grams) were used in this fourth experiment. Each animal was bilaterally nephrectomized immediately before it received an intraperitoneal injection: controls received NaCl; treated animals received NH_4Cl (4.70 mmol/kg); both solutions contained ^3H-raffinose (0.015 mCi/ml; a dose of 0.3 mCi/kg). Sixty minutes later, a sample of blood and of cerebrospinal fluid were taken, and the 4VCP, 3VCP and LVCP's were removed from each animal.

Sampling Procedures

Arterial Blood. Sampling was initiated by opening the abdominal cavity, quickly draining and drying the cavity of any residual fluid from the injection, and exposing the abdominal aorta. After withdrawing a 4-ml sample of blood from the abominal aorta near the femoral bifurcation, the aorta was clamped proximal to the inserted needle. The needle was then withdrawn from the artery and removed from the syringe. The syringe was capped and gently mixed; thereafter, a 2-ml aliquot of blood was transferred into a Falcon tube. The remaining blood, after syringe recapping, was placed into an ice bath for later acid-base and blood-gas analyses. Centrifugation of the 2-ml aliquot of blood was begun while further sampling continued.

Cerebrospinal Fluid. After removing the scalp, the animal was exsanguinated (killed) by incising the abdominal aorta proximal to the aforementioned clamp. A sample of CSF was then

aspirated from the cisterna magna after penetrating the muscle tissue above the foramen magnum with a specially prepared micropipette (see below). The CSF sample was transferred onto a piece of Parafilm; a 0.025-ml aliquot of CSF was immediately taken from the Parafilm and transferred into a tared Falcon tube for later weighing. CSF samples contaminated with blood or muscle tissue were not used.

The micropipettes used to sample CSF were prepared by pulling their heated ends to a rapidly narrowing, yet patent, bore. A vertical pipette puller was used to ensure consistency of manufacture. This pulling-induced, funnel-shaped segment of tubing was scored at its narrowest diameter and broken along the score to obtain a minimally jagged fluid-sampling insertion tip.

Choroid Plexuses. The brain was removed and placed on normal saline-wetted filter paper. With the aid of a stereotaxic microscope, the fourth (4VCP), third (3VCP) and both lateral (LVCP) ventricular choroid plexuses, in the above order, were dissected free from the brain and placed on tared weighing boats for drying and later weighing on an electrobalance.

The weighing boats were 1/4 x 1/2-inch rectangles of aluminum foil; each weighed 4.0 mg. Before use, these weighing boats were thoroughly rinsed in deionized water and air-dried after a final rinse in acetone. Micropipettes were similarly cleaned.

Plasma. Lastly, a 0.025-ml sample of plasma, taken from

the previously centrifuged 2-ml aliquot of blood, was transferred into a tared Falcon tube for later weighing.

Analyses and Calculations

Arterial Blood. All blood samples for acid-base and gas analysis were withdrawn into sterile heparinized glass syringes where the syringe and needle dead space was filled with heparin (see Methods). If small bubbles were observed in the blood after sampling, they were displaced from the syringe before placing the capped sample into an ice bath. Generally, all sealed blood samples remained in an ice bath no longer than 20 minutes before their analysis with a Radiometer ABL2 Laboratory at 37°C; such a 20-minute wait in an ice bath does not significantly alter pH, pCO_2 or pO_2 values.

Cerebrospinal and Plasma Fluids. Cerebrospinal and plasma fluid samples were weighed to the nearest 0.0001 gram; they were then diluted 1:200 by the addition of 5 ml of a solution (hereafter called LH) containing 15.0 mM LiCl and 0.02 N HNO_3. After mixing, the diluted samples were set aside for later [K] and [Na] analysis. The sample contents of [K] and [Na] obtained from flame photometry were corrected for sample and extraction-fluid weights. These values, as mmoles of K or Na per kg of fluid, were further corrected for the contributory weight of CSF or plasma proteins; thus, the final CSF and plasma K and Na concentrations are reported as mmoles per kg of water (mmol/kg water). The correction factors used were 0.99 for CSF and 0.92 for plasma; these

factors are those previously reported for the percentages of water in the CSF and plasma of adult rats (Johanson et al., 1976).

Choroid Plexuses. Choroid plexus samples were desiccated, at 60°C for 2-4 hours, and weighed on a Cahn electrobalance; weights were recorded to the nearest 0.001 mg. The weighed choroid plexus and its adhering weighing boat were then placed into a Falcon tube; the extraction of tissue [K] and [Na] commenced by the addition of 2 ml of LH solution. After 12-24 hours of extraction at room temperature, with occasional mixing, the tissue extracts were analyzed for K and Na. The final tissue K and Na concentrations are reported as mmoles per kg of dry tissue (mmol/kg dry tissue).

Extracellular Fluid Volumes of Choroid Plexuses. The steady-state extracellular fluid volumes of distribution of ^{3}H-raffinose were calculated by dividing the activity of raffinose in the wet choroid plexuses by that in plasma.

Statistics. Statistically significant differences between the results of each treatment and the corresponding control(s) were determined with a one-tailed Student's t-test. Two levels of probability are reported, 0.05 or 0.01. With two-factor linear correlation analyses, the slope of each regression line was analyzed for a significant difference from zero with a one-tailed Student's t-test. For linear regression statistics, the coefficient of determination (r^2) and the slope are presented. For each slope, a significant difference from zero is reported at

the 0.05, 0.01, or 0.001, probability levels.

Materials

Analytical grade ammonium chloride and sodium chloride were purchased from Mallinckrodt, Inc. (Paris, KY). All stock solutions of ammonium or sodium chloride were prepared with deionized water. Lipo-Hepin/BL, which contained 1000 units of heparin and 3.4 mg Na per ml, was acquired from Riker Laboratories (Northridge, CA). Ketamine hydrochloride, 100 mg/ml, was obtained from Bristol Laboratories (Syracuse, NY). Disposable 0.01-ml micropipettes were obtained from Dade Diagnostics (Miami, FL). BD Multifit 5-ml glass syringes were acquired from Becton, Dickinson and Company (Rutherford, NJ). Sterile 12 x 75 mm capped polypropylene tubes were procured from Falcon, Incorporated (Oxnard, CA). ^{3}H-raffinose (7.8 mCi/mmol) and Biofluor liquid scintillation cocktail were obtained from New England Nuclear (Boston, MA).

Instrumentation

Blood pH, pCO_2, $[HCO_3]$ and pO_2 values were determined with a Radiometer ABL2 Acid-Base Laboratory (Copenhagen, Denmark). The concentrations of K and Na in the fluid and tissue sample extracts were analyzed with an Instrumentation Laboratories 443 flame photometer (Lexington, MA). Solution osmolality was determined with a Wescor 5100B vapor pressure osmometer (Logan, UT). Choroid plexuses were weighed on a Cahn Instruments 4700 automatic

electrobalance (Cerritos, CA). Constant bore micropipette insertion tips were manufactured with a David Kopf Instruments 700B vertical pipette puller (Tujunga, CA). Fluid and tissue ^{3}H-raffinose radioactivity analyses were performed with a Beckman LS 7500 liquid-scintillation counter (Irvine, CA).

CHAPTER 3

DOSE-RESPONSE AND SIXTY-MINUTE TIME-COURSE ANALYSES OF THE EFFECTS OF NaCl AND NH_4Cl ON [K] AND [Na] IN CHOROID PLEXUS: RESULTS

Dose-Responses

The doses of NH_4Cl used in this first experiment were 0.00 (control), 2.33, 4.67 or 7.00 mmol/kg; 60 minutes after each corresponding injection, the effects reported below were observed.

Acid-Base and Gas Analyses of Arterial Blood. With each of the three increasing doses of NH_4Cl, plasma [H] was augmented from 38 (control) to 45, 49 and 59 nmol/l (Figure 3-1A); plasma [HCO_3] was decreased from 20 (control) to 18, 14 and 13 mmol/l (Figure 3-1B). Plasma [H] values are presented as pH data in Table 3-1. There were no dose-related changes in blood pCO_2 (32 torr, on average) or pO_2 (75 torr) (Table 3-1).

Cerebrospinal Fluid and Plasma [K] and [Na]. Cerebrospinal fluid (CSF) [K] remained stable around 3.02 mmol/kg, even though plasma [K] increased from 4.1 to 4.3, 4.5 and 5.4 mmol/kg (Figure 3-2A). Similarly, CSF [Na] also remained stable during metabolic acidosis (around 156 mmol/kg), even though plasma [Na] decreased from 158 to 154, 151 and 147 mmol/kg (Figure 3-2B).

Choroid Plexus [K] and [Na]. The effects of NH_4Cl on lateral (LVCP) and fourth (4VCP) ventricular choroid plexuses (CP's) were investigated. Progressive augmentations of the dose

Figure 3-1. Dose-response analyses of the effects of NH_4Cl on [H] (A) and [HCO_3] (B) in arterial plasma.

Arterial blood samples were taken 60 minutes after an intraperitoneal injection of 0.00 (control), 2.33, 4.67 or 7.00 mmol/kg NH_4Cl (see Table 2-1 for a description of the contents of each dose). Values shown are means $\pm$ SEM of data from four to six adult male rats. The significance of the induced differences from the corresponding control in each panel was determined with a one-tailed Student's t-test: * indicates $p < 0.05$; ** indicates $p < 0.01$.

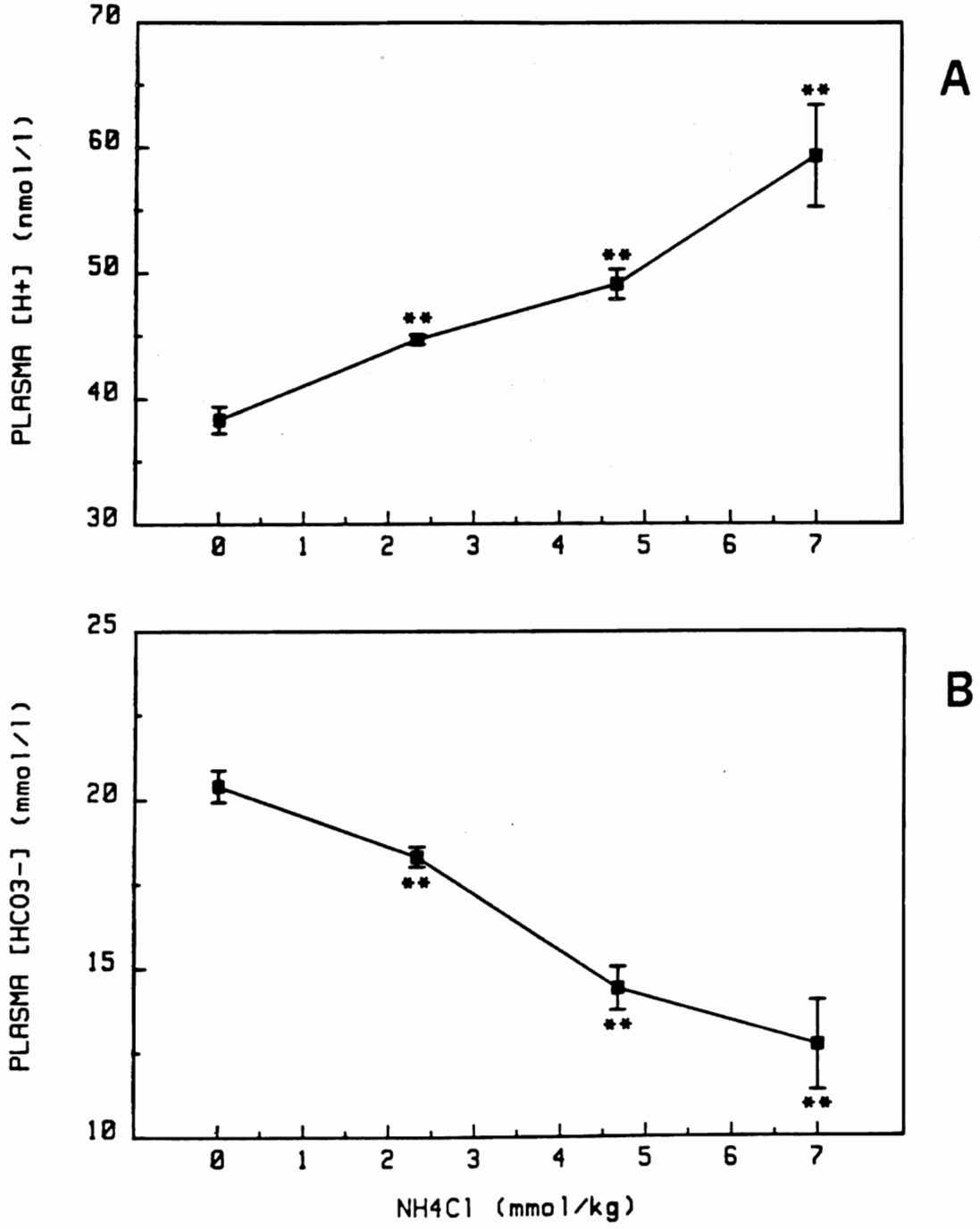

A
PLASMA [H+] (nmol/l)
70
60
50
40
30
0
1
2
3
4
5
6
7
**
B
PLASMA [HCO3-] (mmol/l)
25
20
15
10
NH4Cl (mmol/kg)

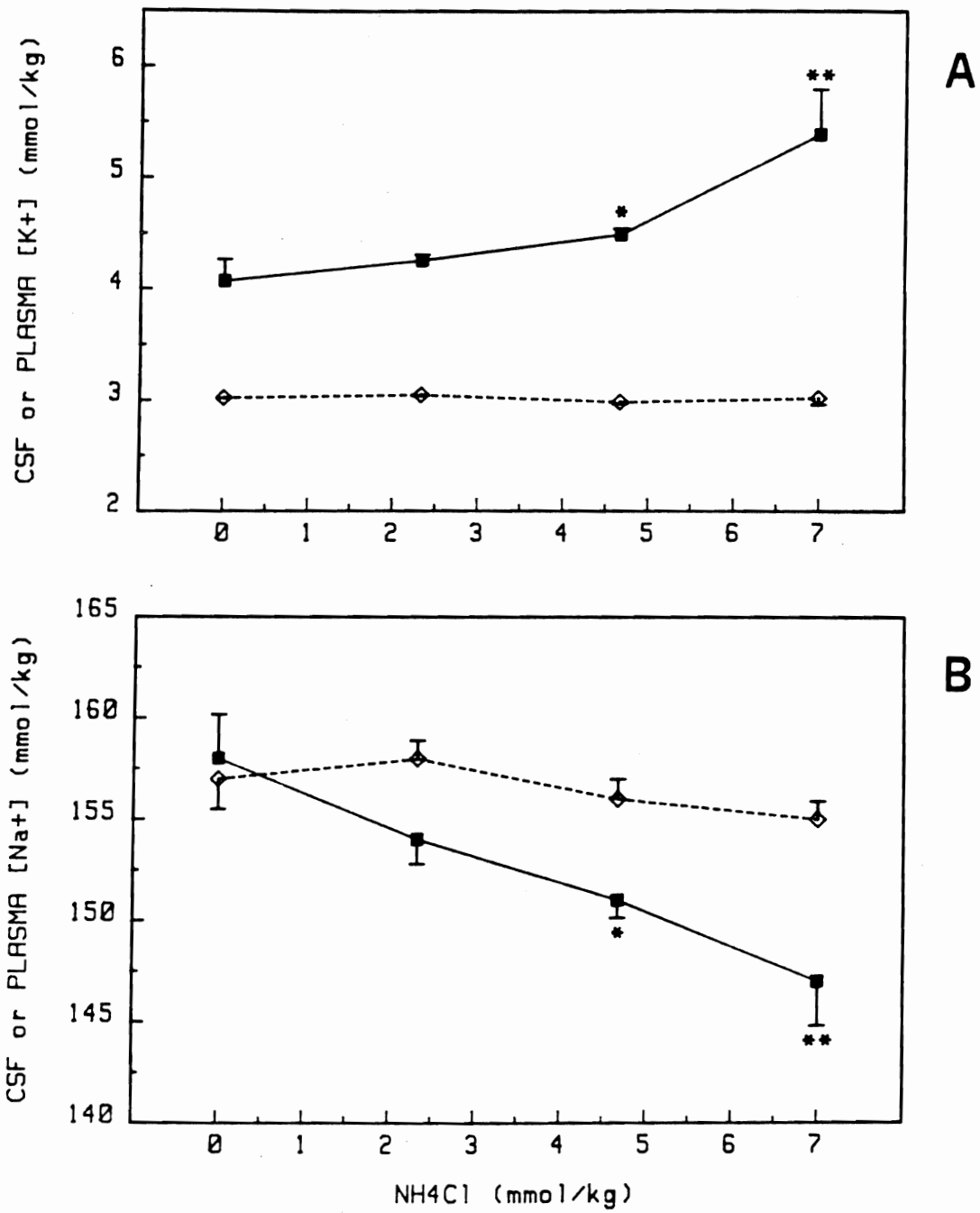
Table 3-1. DOSE-RESPONSE ANALYSES OF THE EFFECTS OF NH_4Cl ON pH, pCO_2 AND pO_2 IN ARTERIAL BLOOD

DOSE (mmol/kg):	0.00	2.33	4.67	7.00
pH	7.42 ± .01	7.35 ± .01**	7.31 ± .01**	7.23 ± .03**
pCO_2	33.5 ± .35	33.9 ± .66	29.4 ± 1.3*	31.4 ± 2.1
pO_2	72.2 ± 1.5	68.3 ± 2.5	78.1 ± 3.8	79.4 ± 4.5

Blood gas concentrations are in torr units. Arterial blood samples were taken 60 minutes after an intraperitoneal injection of 0.00 (control), 2.33, 4.67 or 7.00 mmol/kg NH_4Cl (see Table 2-1 for a description of the contents of each dose). Values shown are means ± SEM of data from three to four adult male rats. The significance of the induced differences from corresponding controls was determined with a one-tailed Student's t-test: * indicates $p < 0.05$; ** indicates $p < 0.01$.

Figure 3-2. Dose-response analyses of the effects of NH_4Cl on [K] (A) and [Na] (B) in cerebrospinal fluid and arterial plasma.

Cerebrospinal fluid (CSF) and arterial blood samples were taken 60 minutes after an intraperitoneal injection of 0.00 (control), 2.33, 4.67 or 7.00 mmol/kg NH_4Cl (see Table 2-1 for a description of the contents of each dose). Values shown are means $\pm$ SEM of data from two to four adult male rats. Unfilled squares connected by a dashed line are CSF values. Filled squares connected by a continuous line are plasma values. The significance of the induced differences from the corresponding controls in each panel was determined with a one-tailed Student's t-test: * indicates $p < 0.05$; ** indicates $p < 0.01$.


A
CSF or PLASMA [K+] (mmol/kg)
6
5
4
3
2
0
1
2
3
4
5
6
7
*
**
B
CSF or PLASMA [Na+] (mmol/kg)
165
160
155
150
145
140
0
1
2
3
4
5
6
7
*
**
NH4Cl (mmol/kg)

of NH_4Cl induced progressive increases in CP [K] (Figures 3-3A,3B): LVCP [K] increased from 500 (control) to 552 (+10%), 617 (+23%) and 664 (+33%) mmol/dry kg; 4VCP [K] increased from 493, to 519 (+5%), 539 (+9%) and 555 (+13%) mmol/dry kg. LVCP demonstrated greater increases in [K] than did 4VCP. LVCP and 4VCP [Na] tended to decrease with the augmented doses of NH_4Cl; notably, LVCP [Na] decreased from 276 to 237 mmol/dry kg (- 14%) after the 4.67 mmol/kg dose ($p < 0.05$).

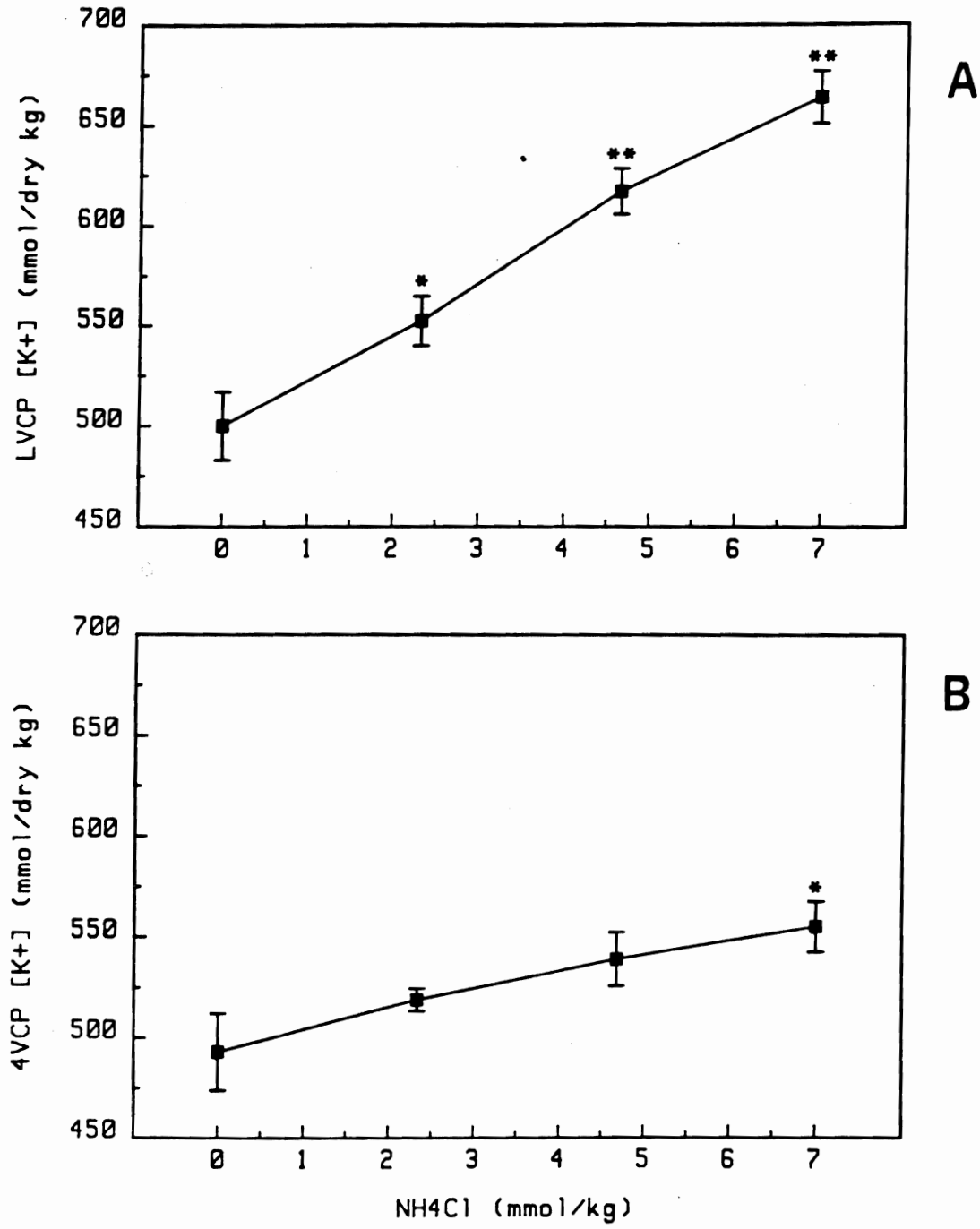
Summary. The greatest treatment-induced alterations of CP [K] and [Na] occurred after the 4.67 and 7.00 mmol/kg doses of NH_4Cl. Whereas animal morbidity was associated with the 7.00 mmol/kg dose of NH_4Cl, the next lower dose given (4.67 mmol/kg) was chosen for use in all of the remaining experiments in this chapter. Animals receiving 4.67 mmol/kg were slightly sedated 10-20 minutes after its injection; the duration of this sedation was 10-20 minutes.

Sixty-Minute Time-Courses

In this second experiment, except where noted, all of the effects described below will refer to NH_4Cl-induced increases above or decreases below time-matched control data. The terms baseline and treatment are adjectives which refer to the effects observed after an injection of either NaCl or NH_4Cl during the time-period being investigated, i.e., 5-60 minutes. Mean-baseline or mean-treatment are adjectives which refer to the average of baseline or treatment data; mean-treatment increase or

Figure 3-3. Dose-response analyses of the effect of NH_4Cl on [K] in LVCP (A) and 4VCP (B).

Lateral (LVCP) and fourth (4VCP) ventricular choroid plexus samples were taken 60 minutes after an intraperitoneal injection of 0.00 (control), 2.33, 4.67 or 7.00 mmol/kg NH_4Cl (see Table 2-1 for a description of the contents of each dose). Values shown are means $\pm$ SEM of data from two to four adult male rats. The significance of the induced differences from the corresponding control in each panel was determined with a one-tailed Student's t-test: * indicates $p < 0.05$; ** indicates $p < 0.01$.


A
LVCP [K+] (mmol/dry kg)
700
650
600
550
500
450
0
1
2
3
4
5
6
7
*
**
**
B
4VCP [K+] (mmol/dry kg)
700
650
600
550
500
450
0
1
2
3
4
5
6
7
*
NH4Cl (mmol/kg)

mean-treatment decrease refers to the difference between mean-baseline and mean-treatment data.

Acid-Base and Gas Analyses of Arterial Blood. Plasma [H] was increased maximally from 38 to 51 nM at 30 minutes (Figure 3-4A); this increase corresponds to a decrease in pH from 7.42 to 7.29, a change of 0.13 pH unit (Table 3-2). In controls, NaCl caused a time-related reduction in baseline plasma [H]; this decrease may have been related to a plasma dilution caused by the the volume of the injected solutions (2 ml per 100 grams of body weight). Plasma [HCO_3] was reduced below baseline at least 4 mmol/l during metabolic acidosis; a maximal reduction from 21 to 14 mmol/l occurred at 30 minutes (Figure 3-4B). Blood pCO_2 and pO_2 data are shown in Table 3-2. Neither pCO_2 nor pO_2 was greatly affected by NH_4Cl; baseline and treatment values tended to remain between 30-35 torr (CO_2) and 70-80 torr (O_2). The arterial hematocrit (HCT) was not significantly altered; the HCT remained around 39%.

Plasma [K] and [Na]. Plasma [K] was increased maximally from 3.6 to 5.3 mmol/kg at 5 minutes (Figure 3-5A); beyond 15 minutes treatment [K] continuously declined but was always greater than corresponding baseline values. Baseline [K] remained steady around 3.7 mmol/kg. Baseline [Na] was approximately 156 mmol/kg; a treatment-induced maximal decrease of 7 mmol/kg occurred at 30 minutes (Figure 3-5B).

Choroid Plexus [K] and [Na]. All three regions of the

Figure 3-4. Time-course analyses of the effects of NaCl and NH_4Cl on [H] (A) and [HCO_3] (B) in arterial plasma.

Arterial blood samples were taken 5, 15, 30 or 60 minutes after an intraperitoneal injection (4.67 mmol/kg) of either NaCl (control) or NH_4Cl (treatment). Values shown are means $\pm$ SEM of data from three to five adult male rats. Filled squares connected by continuous line are control values. Unfilled squares connected by a dashed line are treatment values. The significance of the induced differences from time-matched controls was determined with a one-tailed Student's t-test: * indicates $p < 0.05$; ** indicates $p < 0.01$. In panel B, all of the treatment values shown are significantly different from their time-matched controls ($p < 0.01$).

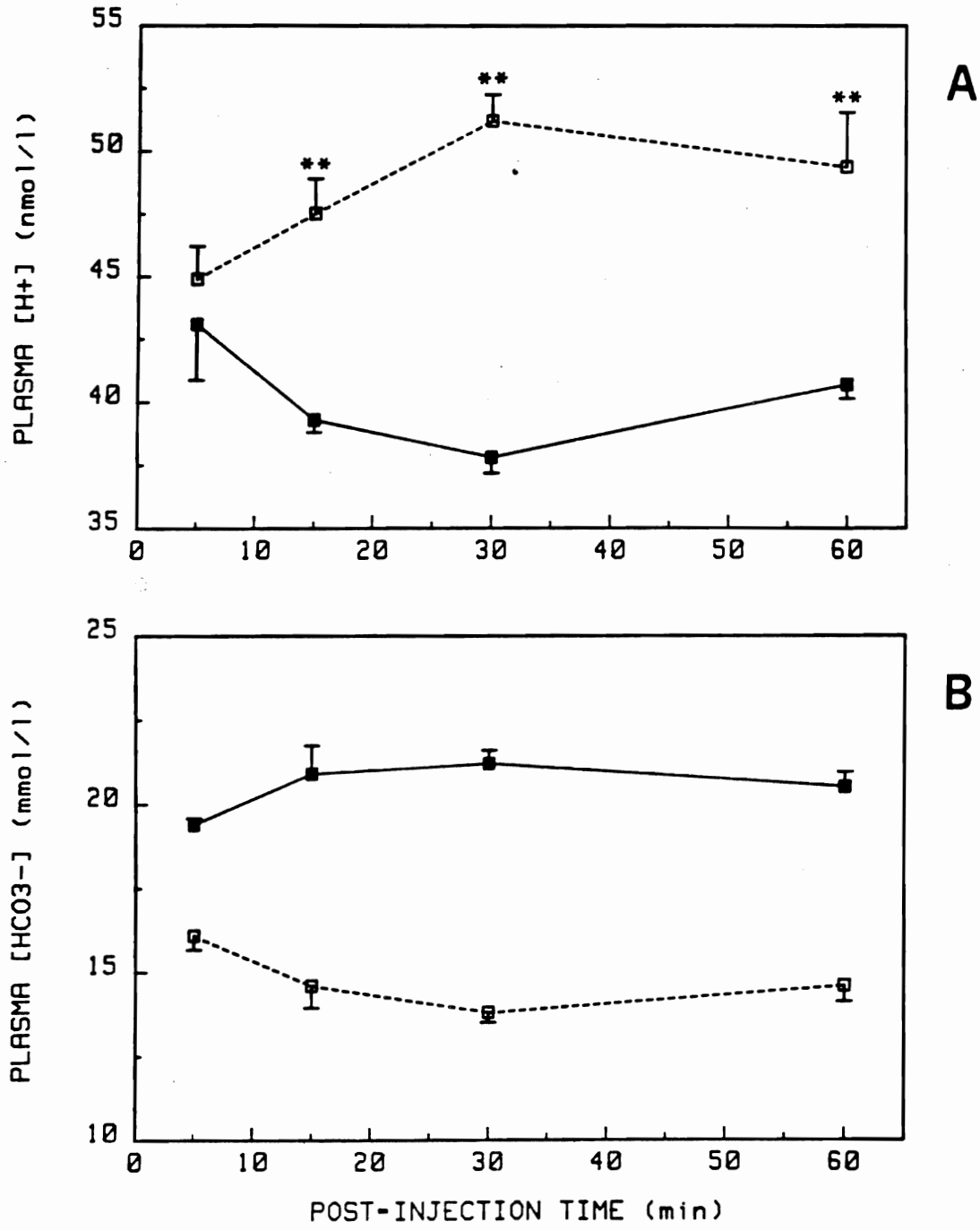

A
B
PLASMA [H+] (nmol/l)
PLASMA [HCO3-] (mmol/l)
POST-INJECTION TIME (min)
55
50
45
40
35
25
20
15
10
0
10
20
30
40
50
60
**
**
**

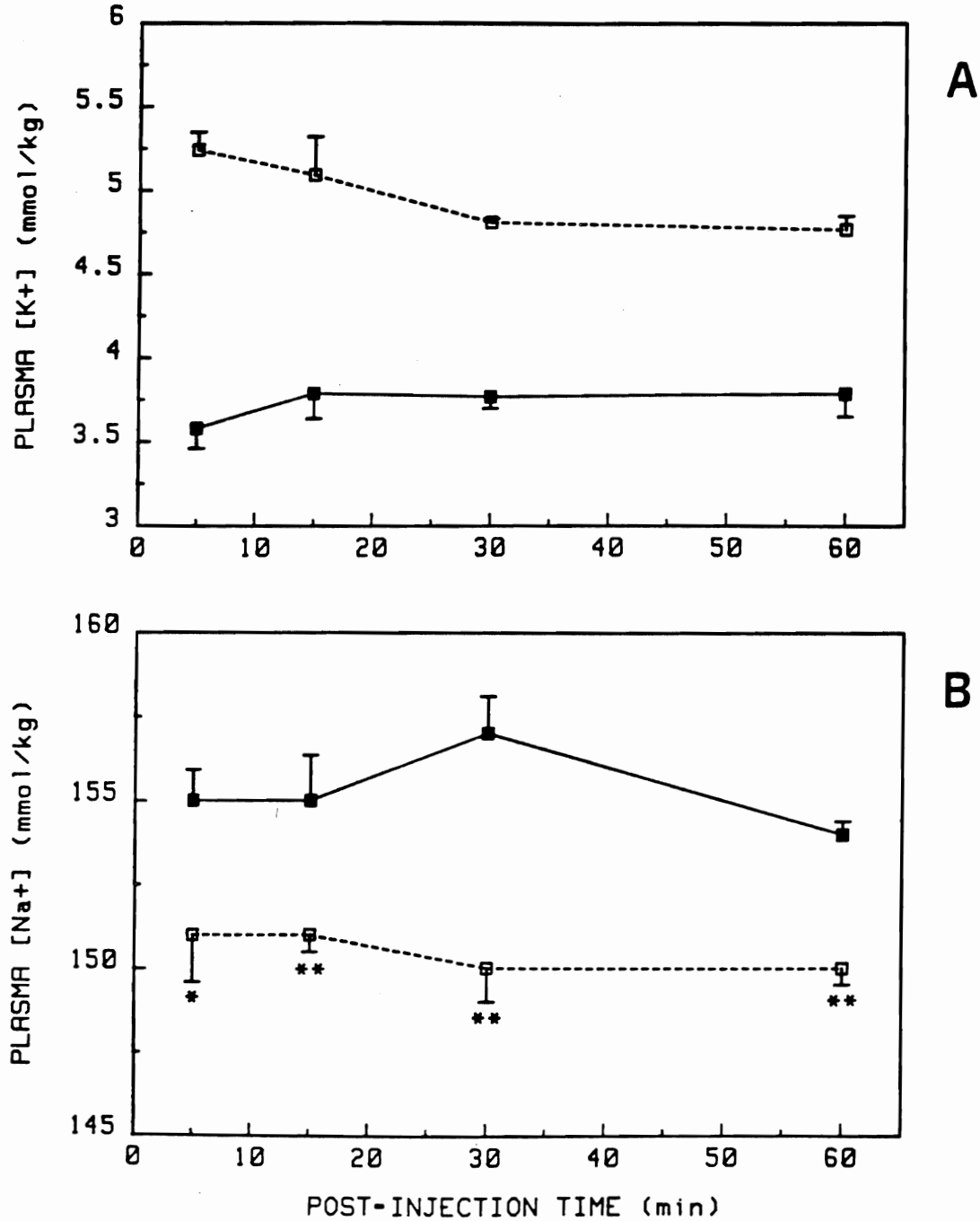
Table 3-2. TIME-COURSE ANALYSES OF THE EFFECTS OF NaCl AND NH_4Cl ON pH, pCO_2 AND pO_2 IN ARTERIAL BLOOD

MINUTES:	5	15	30	60
		CONTROL		
pH	7.37 ± .02	7.41 ± .01	7.42 ± .01	7.39 ± .01
pCO_2	34.7 ± 2.1	33.9 ± 1.7	32.0 ± .49	34.4 ± .77
pO_2	71.3 ± 2.3	70.1 ± 3.1	73.0 ± 1.8	78.7 ± .57
		TREATMENT		
pH	7.35 ± .01	7.32 ± .01**	7.29 ± .01**	7.31 ± .02
pCO_2	30.0 ± 1.1	31.0 ± 1.8	31.8 ± 1.3	30.1 ± 1.6
pO_2	79.6 ± 1.6*	80.0 ± 2.5*	77.3 ± 3.2	80.5 ± 2.0

Blood gas concentrations in torr units. Arterial blood samples were taken 5, 15, 30 or 60 minutes after an intraperitoneal injection (4.67 mmol/kg) of either NaCl (control) or NH_4Cl (treatment) (see Table 2-1 for a description of the contents of each dose). Values shown are means ± SEM of data from three to five adult male rats. The significance of the induced differences from time-matched controls was determined with a one-tailed Student's t-test: * indicates $p < 0.05$; ** indicates $p < 0.01$.

Figure 3-5. Time-course analyses of the effects of NaCl and NH_4Cl on [K] (A) and [Na] (B) in arterial plasma.

Arterial blood samples were taken 5, 15, 30 or 60 minutes after an intraperitoneal injection (4.67 mmol/kg) of either NaCl (control) or NH_4Cl (treatment). Values shown are means $\pm$ SEM of data from three to five adult male rats. Filled squares connected by continuous line are control values. Unfilled squares connected by a dashed line are treatment values. The significance of the induced differences from time-matched controls was determined with a one-tailed Student's t-test: * indicates $p < 0.05$; ** indicates $p < 0.01$. In panel A, all of the treatment values shown are significantly different from their time-matched controls ($p < 0.01$).


A
PLASMA [K+] (mmol/kg)
6
5.5
5
4.5
4
3.5
3
0
10
20
30
40
50
60
B
PLASMA [Na+] (mmol/kg)
160
155
150
145
*
**
**
**
POST-INJECTION TIME (min)

blood-CSF barrier have been analysed during NH_4Cl-induced metabolic acidosis. These regions, the lateral (LVCP), third (3VCP) and fourth (4VCP) ventricular choroid plexuses (CP's), responded to NH_4Cl with increases in [K] which were maximal at 30 minutes; these consistent increases in mean [K] were with the order LVCP [103] > 4VCP [81] > 3VCP [48] (here and below, values in brackets are mmol/kg dry tissue) (see Figures 3-6A,6B,6C). None of the observed increases in [K] returned to baseline by 60 minutes. It is of interest that mean-baseline [K] differed with the order LVCP [505] > 4VCP [480] > 3VCP [446]. [Na] in the CP was reduced generally by NH_4Cl (Figures 3-7A,7B,7C). In control CP's, the following mean-baseline [Na] values were observed: LVCP [249] < 4VCP [266] < 3VCP [336]. There were differences in mean-baseline [K] and [Na] among the control CP's and in the degree to which each tissue responded to treatment. If [K] and [Na] are related to each other as a ratio, i.e., [K]/[Na], there is a relationship between the corresponding ratios of each CP after NaCl and NH_4Cl; the greatest increases in [K]/[Na] after an injection of NH_4Cl occurred in those CP's having the greatest ratio of [K]/[Na] after NaCl (control) (Figure 3-8).

Summary. Alterations in [K] and [Na] in CP occurred as early as 5 minutes after an injection of NH_4Cl. The treatment-induced increases in CP [K] and decreases in CP [Na] were maximal at 30 minutes, repectively; [K] was still significantly elevated at 60 minutes (Figures 3-9A,9B,9C). Among

Figure 3-6. Time-course analyses of the effect of NaCl and NH_4Cl on [K] in LVCP (A), 3VCP (B) and 4VCP (C).

Lateral (LVCP), third (3VCP) and fourth (4VCP) ventricular choroid plexus samples were taken 5, 15, 30 or 60 minutes after an intraperitoneal injection (4.67 mmol/kg) of either NaCl (control) or NH_4Cl (treatment). Values shown are means $\pm$ SEM of data from three to five adult male rats. Filled squares connected by continuous line are control values. Unfilled squares connected by a dashed line are treatment values. The significance of the induced differences from time-matched controls was determined with a one-tailed Student's t-test: * indicates $p < 0.05$; ** indicates $p < 0.01$.

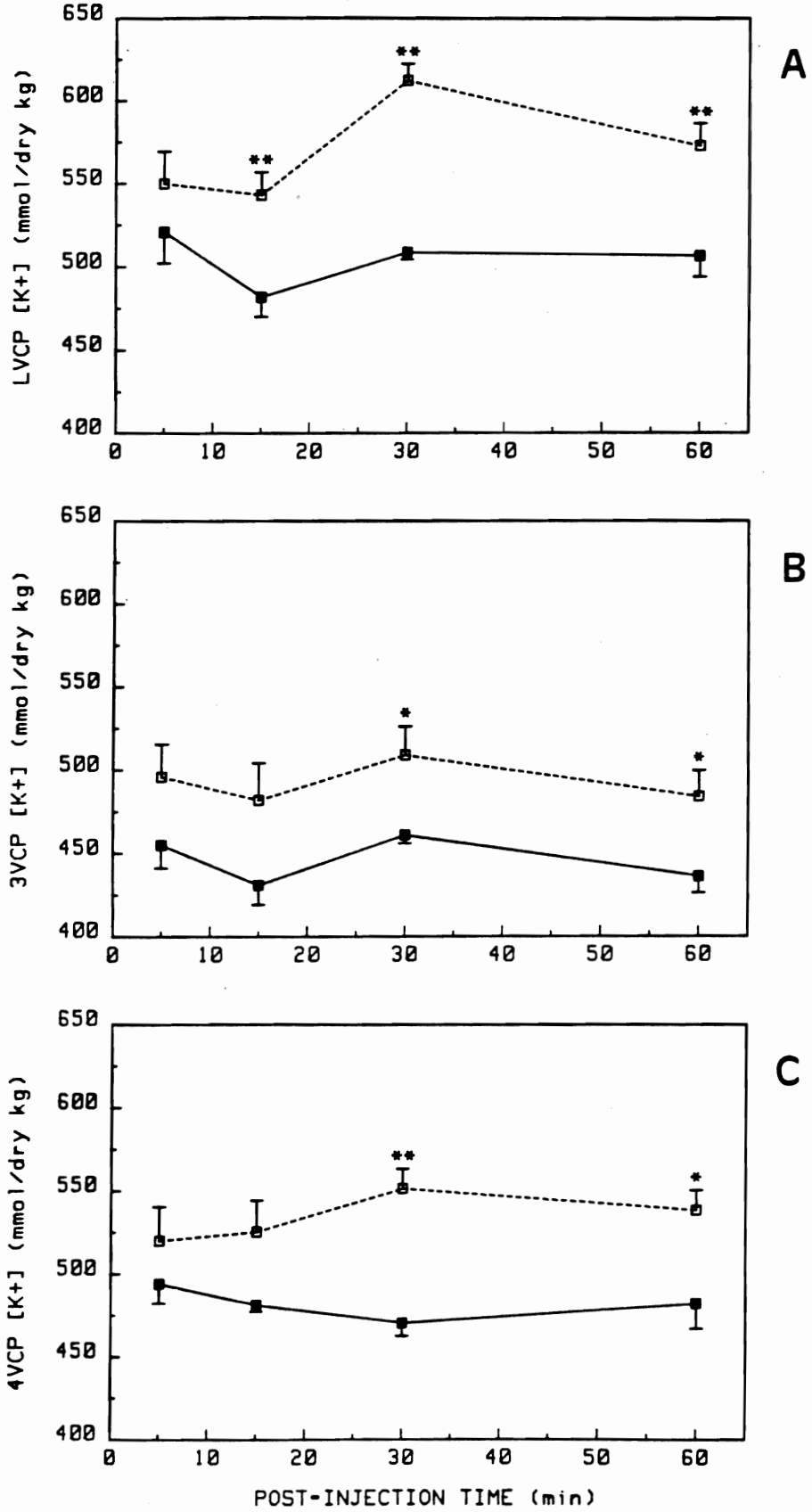

A
B
C
LVCP [K+] (mmol/dry kg)
3VCP [K+] (mmol/dry kg)
4VCP [K+] (mmol/dry kg)
650
600
550
500
450
400
0
10
20
30
40
50
60
**
*
POST-INJECTION TIME (min)

Figure 3-7. Time-course analyses of the effect of NaCl and NH_4Cl on [Na] in LVCP (A), 3VCP (B) and 4VCP (C).

Lateral (LVCP), third (3VCP) and fourth (4VCP) ventricular choroid plexus samples were taken 5, 15, 30 or 60 minutes after an intraperitoneal injection (4.67 mmol/kg) of either NaCl (control) or NH_4Cl (treatment). Values shown are means $\pm$ SEM of data from three to five adult male rats. Filled squares connected by continuous line are control values. Unfilled squares connected by a dashed line are treatment values. The significance of the induced differences from time-matched controls was determined with a one-tailed Student's t-test: * indicates $p < 0.05$; ** indicates $p < 0.01$.

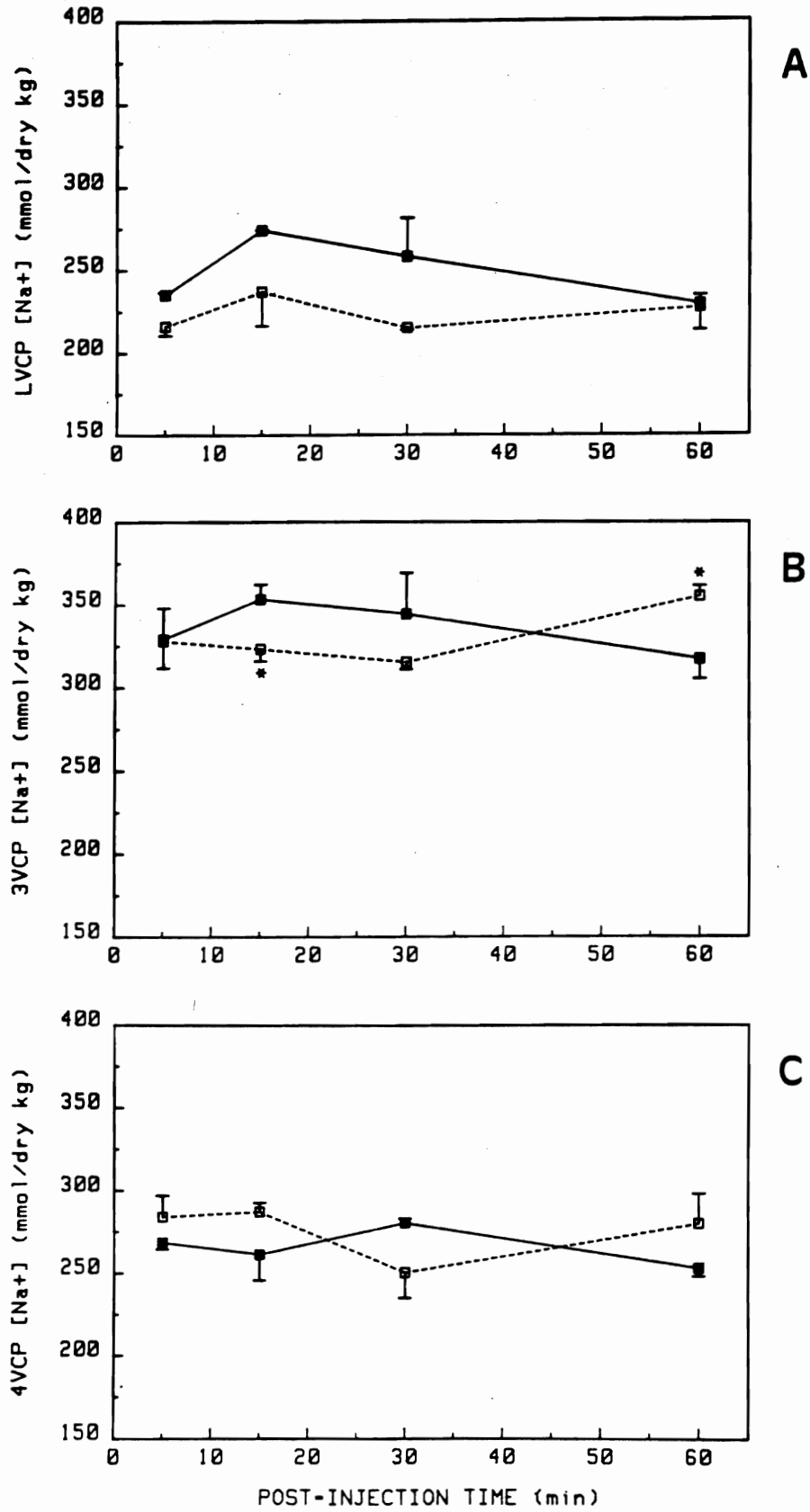

A
B
C
LVCP [Na+] (mmol/dry kg)
3VCP [Na+] (mmol/dry kg)
4VCP [Na+] (mmol/dry kg)
400
350
300
250
200
150
0
10
20
30
40
50
60
POST-INJECTION TIME (min)
*
*

Figure 3-8. Effect of NaCl and NH_4Cl on [K]/[Na] in LVCP, 3VCP and 4VCP.

Lateral (LVCP), third (3VCP) and fourth (4VCP) ventricular choroid plexus samples were taken 30 minutes after an intraperitoneal injection (4.67 mmol/kg) of either NaCl (control) or NH_4Cl (treatment). [K]/[Na] refers to the ratio of the contents of K and Na in dry choroid plexus samples. Values shown are means $\pm$ SEM of data from three to five adult male rats. Unhatched bars represent the control ratios. Hatched bars represent the ratios after the treatment. The significance of the induced differences from corresponding controls was determined with a one-tailed Student's t-test: * indicates $p < 0.05$; ** indicates $p < 0.01$.

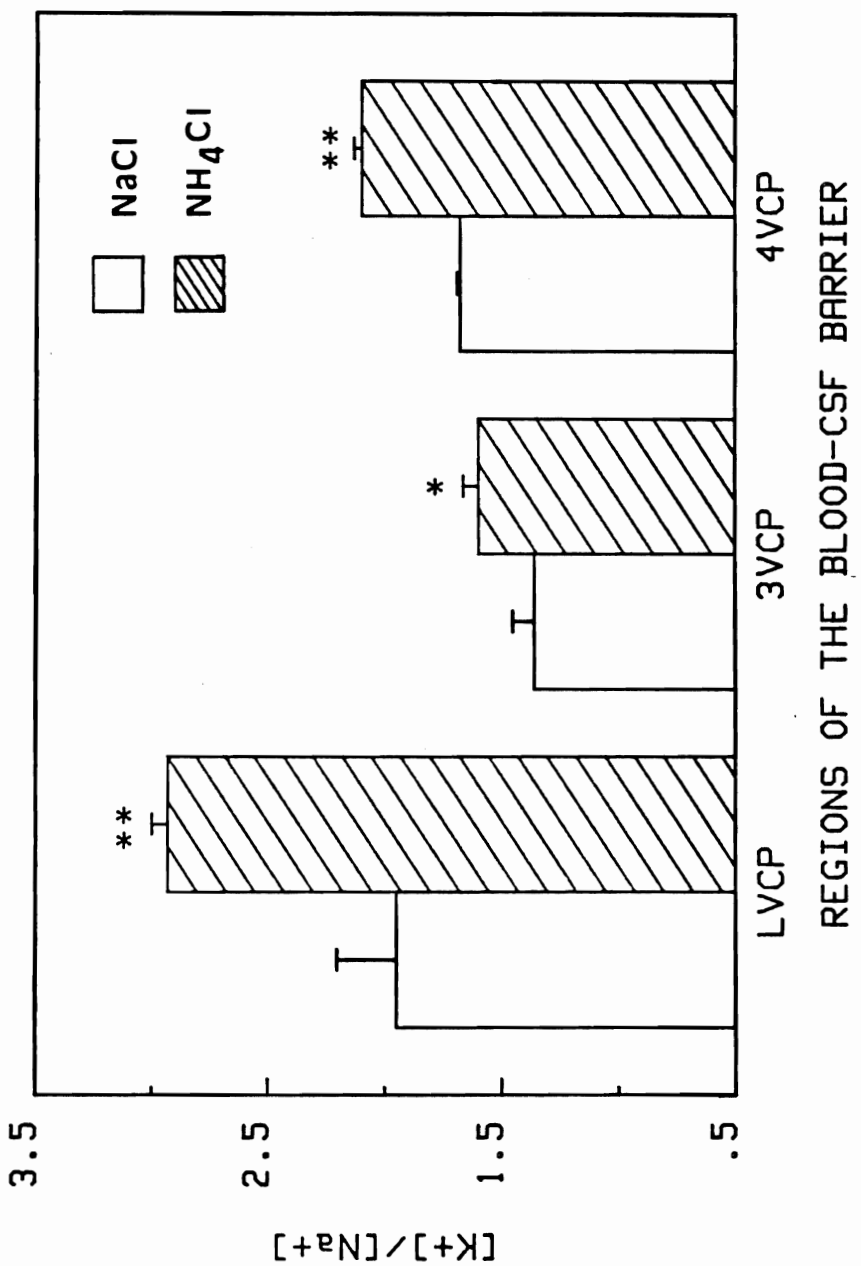
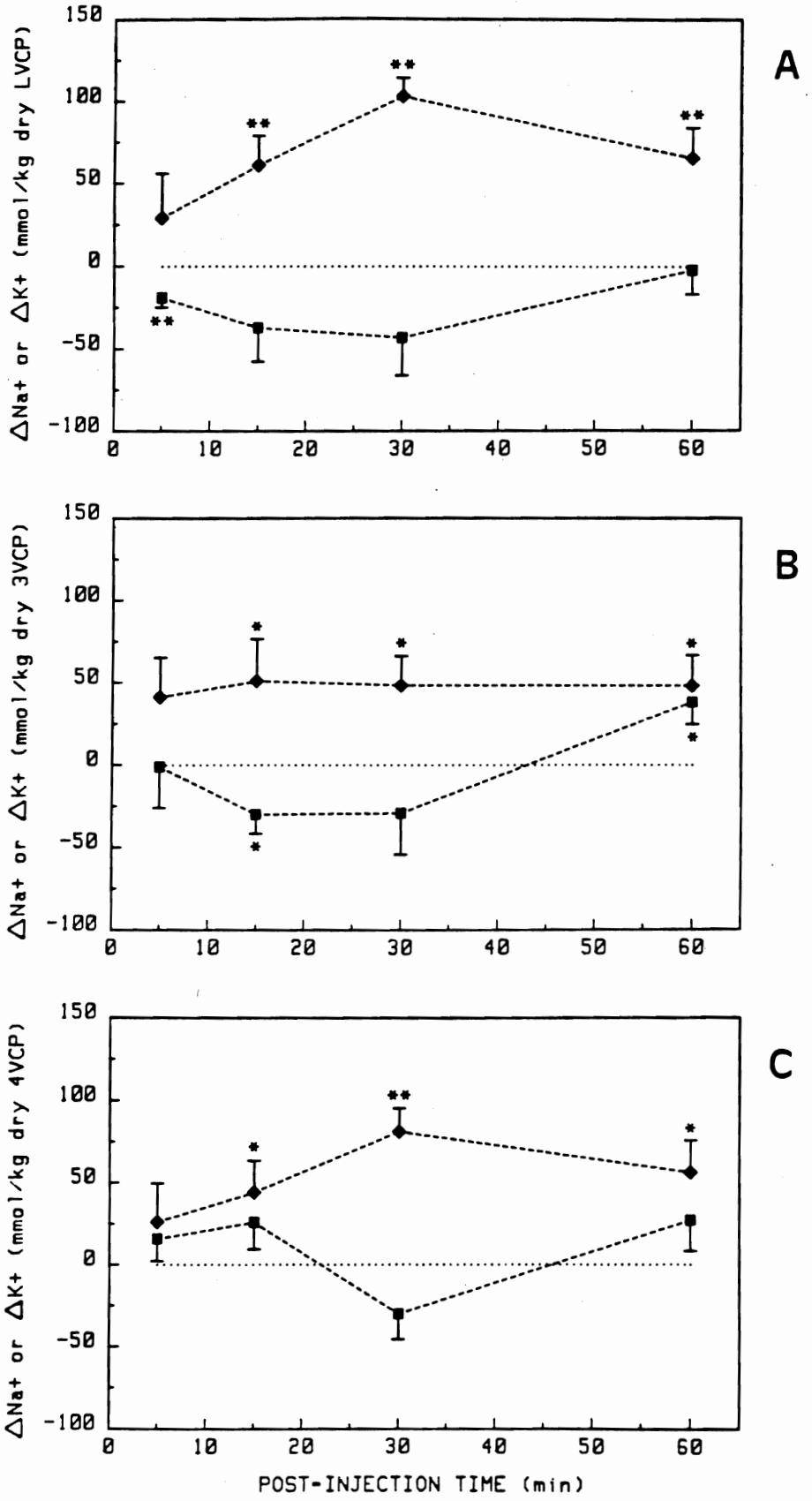

NaCl
NH_4Cl
**
*
**
LVCP
3VCP
4VCP
REGIONS OF THE BLOOD-CSF BARRIER
[K+]/[Na+]
3.5
2.5
1.5
.5

Figure 3-9. Time-course analyses of the changes induced from time-matched controls by NH_4Cl on [K] and [Na] in LVCP (A), 3VCP (B) and 4VCP (C).

Lateral (LVCP), third (3VCP) and fourth (4VCP) ventricular choroid plexus samples were taken 5, 15, 30 or 60 minutes after an intraperitoneal injection (4.67 mmol/kg) of either NaCl (control) or NH_4Cl (treatment). Values shown are mean $\pm$ SEM of data from six to ten adult male rats. Filled diamonds represent tissue [K] and filled squares represent tissue [Na]. The significance of the induced differences from time-matched controls was determined with a one-tailed Student's t-test: * indicates $p < 0.05$; ** indicates $p < 0.01$.


A
B
C
ΔNa+ or ΔK+ (mmol/kg dry LVCP)
ΔNa+ or ΔK+ (mmol/kg dry 3VCP)
ΔNa+ or ΔK+ (mmol/kg dry 4VCP)
150
100
50
0
-50
-100
0
10
20
30
40
50
60
POST-INJECTION TIME (min)

the three CP controls, there appeared to be an inverse relationship between mean-baseline [K] and [Na]. The control CP with the greatest ratio of [K]/[Na] responded the most to NH_4Cl.

Regional Analyses of Choroid Plexus [K] and [Na]

The results of this study indicate that there is no significant difference of [K] and [Na] in the anterior versus posterior halves of the LVCP. For comparative purposes, 3VCP and 4VCP were also sampled and analyzed. As observed in controls in the Time-Course study (above), the concentration of [K] (LVCP > 4VCP > 3VCP), was reciprocally associated with that of [Na] (LVCP < 4VCP < 3VCP); see Table 3-3.

Extracellular Fluid Volumes of Choroid Plexuses

NH_4Cl did not significantly alter the extracellular fluid volumes of LVCP, 3VCP or 4VCP (Table 3-4). The extracellular fluid volumes of each tissue were estimated with the steady-state distribution of ^3H-raffinose.

Table 3-3. ANALYSES OF TISSUE [K] AND [Na] IN FOUR CHOROID PLEXUS REGIONS

REGION:	3VCP	4VCP	A/LVCP	P/LVCP
[K]	441 ± 9.4	450 ± 4.7	478 ± 8.4*	460 ± 6.8
[Na]	364 ± 25.5	294 ± 4.8*	239 ± 5.1**	246 ± 6.0**

[K] and [Na] are in mmol/kg dry tissue units. After the lateral (LVCP) ventricular choroid plexus tissues were dissected out of the brain, they were sectioned into anterior (A/LVCP) and posterior (P/LVCP) halves before being analyzed for [K] and [Na]. The third (3VCP) and fourth (4VCP) ventricular choroid plexus tissues were also removed and analysed. All choroid plexus samples were taken from untreated animals. Values shown are means ± SEM of data from three to four adult male rats. With a one-tailed Student's t-test, it was determined that [K] and [Na] in A/LVCP did not significantly differ from that in P/LVCP; however, [K] and [Na] in the 3VCP significantly differed from that in other regions: * indicates $p < 0.05$; ** indicates $p < 0.01$.

Table 3-4. EFFECT OF NH_4Cl ON THE V_d OF ^3H-RAFFINOSE IN THREE CHOROID PLEXUS REGIONS AND CEREBROSPINAL FLUID

REGION:	3VCP	LVCP	4VCP	CSF
NaCl	12.9 ± 1.0	14.9 ± 0.9	16.3 ± 0.5	1.50 ± 0.12
NH_4Cl	14.8 ± 2.4	14.3 ± 1.1	15.6 ± 0.8	1.18 ± 0.10*

Lateral (LVCP), third (3VCP) and fourth (4VCP) ventricular choroid plexus, and cerebrospinal fluid (CSF) samples were taken 60 minutes after an intraperitoneal injection (4.70 mmol/kg) of either NaCl (control) or NH_4Cl (treatment); ^3H-raffinose (0.3 mCi/kg) was formulated into each solution. Values shown are means ± SEM of data from three to four bilaterally nephrectomized adult male rats. The effect of NH_4Cl on the volumes of distribution (V_d) of ^3H-raffinose were compared to corresponding controls with a one-tailed Student's t-test: * indicates $p < 0.05$; ** indicates $p < 0.01$.

CHAPTER 4

EFFECTS OF UNILATERAL SUPERIOR CERVICAL GANGLIONECTOMY AND BILATERAL ADRENALECTOMY ON THE EFFECTS OF NaCl AND NH_4Cl ON [K] AND [Na] IN CHOROID PLEXUS: METHODS

Experimental Protocols

Eighteen male Sprague-Dawley rats were used in the studies described below. Prior to each experiment, animals were housed in cages wherein they had free access to food and water, and were regularly exposed to alternating 12-hour periods of darkness or overhead fluorescent lighting. During the experiments, rats had continued free access to food and water in cages receiving room-light. Five minutes before fluid sampling and choroid plexus removal were initiated, each rat was injected intraperitoneally with 80 mg/kg ketamine hydrochloride. This anesthetic dose of ketamine, which did not change the animal's rate of respiration, required 5 minutes to effect sedation. Each rat was killed by exsanguination after the blood sample was taken. In the experiments described below, the following were measured: plasma [H], [HCO_3], [K] and [Na]; blood pCO_2 and pO_2; and choroid plexus [K] and [Na]. In one of the experiments, the concentrations of K and Na in the cerebrospinal fluid were also measured.

Unilateral Superior Cervical Ganglionectomy. Twelve 7-8 week-old rats (225-325 grams) were used in this first experiment.

Six days prior to the experiment, each animal was prepared for surgery with an intraperitoneal injection of ketamine hydrochloride (100 mg/kg). With the aid of a stereotaxic microscope, both superior cervical ganglia, in each animal, were surgically exposed and gently separated from the surrounding fascia. After removing only one ganglion per rat, each animal was sutured and placed into one of two designated post-operative recovery cages. The six animals which had their right superior cervical ganglion excised were placed into a cage designated R-SCGX; the six animals which had their left superior cervical ganglion removed were placed into a cage designated L-SCGX. In each R-SCGX and L-SCGX group, the unremoved ganglion, in each animal, served as a sham-operated control. An operation was considered a success if a unilateral ptosis developed only in the eye located on the same side as the ganglionectomy. Six days after the surgical ganglionectomy (SCGX), three animals from each R-SCGX and L-SCGX cage were placed into one of two injection groups: controls received NaCl; treated animals received NH_4Cl (4.70 mmol/kg, intraperitoneally). Each of these two injection groups had three R-SCGX and L-SCGX representatives; therefore, possible variations in the sidedness of the effect of SCGX on choroid plexus [K] and [Na] was controlled. Thirty minutes after injection, a sample of blood was taken, and the fourth, third and lateral ventricular choroid plexuses were removed, in the order mentioned.

Bilateral Adrenalectomy. Six 6-7 week-old Sprague-Dawley rats (190-230 grams) were used in this second experiment. One day prior to the experiment, animals were prepared for surgery with an intraperitoneal injection of ketamine hydrochloride (100 mg/kg). In each animal, both adrenal glands were surgically exposed and removed from their superior renal positions. Following each bilateral adrenalectomy, all incisions were sutured, and animals were placed into post-operative recovery cages wherein they had continued free access to food and normal saline (0.9% NaCl). One day after the bilateral adrenalectomy (ADRNx), the animal's were injected. Controls received NaCl; treated animals received NH_4Cl (4.70 mmol/kg, intraperitoneally). Thirty minutes after injection, a sample of blood was taken, and the fourth, third and lateral ventricular choroid plexuses were removed, in the order mentioned.

The materials, instruments and procedures used in the sampling, analysis and calculation of blood pH, pCO_2 and pO_2, and fluid and choroid plexus [K] and [Na], have been described in Chapter 2.

CHAPTER 5

EFFECTS OF UNILATERAL SUPERIOR CERVICAL GANGLIONECTOMY AND BILATERAL ADRENALECTOMY ON THE EFFECTS OF $NaCl$ AND NH_4Cl ON [K] AND [Na] IN CHOROID PLEXUS: RESULTS

Unilateral Superior Cervical Ganglionectomy

In this first experiment, the effect of a unilateral superior cervical ganglionectomy (SCGX) on the response of the lateral (LVCP) ventricular choroid plexus (CP) to the effects of an intraperitoneal injection of NH_4Cl were investigated; control animals received an injection of NaCl. In comparison to control animals, NH_4Cl induced the following changes.

Acid-Base and Gas Analyses of Arterial Blood. Plasma [H] was increased from 40 to 57 nmol/l (Table 5-1), a decrease in pH from 7.40 to 7.24 (Table 5-2). Plasma [HCO_3] was decreased 6 mmol/l (from 21 to 15 mmol/l). Both control and treatment group mean pCO_2 remained at 36 torr. Some enhancement of respiration was suggested by an increase of 10 torr in blood pO_2 (from 67 to 77 torr). No change in the arterial hematocrit (HCT) was observed; the HCT in both groups was 41%.

Cerebrospinal Fluid and Plasma [K] and [Na]. Cerebrospinal fluid (CSF) [K] decreased from 3.08 to 2.94 mmol/kg (Table 5-3), even though plasma [K] increased from 3.7 to 4.5 mmol/kg (Table 5-1). CSF [Na] significantly decreased from 157 to 153

Table 5-1. EFFECT OF UNILATERAL SUPERIOR CERVICAL GANGLIONECTOMY ON THE EFFECTS OF NaCl AND NH_4Cl ON [H], [HCO_3], [K] AND [Na] IN ARTERIAL PLASMA

PLASMA	NaCl	NH_4Cl
[H]	40.4 ± 1.49	57.0 ± 0.92**
[HCO_3]	21.3 ± 0.73	15.0 ± 0.42**
[K]	3.68 ± 0.11	4.48 ± 0.06**
[Na]	141 ± 0.60	134 ± 0.33**

[H], [HCO_3], and [K] and [Na], are in the following respective units: nmol/l, mmol/l, and mmol/kg water. Arterial blood samples were taken 30 minutes after an injection (4.70 mmol/kg) of either NaCl (control) or NH_4Cl (treatment). Values shown are means ± SEM of data from six unilaterally superior cervical ganglionectomized adult male rats. The significance of the induced differences from corresponding controls was determined with a one-tailed Student's t-test: * indicates $p < 0.05$; ** indicates $p < 0.01$.

Table 5-2. EFFECT OF UNILATERAL SUPERIOR CERVICAL GANGLIONECTOMY ON THE EFFECTS OF NaCl AND NH_4Cl ON pH, pCO_2 AND pO_2 IN ARTERIAL BLOOD

BLOOD	NaCl	NH_4Cl
pH	7.40 ± .016	7.24 ± .007**
pCO_2	35.5 ± 1.90	35.9 ± 1.10
pO_2	67.3 ± 3.50	76.5 ± 2.40*

Blood gases are in torr units. Arterial blood samples were taken 30 minutes after an injection (4.70 mmol/kg) of either NaCl (control) or NH_4Cl (treatment). Values shown are means ± SEM of data from six unilaterally superior cervical ganglionectomized adult male rats. The significance of the induced differences from corresponding controls was determined with a one-tailed Student's t-test: * indicates $p < 0.05$; ** indicates $p < 0.01$.

Table 5-3. EFFECT OF UNILATERAL SUPERIOR CERVICAL GANGLIONECTOMY ON THE EFFECTS OF NaCl AND NH_4Cl ON [K] AND [Na] IN CEREBROSPINAL FLUID

CEREBROSPINAL FLUID	NaCl	NH_4Cl
[K]	3.08 ± 0.02	2.94 ± 0.04**
[Na]	157 ± 0.33	153 ± 0.45**

[K] and [Na] values are in mmol/kg water units. Cerebrospinal fluid (C^F) samples were taken 30 minutes after an injection (4.70 mmol/kg) of either NaCl (control) or NH_4Cl (treatment). Values shown are means ± SEM of data from six unilaterally superior cervical ganglionectomized adult male rats. The significance of the induced differences from corresponding controls was determined with a one-tailed Student's t-test: * indicates $p < 0.05$; ** indicates $p < 0.01$.

mmol/kg; plasma [Na] decreased from 141 to 134 mmol/kg. All of the above CSF and plasma changes are statistically significant.

Lateral Ventricular Choroid Plexus [K] and [Na]. The sham SCGX-LVCP response to NH_4Cl was similar to the choroid plexus (CP) responses seen in Chapter 1: CP [K] increased (from 458 to 595 mmol/dry kg); and, CP [Na] decreased (from 277 to 256 mmol/dry kg); see Figures 5-1A,1B. In the SCGX-LVCP, NH_4Cl also induced an increase in [K] from 455 to 594 mmol/dry kg and a decrease in [Na] from 282 to 241 mmol/dry kg. These results indicate that the responses to NH_4Cl observed in sham SCGX-LVCP were the same as those observed in SCGX-LVCP. Thus, innervation to the LVCP through the superior cervical ganglion does not appear to be a requirement for NH_4Cl-induced effects on [K] and [Na] in the CP's.

Bilateral Adrenalectomy

This second experiment investigated the effect of bilateral adrenalectomy on the effect of NaCl (control) and NH_4Cl (treatment) on [K] and [Na] in the lateral (LVCP), third (3VCP) and fourth (4VCP) ventricular CP's of adult male rats. In comparison to controls, NH_4Cl (treatment) induced the results described below.

Acid-Base and Gas Analyses of Arterial Blood. Plasma [H] was increased from 38 to 68 nmol/l (Table 5-4), a decrease in pH from 7.43 to 7.17 (Table 5-5). Plasma [HCO_3] was decreased from 21 to 15 mmol/l. NH_4Cl augmented pCO_2 from 33 to 42 torr; blood pO_2 decreased from 74 to 57 torr. It is noted that

Figure 5-1. Effect of unilateral superior cervical ganglionectomy on the effects of NaCl and NH_4Cl on [K] (A) and [Na] (B) in LVCP.

Lateral (LVCP) ventricular choroid plexus samples were taken 30 minutes after an intraperitoneal injection (4.67 mmol/kg) of either NaCl (control) or NH_4Cl (treatment). Values shown are means $\pm$ SEM of data from six adult male rats. Unhatched bars represent control values. Hatched bars represent concentrations of tissue electrolytes after treatment. The significance of the induced differences from corresponding controls was determined with a one-tailed Student's t-test: * indicates $p < 0.05$; ** indicates $p < 0.01$.

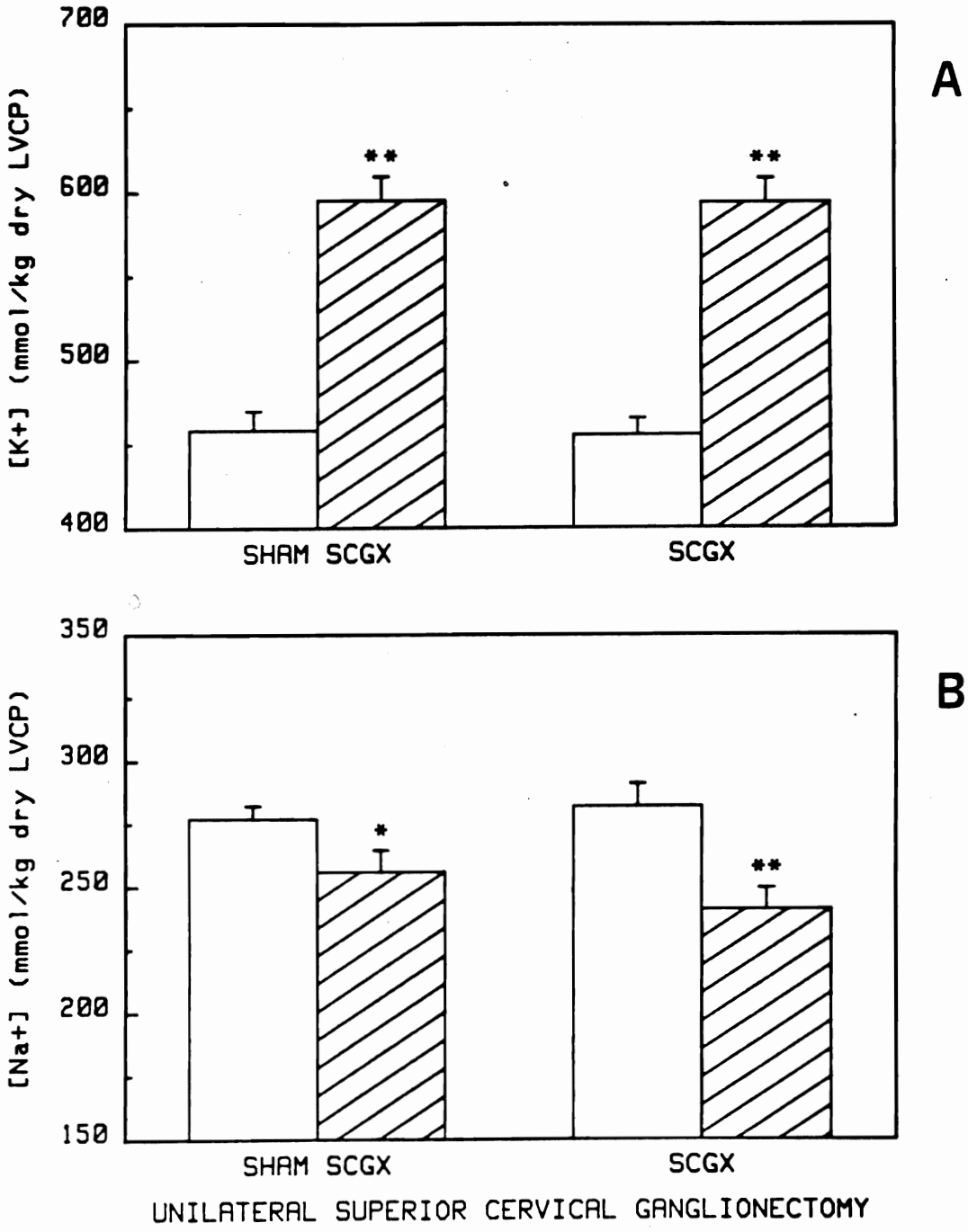

A
700
600
500
400
[K+] (mmol/kg dry LVCP)
**
**
SHAM SCGX
SCGX
B
350
300
250
200
150
[Na+] (mmol/kg dry LVCP)
*
**
SHAM SCGX
SCGX
UNILATERAL SUPERIOR CERVICAL GANGLIONECTOMY

Table 5-4. EFFECT OF BILATERAL ADRENALECTOMY ON THE EFFECTS OF NaCl AND NH_4Cl ON [H], [HCO_3], [K] AND [Na] IN ARTERIAL PLASMA

PLASMA	NaCl	NH_4Cl
[H]	37.5 ± 1.16	67.8 ± 4.31**
[HCO_3]	21.4 ± 1.34	14.6 ± 0.50**
[K]	4.45 ± 0.16	5.39 ± 0.14**
[Na]	153 ± 1.20	142 ± 1.20**

[H], [HCO_3], and [K] and [Na] are in the following units: nmol/l, mmol/l, and mmol/kg water. Arterial blood samples were taken 30 minutes after an injection (4.70 mmol/kg) of either NaCl (control) or NH_4Cl (treatment). Values shown are means ± SEM of data from three bilaterally adrenalectomized adult male rats. The significance of the induced differences from corresponding controls was determined with a one-tailed Student's t-test: * indicates $p < 0.05$; ** indicates $p < 0.01$.

Table 5-5. EFFECT OF BILATERAL ADRENALECTOMY ON THE EFFECTS OF NaCl AND NH_4Cl ON pH, pCO_2 and pO_2 IN ARTERIAL PLASMA

BLOOD	NaCl	NH_4Cl
pH	7.43 ± .014	7.17 ± .027**
pCO_2	33.2 ± 2.92	41.6 ± 1.40*
pO_2	73.9 ± 1.68	56.5 ± 6.92*

Blood gases are in torr units. Arterial blood samples were taken 30 minutes after an injection (4.70 mmol/kg) of either NaCl (control) or NH_4Cl (treatment). Values shown are means ± SEM of data from three bilaterally adrenalectomized adult male rats. The significance of the induced differences from corresponding controls was determined with a one-tailed Student's t-test: * indicates $p < 0.05$; ** indicates $p < 0.01$.

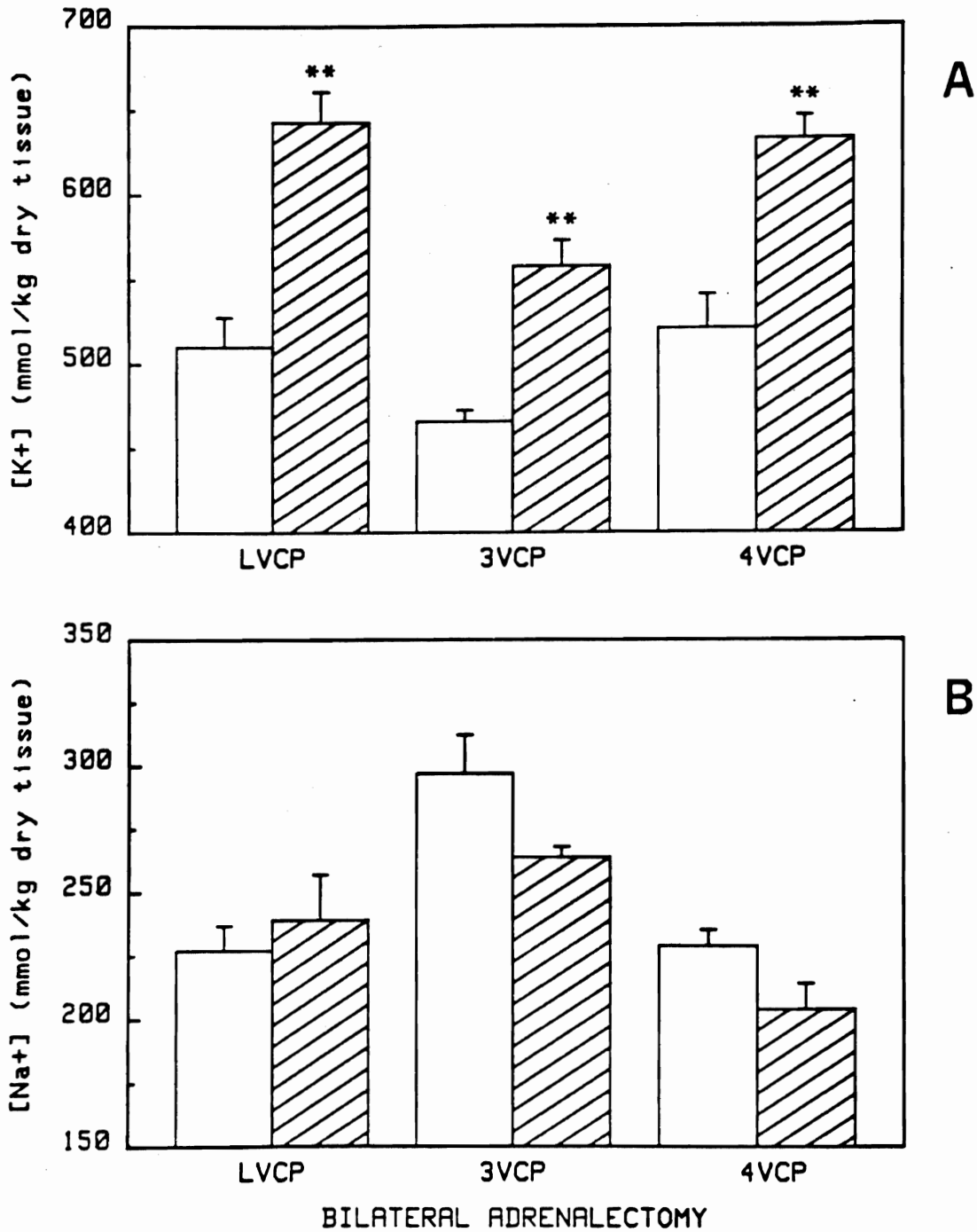
adrenalectomized animals did not tolerate very well the injection of NH_4Cl; in general their rates of respiration were depressed. No change in the arterial hematocrit (HCT) was observed; in both groups it was about 40%.

Plasma Electrolytes. Plasma [K] increased from 4.4 to 5.4 mmol/kg (Table 5-4); plasma [Na] decreased from 153 to 142 mmol/kg.

Choroid Plexus [K] and [Na]. NH_4Cl induced a substantial increase in [K] in the LVCP, 3VCP and 4VCP (Figure 5-2A). LVCP [K] increased from 510 to 643 mmol/dry kg; 3VCP [K] increased from 466 to 558 mmol/dry kg; and 4VCP [K] increased from 521 to 633 mmol/dry kg. [Na] in the CP's was not significantly affected (Figure 5-2B). The results indicate that bilateral adrenalectomy does not block the effect of NH_4Cl to increase CP [K]. Thus, a release of hormones or other substances from the adrenal glands does not appear to be a requirement for the NH_4Cl-induced increase in CP [K].

Figure 5-2. Effect of bilateral adrenalectomy on the effects of NaCl and NH_4Cl on [K] (A) and [Na] (B) in LVCP, 3VCP and 4VCP

Lateral (LVCP), third (3VCP) and fourth (4VCP) ventricular choroid plexus samples were taken 30 minutes after an intraperitoneal injection (4.67 mmol/kg) of either NaCl (control) or NH_4Cl (treatment). Values shown are means $\pm$ SEM of data from three adult male rats. Unhatched bars represent control values. Hatched bars represent concentrations of tissue electrolytes after the treatment. The significance of the induced differences from corresponding controls was determined with a one-tailed Student's t-test: * indicates $p < 0.05$; ** indicates $p < 0.01$.


700
600
500
400
[K+] (mmol/kg dry tissue)
**
**
**
A
LVCP
3VCP
4VCP
350
300
250
200
150
[Na+] (mmol/kg dry tissue)
B
LVCP
3VCP
4VCP
BILATERAL ADRENALECTOMY

CHAPTER 6

EIGHT-HOUR TIME-COURSE ANALYSES OF THE EFFECTS OF NaCl AND NH_4Cl ON [K] AND [Na] IN CHOROID PLEXUS: METHODS

Experimental Protocols

Sixty-seven 6-8 week-old male Sprague-Dawley rats (165-300 grams) were used in the studies described below. Prior to each experiment, the animals were housed in cages wherein they had free access to food and water and were regularly exposed to alternating 12-hour periods of darkness or overhead-fluorescent lighting. For the experiment, rats were separated into two groups: controls received NaCl; treated animals received NH_4Cl (4.70 mmol/kg, intraperitoneally). During the experiment rats had continued free access to food and water in cages receiving room-light. One-quarter, 0.5, 1, 2, 4 or 8 hours after injection, a sample of blood and of cerebrospinal fluid were taken, and the fourth (4VCP), third (3VCP) and lateral (LVCP) ventricular choroid plexuses were removed from each rat, all in the order mentioned. Five minutes before fluid sampling and choroid plexus removal were initiated, each animal was injected intraperitoneally with 80 mg/kg ketamine hydrochloride. This anesthetic dose of ketamine, which did not change the animal's rate of respiration, required 5 minutes to effect sedation. Each rat was killed by exsanguination after the blood sample was taken. In the experiments described

below, the following were measured: plasma [H], [HCO_3], [K] and [Na], blood pCO_2 and pO_2, choroid plexus [K] and [Na], and cerebrospinal fluid [K] and [Na].

Technique of Brain-Blood Washout

The choroid plexus (CP) is a very vascular and predominantly epithelial tissue (Dohrmann, 1970). Whole blood represents about 15% of the total wet weight of the CP (Johanson et al., 1974; 1976). Epithelial cells represent about 50-60% of the total wet weight of the CP (Quay, 1972; Johanson et al., 1974). When a CP sample is excised from an animal's brain and analyzed for [K] and [Na], it must be realized that a portion of the total [K] and [Na] detected is contributed by the blood present in the vasculature of this tissue. In order to minimize the blood, and thus maximize the epithelial cell, contributions of K and Na in each CP sample, blood was washed out of the CP's before they were removed from the brain. In this study, residual blood was displaced by means of a brain-blood washout. The washout immediately followed the acquisition of a cisternal CSF sample: a drainage port was incised into the inferior vena cava; with the abdominal aortic clamp still in place (see Sampling Procedures), 18 ml of a 280 mM chilled sucrose solution was perfused (with a peristaltic pump through a 25-gauge venoset) into the left ventricle of the heart over a 1-minute period; as the sucrose solution moved from the left ventricle to the brain and then to the drainage port in the inferior vena cava, the blood in

the vasculature of the choroid plexuses was almost totally displaced. The sucrose perfusion-fluid also contained bovine serum albumin (4% w/v) in order to simulate the normal oncotic pressure present in blood. Under similar washout conditions with ^{51}Cr-tagged erythrocytes, the percentage of blood removed from the CP was determined to be 95-99% (Murphy, unpublished data).

Sampling Procedures

Arterial Blood. Sampling was initiated by opening the abdominal cavity, quickly draining and drying the cavity of any residual fluid from the injection, and exposing the abdominal aorta. After withdrawing a 4-ml sample of blood from the abominal aorta near the femoral bifurcation, the aorta was clamped proximal to the inserted needle. The needle was then withdrawn from the artery and removed from the syringe. The syringe was capped and gently mixed; thereafter, a 2-ml aliquot of blood was transferred into a Falcon tube. The remaining blood, after syringe recapping, was placed into an ice bath for later acid-base and blood-gas analyses. Centrifugation of the 2 ml-aliquot of blood was begun while further sampling continued.

Cerebrospinal Fluid. After the scalp was removed, the animal was exsanguinated (killed) by incising the abdominal aorta above the aforementioned clamp. A sample of CSF was then aspirated from the cisterna magna by penetrating the muscle tissue above the foramen magnum with a specially prepared micropipette. The CSF sample was transferred onto a piece of Parafilm;

immediately, a 0.025-ml aliquot of CSF was transferred from the Parafilm into a tared Falcon tube for later weighing. CSF samples contaminated with blood or muscle tissue were not analyzed.

Choroid Plexuses. The washed-out brain was removed and placed on normal saline-wetted filter paper. With the aid of a stereotaxic microscope, the fourth (4VCP), third (3VCP) and both lateral (LVCP) ventricular choroid plexuses, in the above order, were dissected free from the brain and placed on tared, 4.0-mg, aluminum foil weighing boats for drying and later weighing on an electrobalance.

Plasma. Lastly, three plasma samples were taken from the previously centrifuged 2-ml aliquot of blood: (1) a 0.025-ml sample was transferred into a tared Falcon tube for a later weighing, (2) a 0.70-ml sample was drawn into and capped in a sterile 1-ml tuberculin syringe and immediately placed into an ice bath for a later analysis of ammonia (see below), and finally, (3) a 0.50-ml sample was transferred into a Falcon tube for a later determination of osmolality.

Analyses and Calculations

Plasma Ammonia Analysis. The Automatic Clinical Analyzer (ACA) method of plasma ammonia analysis, by DuPont, uses an adaptation of the glutamate dehydrogenase (GLDH) enzymatic method of van Anken and Schiphorst (1974). This plasma ammonia analysis does not distinguish NH_4 from NH_3. Therefore, the term ammonia in this text will refer to the sum of plasma $[NH_4]$ and $[NH_3]$.

Individual ionized or un-ionized forms of ammonia will be referred to specifically. All blood samples taken for plasma ammonia analysis were immediately centrifuged. Following centrifugation, 0.70 ml of plasma was aspirated into a sterile 1-ml tuberculin syringe that was immediately capped and placed into an ice bath. Each sample was analyzed 20-60 minutes later. The ACA method of plasma ammonia analysis has a company-tested reliability range of 0-1000 micromol/l. I determined that the ACA was capable of reliably analysing sample ammonia concentrations in excess of 2000 micromol/l. Since all of the samples were well below this new upper limit, no plasma samples were diluted. Thus, microorganisms had no opportunity to contaminate a plasma sample before its analysis: blood samples were aseptically drawn into heparinized sterile glass syringes; aliquots were placed into capped sterile Falcon tubes for centrifugation; plasma samples were drawn into sterile tuberculin syringes, aseptically sealed and placed into an ice bath; plasma samples were injected into sampling reservoirs for ACA analysis. The plasma concentrations of NH_4 and NH_3, at each time investigated, were calculated from plasma [H] data; plasma ammonia data; and the acid dissociation constant for NH_4 (Ka). The Ka value and equations used are in Table 6-1.

Arterial Blood. All blood samples for acid-base and gas analysis were withdrawn into sterile, heparinized, glass syringes where the syringe and needle dead space was filled with Lipo-Hepin/BL (total dead space volume was about 0.075 ml). If

Table 6-1. TERMS, CONSTANTS AND EQUATIONS USED IN THE CALCULATION OF PLASMA [NH_4] AND [NH_3]

Terms

CA = Concentration of Acid: NH_4
CB = Concentration of Base: NH_3
CT = Concentration Total: [NH_4] + [NH_3]
[H] = Plasma Concentration of Hydrogen Ion at 37°

Constants

Ka = Dissociation Constant of NH_4 at 37°: 1.288×10^{-9}
Kw = Dissociation Constant of H_2O at 37°: 2.388×10^{-14}

General Acid-Base Equation

$$Ka = \frac{[H](CB + [H] - [OH])}{CA - [H] + [OH]} \qquad [OH] = Kw/[H]$$

Plasma Ammonium Equation

$$[NH_4] = \frac{[H](CT + [H]) - Kw + Ka([H] - Kw/[H])}{Ka + [H]}$$

Plasma Ammonia Equation

$$[NH_3] = \frac{Ka(CT + Kw/[H]) + Kw - [H](Ka + [H])}{Ka + [H]}$$

---

small bubbles were observed in the blood after sampling, they were displaced from the syringe before placing the capped sample into an ice bath. Generally, all blood samples remained in an ice bath no longer than 20 minutes before their analysis with a Radiometer ABL2 Laboratory; such a 20-minute wait does not significantly alter pH, pCO_2 or pO_2 values.

Cerebrospinal and Plasma Fluids. Cerebrospinal and plasma fluid samples (0.025 ml) were placed into tared Falcon tubes. They were weighed to the nearest 0.0001 gram and were then diluted 1:200 by the addition of 5 ml of a solution (hereafter called LH) containing 15.0 mM LiCl and 0.02 N HNO_3. After mixing, the diluted samples were set aside for later [K] and [Na] analysis. The sample contents of [K] and [Na] obtained from flame photometry were corrected for sample and extraction-fluid weights. These values, as mmoles of K or Na per kg of fluid, were further corrected for the contributory weight of CSF or plasma proteins; thus, the final CSF and plasma K and Na concentrations are reported as mmoles per kg of water (mmol/kg water). The correction factors used were 0.99 for CSF and 0.92 for plasma; these factors are those previously reported for the percentages of water in the CSF and plasma of adult rats (Johanson et al., 1976).

Choroid Plexuses. Choroid plexus samples were desiccated, at 60°C for 2-4 hours, and weighed on a Cahn electrobalance; weights were recorded to the nearest 0.001 mg. The weighed choroid plexus and its adhering weighing boat were then placed

into a Falcon tube. The extraction of tissue K and Na was commenced with the addition of 2 ml of LH solution. After 12-24 hours of extraction at room temperature with occasional mixing, the tissue-extracts were analyzed for [K] and [Na]. The final tissue K and Na concentrations are reported as mmoles per kg of dry tissue (mmol/kg dry tissue).

Statistics. Statistically significant differences between the results of each treatment and the corresponding control(s) were determined with a one-tailed Student's t-test. Two levels of probability are reported, 0.05 or 0.01. With two-factor linear correlation analyses, the slope of each regression line was analyzed for a significant difference from zero with a one-tailed Student's t-test. For linear regression statistics, the coefficient of determination (r^2) and the slope are presented. For each slope, a significant difference from zero is reported at the 0.05, 0.01, or 0.001, probability levels.

Materials

Grade-1 sucrose and bovine serum albumin were obtained from Sigma Chemical Company (St. Louis, MO). Analytical reagent grade ammonium chloride and sodium chloride were purchased from Mallinckrodt, Inc. (Paris, KY). All stock solutions of ammonium or sodium chloride were prepared with deionized water. Lipo-Hepin/BL, which contained 1000 units of heparin and 3.4 mg Na per ml, was acquired from Riker Laboratories (Northridge, CA). Ketamine hydrochloride, 100 mg/ml, was obtained from Bristol

Laboratories (Syracuse, NY). Disposable 0.01-ml micropipettes were obtained from Dade Diagnostics (Miami, FL). BD Multifit 5-ml glass syringes were acquired from Becton, Dickinson and Company (Rutherford, NJ). Sterile 12 x 75 mm capped polypropylene tubes were procured from Falcon, Incorporated (Oxnard, CA).

Instrumentation

Plasma ammonia levels were analyzed with a Dupont ACA-III Automatic Clinical Analyzer (Wilmington, DE). Brain perfusion was accomplished with the use of a Sage Instruments 375A peristaltic tubing pump (Cambridge, MA). Blood pH, pCO_2, $[HCO_3]$ and pO_2 values were determined with a Radiometer ABL2 Acid-Base Laboratory (Copenhagen, Denmark). Fluid and tissue-extract [K] and [Na] were analyzed with an Instrumentation Laboratories 443 flame photometer (Lexington, MA). Solution osmolality was determined with a Wescor 5100B vapor pressure osmometer (Logan, UT). Choroid plexus tissues were weighed on a Cahn Instruments 4700 automatic electrobalance (Cerritos, CA). Constant bore micropipette insertion tips were manufactured with a David Kopf Instruments 700B vertical pipette puller (Tujunga, CA).

CHAPTER 7

EIGHT-HOUR TIME-COURSE ANALYSES OF THE EFFECTS OF NaCl AND NH_4Cl ON [K] AND [Na] IN CHOROID PLEXUS: RESULTS

Eight-Hour Time-Courses

The effects of ammonium chloride on (1) the concentration of plasma ammonia, (2) pH, pCO_2 and pO_2 in the arterial blood, (3) [K] and [Na] in cerebrospinal fluid (CSF) and plasma, (4) [K] and [Na] in the lateral (LVCP), third (3VCP) and fourth (4VCP) ventricular choroid plexuses (CP's) and (5) blood hematocrit and plasma osmolality, have been investigated. A time-course study was devised whereby the effects of an ammonium chloride injection were temporally compared to the effects of sodium chloride at six times during an eight-hour period: 0.25, 0.5, 1, 2, 4 and 8 hours. Except where noted, all of the effects described below will refer to NH_4Cl-induced increases above or decreases below time-matched control data. The terms baseline and treatment are adjectives which refer to the effects observed after an injection of either NaCl or NH_4Cl during the time-period being investigated, i.e., 0.25-8 hours. Mean-baseline or mean-treatment are adjectives which refer to the average of baseline or treatment data; mean-treatment increase or mean-treatment decrease refers to the difference between mean-baseline and mean-treatment data.

Plasma Ammonia. The baseline ammonia concentration was 33

micromol/l (Figure 7-1). At 15 minutes, the earliest time-point measured, plasma ammonia was maximally elevated to 1168 micromol/l. Plasma ammonia rapidly returned to baseline by 1 hour. As determined by first-order kinetics, the half-life of this elimination was 10.4 minutes. Derived plasma [NH_4] and [NH_3] data are shown in Table 7-1. Approximately 95% of the ammonia in plasma existed in the ionized form, NH_4. The concentrations of NH_4 and NH_3 were both maximal at 15 minutes: respectively, 1135 and 32.7 micromol/l.

Acid-Base and Gas Analyses of Arterial Blood. The time-courses of [H] and [HCO_3] are shown in Figure 7-2. Baseline [H] averaged 36 nmol/l (pH 7.45); treatment [H] was maximally increased to 56 nmol/l at 30 minutes (pH 7.26). Baseline [HCO_3] averaged 20.5 mmol/l; treatment [HCO_3] was maximally depressed to 13.7 mmol/l at 30 minutes. At 8 hours, treatment [H] returned to baseline levels, while [HCO_3] was still 1.4 mmol below control ($p < 0.05$). pH data corresponding to the values in Figure 7-2A, and blood-gas data, are shown in Table 7-2. The pCO_2 did not generally differ from a baseline of 30 torr. pO_2 was elevated about 8 torr at 0.25-2 hours.

Cerebrospinal Fluid and Plasma [K] and [Na]. The time-courses of plasma [K] and [Na] are shown in Figure 7-3. Baseline [K] averaged 4 mmol/l; there was a maximal treatment increase of 1.2 mmol/l at 15 minutes. Baseline [Na] averaged 154 mmol/l; [Na] was maximally decreased 9 mmol/l at 15 minutes.

Figure 7-1. Time-course analysis of the effect of NaCl and NH_4Cl on plasma ammonia.

Arterial blood samples were taken 0.25, 0.5, 1, 2, 4 or 8 hours after an intraperitoneal injection (4.70 mmol/kg) of either NaCl (control) or NH_4Cl (treatment). Values shown are means $\pm$ SEM of data from four to six adult male rats. The term ammonia refers to the sum of the combined concentrations of plasma $[NH_4]$ and $[NH_3]$. Filled squares connected by continuous line are control values. Unfilled squares connected by a dashed line are treatment values. The significance of the induced differences from time-matched controls was determined with a one-tailed Student's t-test: * indicates $p < 0.05$; ** indicates $p < 0.01$.

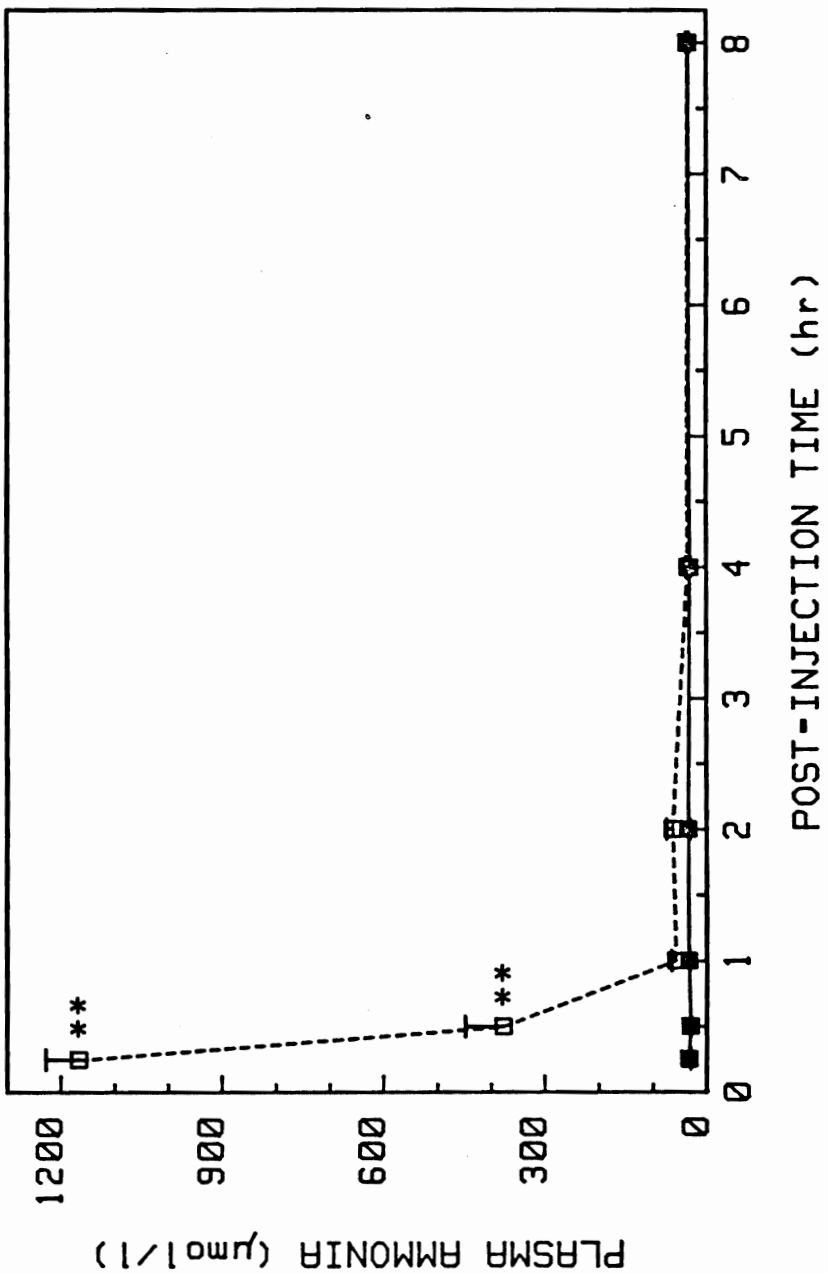

PLASMA AMMONIA (μmol/l)
1200
900
600
300
0
**
**
0
1
2
3
4
5
6
7
8
POST-INJECTION TIME (hr)

Table 7-1. TIME-COURSE ANALYSES OF THE EFFECTS OF NaCl AND NH_4Cl ON $[NH_4]$ AND $[NH_3]$ IN ARTERIAL PLASMA

HOURS:	0.25	0.5	1	2	4	8
			CONTROL			
$[NH_4]$	30.8 ± 3.2	28.5 ± 1.2	31.5 ± 1.6	30.4 ± 2.8	28.7 ± 2.6	33.0 ± 2.4
$[NH_3]$	1.72 ± .11	1.71 ± .10	1.85 ± .08	1.77 ± .13	1.80 ± .07	1.76 ± .06
			TREATMENT			
$[NH_4]$	1135 ± 59**	368 ± 69**	55.3 ± 7.8*	76.2 ± 17*	32.3 ± 3.4	33.8 ± 1.5
$[NH_3]$	32.7 ± 1.9**	9.15 ± 1.8**	2.06 ± .29	2.59 ± .50	1.68 ± .09	1.72 ± .06

$[NH_4]$ and $[NH_3]$ are in micromol/l plasma units. $[NH_4]$ and $[NH_3]$ were derived from plasma [H] data (see Figure 7-2), arterial plasma total ammonia data (see Figure 7-1) and the ammonium dissociation constant (see Table 6-1). Arterial blood samples were taken 0.25, 0.5, 1, 2, 4 or 8 hours after an intraperitoneal injection of either NaCl (control) or NH_4Cl (treatment). Values shown are means ± SEM of data from four to five adult male rats. The significance of induced differences from time-matched controls was determined with a one-tailed Student's t-test: * indicates $p < 0.05$; ** indicates $p < 0.01$.

Figure 7-2. Time-course analyses of the effects of NaCl and NH_4Cl on [H] (A) and [HCO_3] (B) in arterial plasma.

Arterial blood samples were taken 0.25, 0.5, 1, 2, 4 or 8 hours after an intraperitoneal injection (4.70 mmol/kg) of either NaCl (control) or NH_4Cl (treatment). Values shown are means $\pm$ SEM of data from four to six adult male rats. Filled squares connected by continuous line are control values. Unfilled squares connected by a dashed line are treatment values. The significance of the induced differences from time-matched controls was determined with a one-tailed Student's t-test: * indicates $p < 0.05$; ** indicates $p < 0.01$.

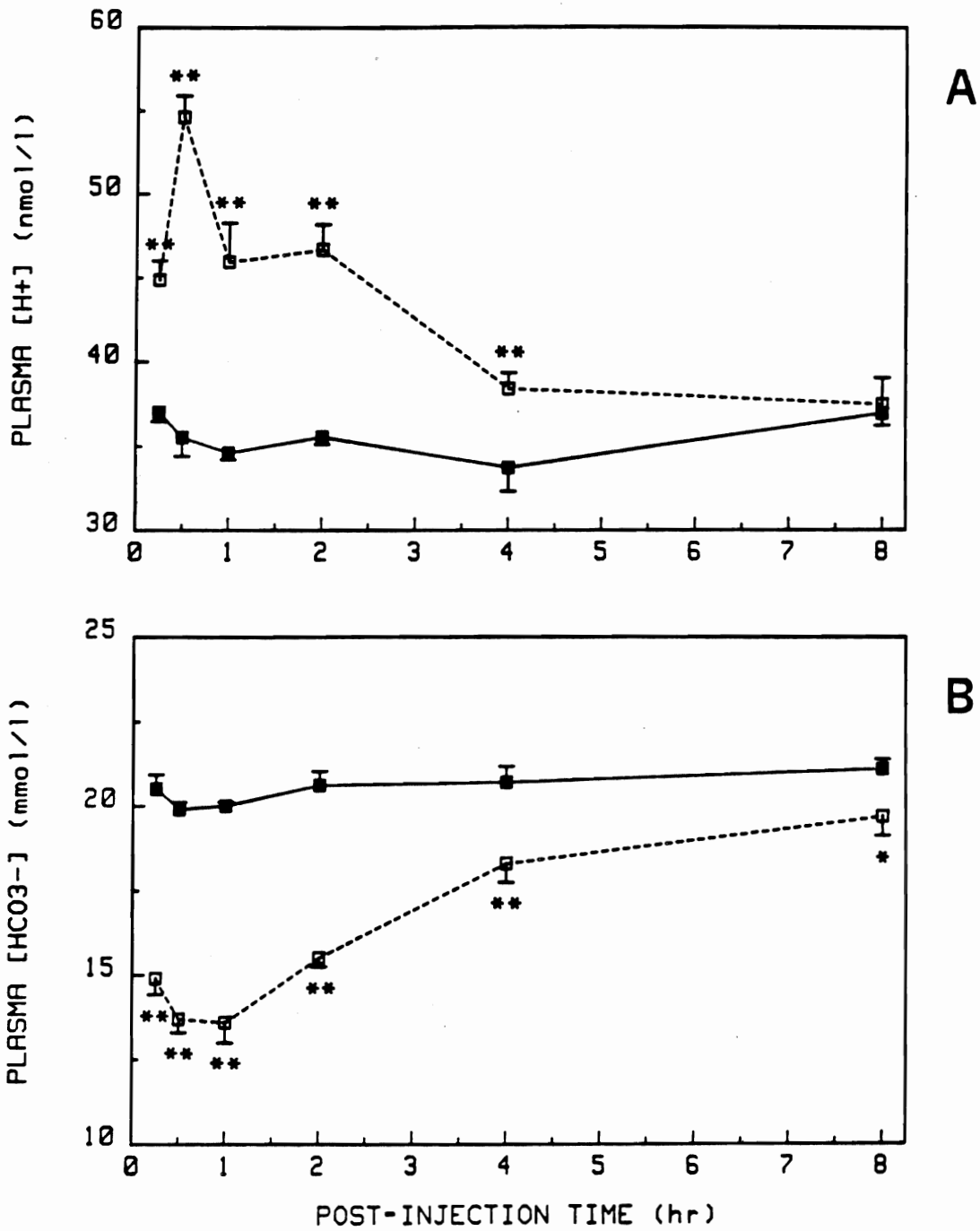

A
B
PLASMA [H+] (nmol/l)
PLASMA [HCO3-] (mmol/l)
POST-INJECTION TIME (hr)
60
50
40
30
25
20
15
10
0
1
2
3
4
5
6
7
8

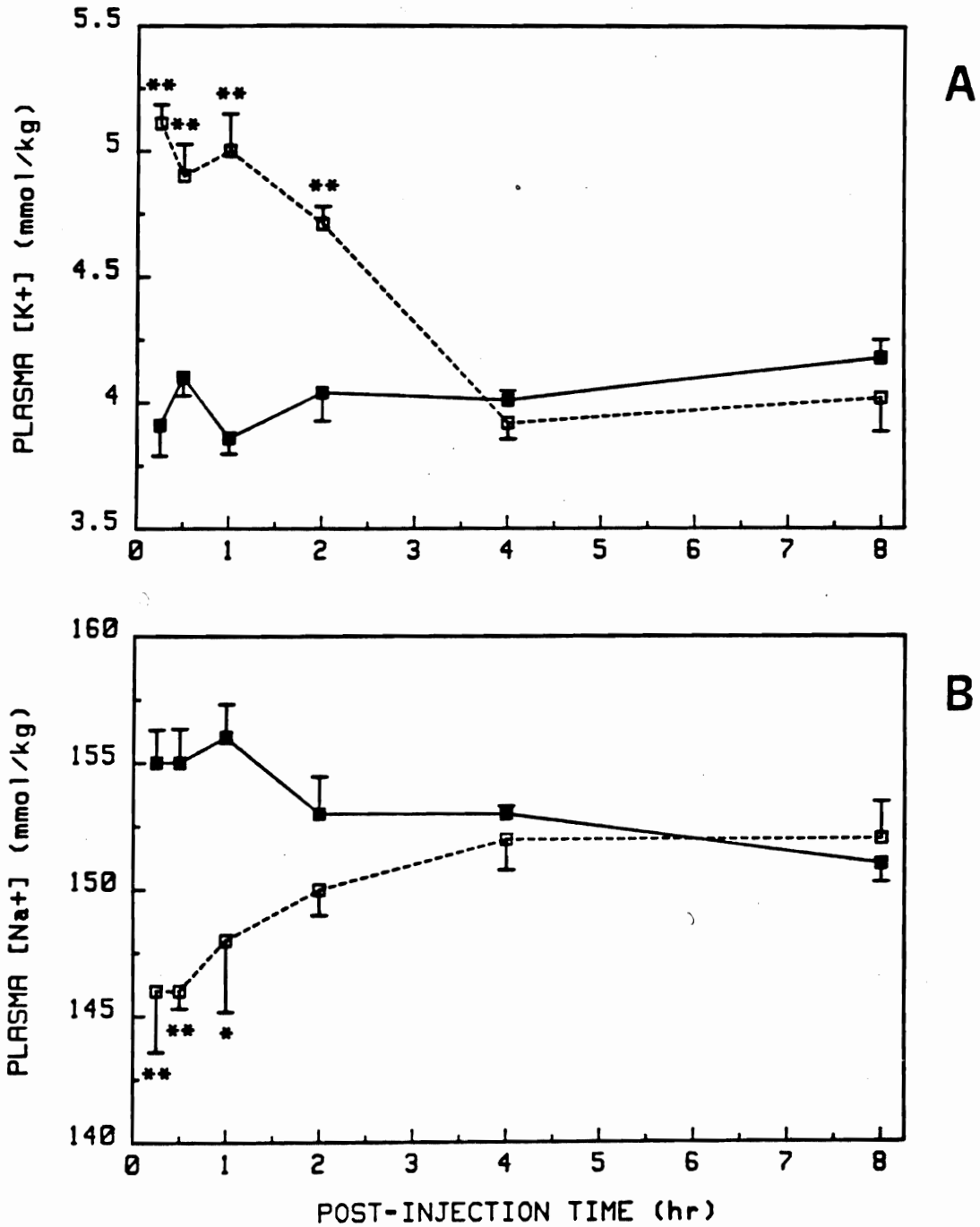
Table 7-2. TIME-COURSES ANALYSES OF THE EFFECTS OF NaCl AND NH_4Cl ON pH, pCO_2 AND pO_2 IN ARTERIAL BLOOD

HOURS:	0.25	0.5	1	2	4	8
			CONTROL			
pH	7.43 ± .01	7.45 ± .01	7.46 ± .01	7.45 ± .01	7.47 ± .02	7.43 ± .01
pCO_2	31.2 ± .46	28.9 ± .86	28.3 ± .21	30.0 ± .71	28.7 ± 1.8	32.1 ± .55
pO_2	74.3 ± 1.7	74.3 ± 2.8	74.1 ± 1.5	69.9 ± 1.8	75.0 ± 3.6	74.9 ± 1.2
			TREATMENT			
pH	7.35 ± .01**	7.26 ± .01**	7.34 ± .02**	7.33 ± .01**	7.42 ± .01*	7.43 ± .02
pCO_2	27.7 ± .74**	31.2 ± .76*	26.0 ± 2.5	29.9 ± .61	28.9 ± .68	30.3 ± 1.0
pO_2	82.8 ± 2.7*	81.4 ± 1.9*	82.7 ± 5.2	77.5 ± 1.8**	76.3 ± 2.2	73.9 ± 1.1

Blood gases are in torr units. Arterial blood samples were taken 0.25, 0.5, 1, 2, 4 or 8 hours after an injection (4.70 mmol/kg) of either NaCl (control) or NH_4Cl (treatment). Values shown are means ± SEM of data from four to five adult male rats. The significance of the induced differences from time-matched controls was determined with a one-tailed Student's t-test: * indicates $p < 0.05$; ** indicates $p < 0.01$.

Figure 7-3. Time-course analyses of the effects of NaCl and NH_4Cl on [K] (A) and [Na] (B) in arterial plasma.

Arterial blood samples were taken 0.25, 0.5, 1, 2, 4 or 8 hours after an intraperitoneal injection (4.70 mmol/kg) of either NaCl (control) or NH_4Cl (treatment). Values shown are means $\pm$ SEM of data from four to six adult male rats. Filled squares connected by continuous line are control values. Unfilled squares connected by a dashed line are treatment values. The significance of the induced differences from time-matched controls was determined with a one-tailed Student's t-test: * indicates $p < 0.05$; ** indicates $p < 0.01$.


A
B
PLASMA [K+] (mmol/kg)
PLASMA [Na+] (mmol/kg)
POST-INJECTION TIME (hr)
5.5
5
4.5
4
3.5
160
155
150
145
140
0
1
2
3
4
5
6
7
8

Unlike the alterations of plasma [H] and [HCO_3], the alterations of plasma [K] and [Na] returned to baseline by 4 hours. The effects of NH_4Cl on CSF and plasma [K], and on the CSF/plasma ratio of [K], are shown in Table 7-3. Likewise, the effects of NH_4Cl on CSF and plasma [Na], and on the CSF/plasma ratio of [Na] are shown in Table 7-4. Although [K] and [Na] in the CSF were not significantly altered, the CSF/plasma ratio of K was significantly decreased during the first 2 hours of acidosis. Additionally, there were significant increases in the CSF/plasma ratio of Na during the first 60 minutes investigated.

Changes in plasma [H] were correlated with plasma [HCO_3], plasma [K] and plasma [Na] by linear regression. As expected, plasma [HCO_3] decreased and plasma [K] increased with the augmentation of plasma [H]; plasma [Na] decreased with increases in plasma [H] (Figures 7-4A,4B,4C).

Blood Hematocrit and Plasma Osmolality. Table 7-5 summarizes plasma osmolality and blood hematocrit (HCT) data. NH_4Cl did not alter plasma osmolality in either the baseline or treatment groups: both groups averaged 289 mOsm/kg. Baseline HCT was 37.5%; the HCT remained fairly stable in both groups at all times except 30 minutes where NH_4Cl caused a significant increase from 38.6 to 42.4% ($p < 0.01$).

Choroid Plexus [K] and [Na]. The time-courses of [K] and [Na] (mmol/kg dry tissue) in LVCP, 3VCP and 4VCP are shown in Figures 7-5 and 7-6. The K and Na concentrations found in these

Table 7-3. TIME-COURSE ANALYSES OF THE EFFECT OF NaCl AND NH_4Cl ON [K] IN CEREBROSPINAL FLUID AND ARTERIAL PLASMA

HOURS:	0.25	0.5	1	2	4	8
			CONTROL			
CSF	3.2 ± .02	3.1 ± .02	3.1 ± .02	3.0 ± .05	3.1 ± .04	3.0 ± .02
PLASMA	3.9 ± .12	4.1 ± .07	3.9 ± .06	4.0 ± .11	4.0 ± .04	4.2 ± .07
RATIO	.81 ± .03	.75 ± .01	.81 ± .02	.74 ± .04	.77 ± .02	.73 ± .01
			TREATMENT			
CSF	3.0 ± .04	3.1 ± .13	3.1 ± .03	3.1 ± .03	3.0 ± .10	3.1 ± .06
PLASMA	5.1 ± .07	4.9 ± .13	5.0 ± .15	4.7 ± .07	3.9 ± .07	4.0 ± .13
RATIO	.60 ± .01**	.64 ± .01**	.63 ± .02**	.65 ± .01*	.78 ± .01	.76 ± .01

[K] is in mmol/kg water units. Arterial blood and cerebrospinal fluid (CSF) samples were taken 0.25, 0.5, 1, 2, 4 or 8 hours after an intraperitoneal injection of either 4.70 mmol/kg NaCl (control) or NH_4Cl (treatment). Values shown are means ± SEM of data from three adult male rats. The significance of induced differences from time-matched controls was determined with a one-tailed Student's t-test: * indicates $p < 0.05$; ** indicates $p < 0.01$.

Table 7-4. TIME-COURSE ANALYSES OF THE EFFECT OF NaCl AND NH_4Cl ON [Na] IN CEREBROSPINAL FLUID AND ARTERIAL PLASMA

HOURS:	0.25	0.5	1	2	4	8
			CONTROL			
CSF	158 ± 1.2	156 ± 2.4	156 ± 1.2	154 ± .89	154 ± 1.9	153 ± .59
PLASMA	155 ± 1.3	155 ± 1.3	156 ± 1.3	153 ± 1.5	153 ± .31	151 ± .74
RATIO	1.02 ± .01	1.01 ± .01	1.00 ± .01	.99 ± .01	1.01 ± .01	1.02 ± .01
			TREATMENT			
CSF	157 ± 2.5	154 ± 1.5	153 ± 1.7	153 ± .89	155 ± .50	152 ± .87
PLASMA	146 ± 2.4	146 ± .70	148 ± 2.8	150 ± 1.0	152 ± 1.2	152 ± 1.5
RATIO	1.07 ± .01*	1.06 ± .01**	1.03 ± .01*	1.02 ± .01	1.01 ± .01	1.01 ± .01

[Na] is in mmol/kg water units. Arterial blood and cerebrospinal fluid (CSF) samples were taken 0.25, 0.5, 1, 2, 4 or 8 hours after an intraperitoneal injection of either 4.70 mmol/kg NaCl (control) or NH_4Cl (treatment). Values shown are means ± SEM of data from three adult male rats. The significance of induced differences from time-matched controls was determined with a one-tailed Student's t-test: * indicates $p < 0.05$; ** indicates $p < 0.01$.

Figure 7-4. Correlation analyses of the effects of NH_4Cl on $[HCO_3]$ (A), [K] (B) or [Na] (C) in plasma, with the effect of NH_4Cl on plasma [H].

Arterial blood samples were taken 0.25, 0.5, 1, 2, 4 or 8 hours after an intraperitoneal injection of NH_4Cl (4.70 mmol/kg). The probability that the slope of each fitted line was statistically different from zero was determined with a one-tailed Student's t-test. The linear regression coefficient of determination, slope, sample population and probability, are listed respectively: (A) $r^2 = 0.659$, $m = -0.310 \pm 0.045$, $n = 27$, $p < 0.001$; (B) $r^2 = 0.516$, $m = 0.056 \pm 0.011$, $n = 25$, $p < 0.001$; (C) $r^2 = 0.309$, $m = -0.323 \pm 0.101$, $n = 25$, $p < 0.01$.

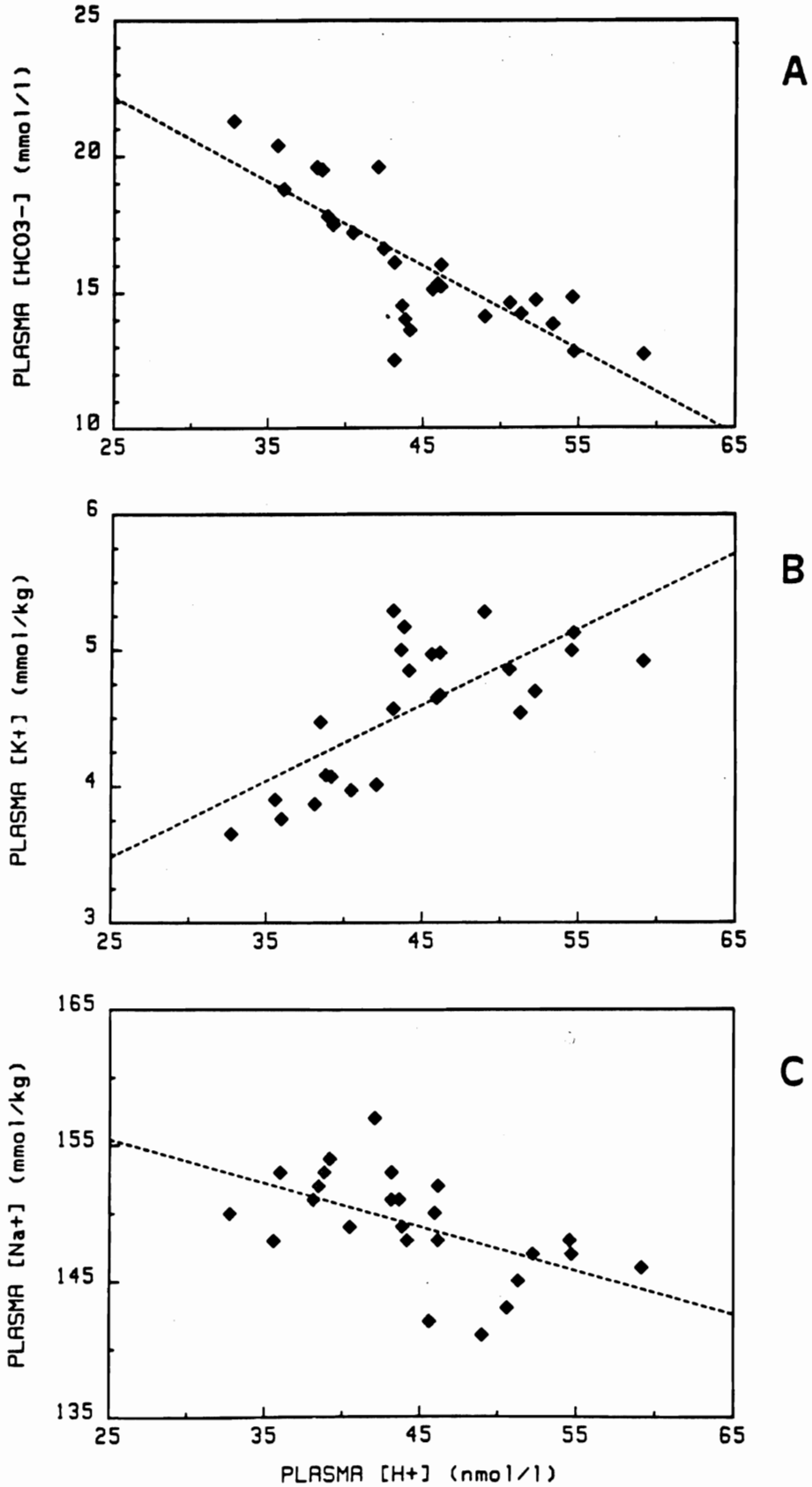

A
PLASMA [HCO3-] (mmol/l)
25
20
15
10
25
35
45
55
65
B
PLASMA [K+] (mmol/kg)
6
5
4
3
25
35
45
55
65
C
PLASMA [Na+] (mmol/kg)
165
155
145
135
25
35
45
55
65
PLASMA [H+] (nmol/l)

Table 7-5. TIME-COURSE ANALYSES OF THE EFFECTS OF NaCl AND NH_4Cl ON HCT AND OSMOLALITY OF ARTERIAL BLOOD

HOURS:	0.25	0.5	1	2	4	8
			CONTROL			
HCT	38.0 ± 1.1	38.6 ± 0.4	36.7 ± 1.2	37.2 ± 1.0	37.0 ± 0.7	37.8 ± 1.0
mOsm/kg	292 ± 1.2	292 ± 1.3	292 ± 1.9	287 ± 0.8	290 ± 2.6	285 ± 1.6
			TREATMENT			
HCT	38.6 ± 1.0	42.4 ± 1.0**	39.0 ± 2.1	37.6 ± 0.9	38.3 ± 1.8	38.4 ± 0.6
mOsm/kg	292 ± 1.5	291 ± 1.3	288 ± 1.4	288 ± 1.4	288 ± 0.3	288 ± 0.4

HCT refers to the hematocrit. Arterial blood samples were taken 0.25, 0.5, 1, 2, 4 or 8 hours after an intraperitoneal injection of either 4.70 mmol/kg NaCl (control) or NH_4Cl (treatment). Values shown are means ± SEM of data from four to five adult male rats. The significance of the induced differences from time-matched controls was determined with a one-tailed Student's t-test: * indicates $p < 0.05$; ** indicates $p < 0.01$.

Figure 7-5. Time-course analyses of the effect of NaCl and NH_4Cl on [K] in LVCP (A), 3VCP (B) and 4VCP (C).

Lateral (LVCP), third (3VCP) and fourth (4VCP) ventricular choroid plexus samples were taken 0.25, 0.5, 1, 2, 4 or 8 hours after an intraperitoneal injection (4.70 mmol/kg) of either NaCl (control) or NH_4Cl (treatment). Values shown are means $\pm$ SEM of data from four to six adult male rats. Filled squares connected by continuous line are control values. Unfilled squares connected by a dashed line are treatment values. The significance of the induced differences from time-matched controls was determined with a one-tailed Student's t-test: * indicates $p < 0.05$; ** indicates $p < 0.01$.

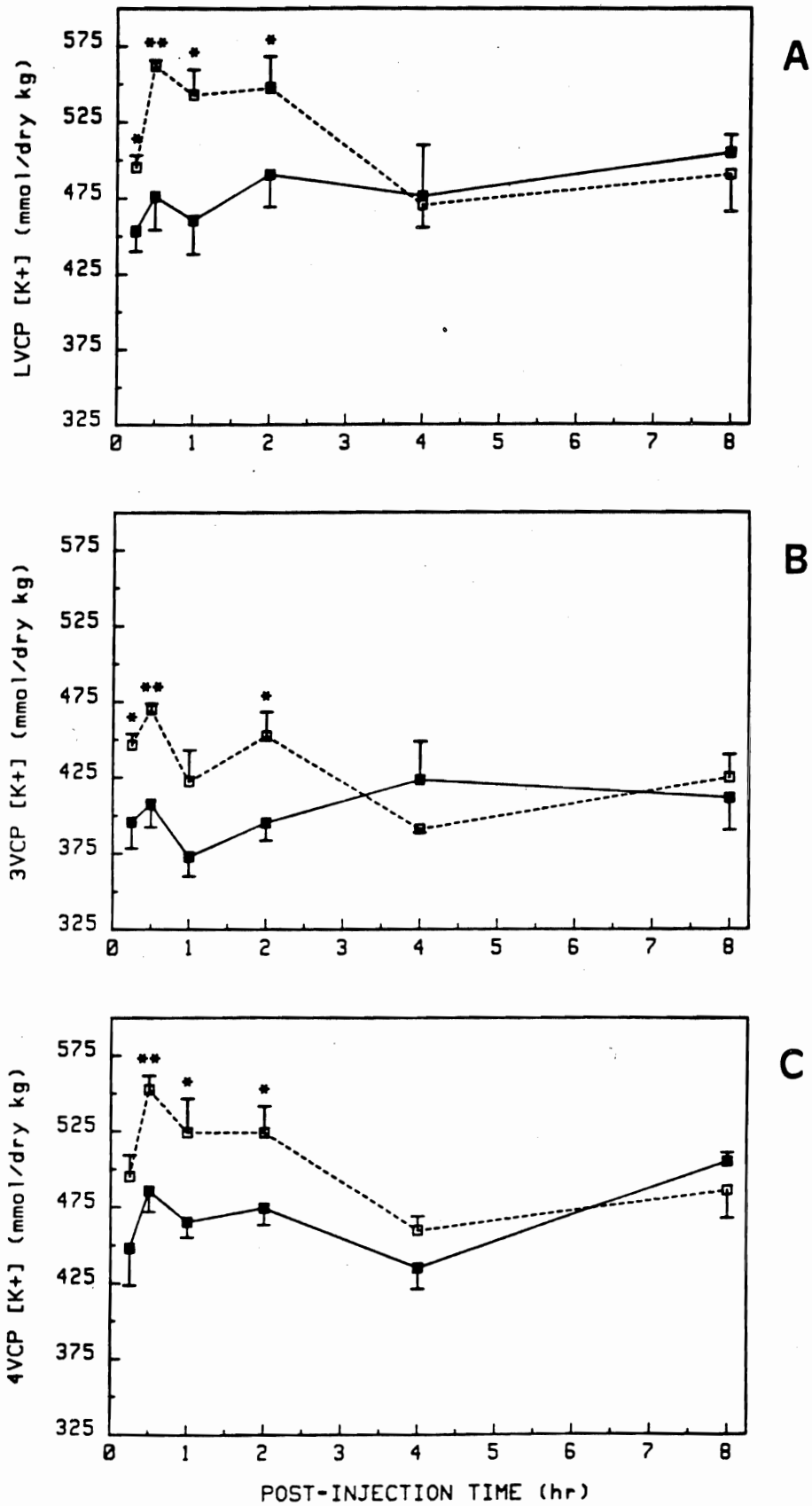
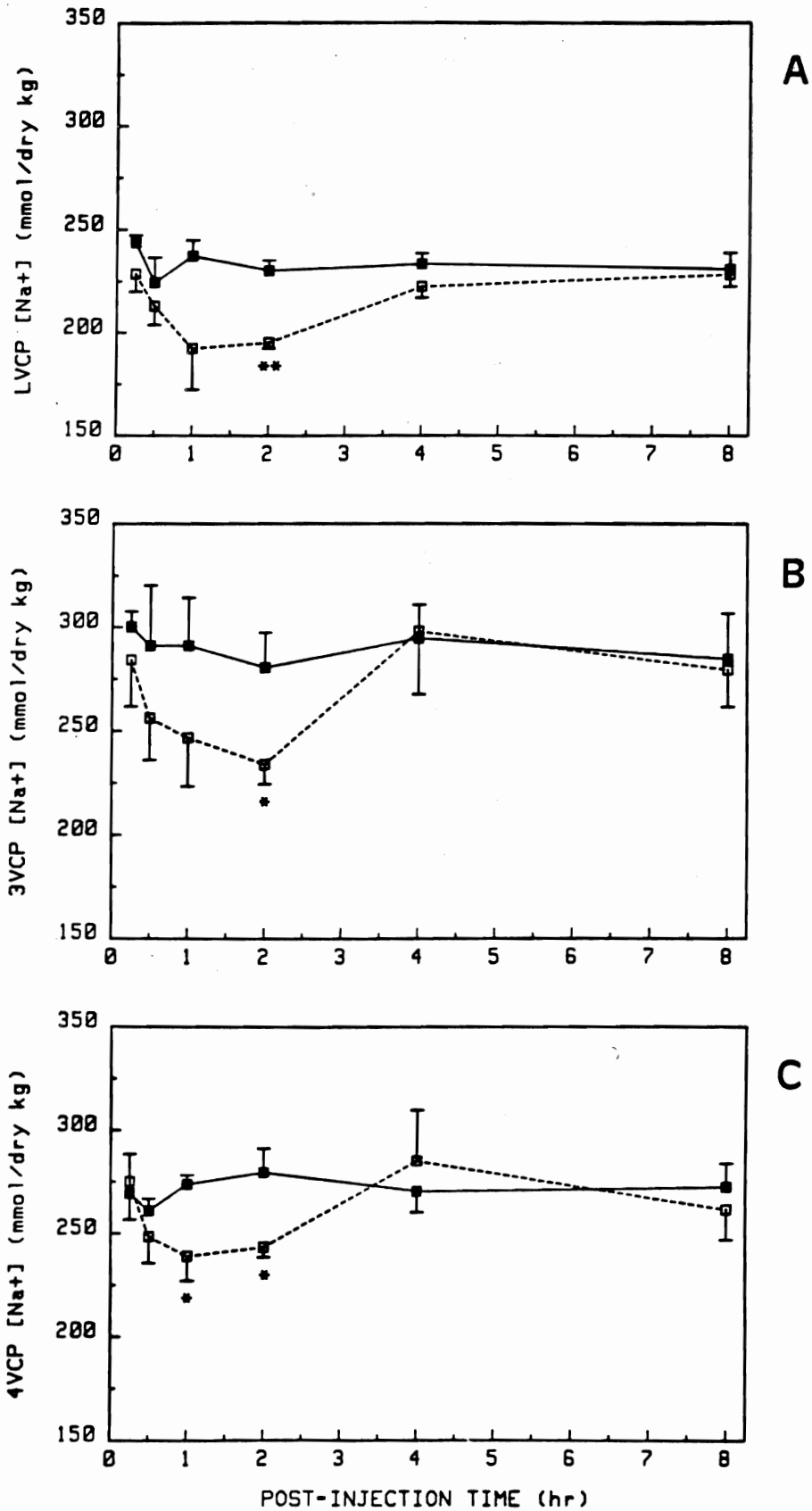

A
B
C
LVCP [K+] (mmol/dry kg)
3VCP [K+] (mmol/dry kg)
4VCP [K+] (mmol/dry kg)
575
525
475
425
375
325
0
1
2
3
4
5
6
7
8
POST-INJECTION TIME (hr)

Figure 7-6. Time-course analyses of the effect of NaCl and NH_4Cl on [Na] in LVCP (A), 3VCP (B) and 4VCP (C).

Lateral (LVCP), third (3VCP) and fourth (4VCP) ventricular choroid plexus samples were taken 0.25, 0.5, 1, 2, 4 or 8 hours after an intraperitoneal injection (4.70 mmol/kg) of either NaCl (control) or NH_4Cl (treatment). Values shown are means $\pm$ SEM of data from four to six adult male rats. Filled squares connected by continuous line are control values. Unfilled squares connected by a dashed line are treatment values. The significance of the induced differences from time-matched controls was determined with a one-tailed Student's t-test: * indicates $p < 0.05$; ** indicates $p < 0.01$.


A
B
C
LVCP [Na+] (mmol/dry kg)
3VCP [Na+] (mmol/dry kg)
4VCP [Na+] (mmol/dry kg)
350
300
250
200
150
0
1
2
3
4
5
6
7
8
**
*
*
*
POST-INJECTION TIME (hr)

tissues are proportional to the concentrations of K and Na in the tissue parenchymal cells. Figures 7-5A,5B,5C, respectively show the induced elevations of [K] in LVCP, 3VCP and 4VCP. [K] was maximally increased by 86 mmol/kg at 30 minutes in the LVCP. In the 3VCP and 4VCP, the maximal increases in [K], also at 30 minutes, were both about 67 mmol/kg. Figures 7-6A,6B,6C show the induced decreases in [Na] below baseline. A maximal decrease in [Na], in LVCP, 3VCP and 4VCP, of about 43 mmol/dry kg occurred at 1 hour. Note that in all three CP's the peak increase in [K] occurred at 30 minutes, whereas, the greatest reduction in [Na] occurred around 60 minutes.

There were differences in the baseline concentrations of CP [K] and [Na] (Figures 7-5 and 7-6). Baseline [K] (mmol/kg dry tissue) differed for each CP with the order [476 (LVCP)] $\geq$ [470 (4VCP)] > [402 (3VCP)]. LVCP had the greatest concentration of K; 3VCP had the least. Baseline [Na] (mmol/kg dry tissue) also differed for each CP: [232 (LVCP)] < [271 (4VCP)] < [288 (3VCP)]. Note that 3VCP had the greatest [Na]; LVCP had the least.

Figures 7-7A,7B,7C summarize the treatment-induced changes in [K] and [Na] from time-matched controls for all three plexus tissues shown in Figures 7-5 and 7-6. All treatment- induced changes in [K] and [Na] returned to control by 4 hours.

Plexus [K] was correlated with plasma [H] in Figure 7-8. Treatment increases in LVCP, 3VCP and 4VCP [K] correlated significantly with treatment increases in plasma [H] (Figure

Figure 7-7. Time-course analyses of the changes induced from time-matched controls by NH_4Cl on [K] and [Na] in LVCP (A), 3VCP (B) and 4VCP (C).

Lateral (LVCP), third (3VCP) and fourth (4VCP) ventricular choroid plexus samples were taken 0.25, 0.5, 1, 2, 4 or 8 hours after an intraperitoneal injection (4.70 mmol/kg) of either NaCl (control) or NH_4Cl (treatment). Values shown are means $\pm$ SEM of data from four to six adult male rats. Filled diamonds represent changes in tissue [K]. Filled squares represent changes in tissue [Na]. The significance of the induced differences from time-matched controls was determined with a one-tailed Student's t-test: * indicates $p < 0.05$; ** indicates $p < 0.01$.

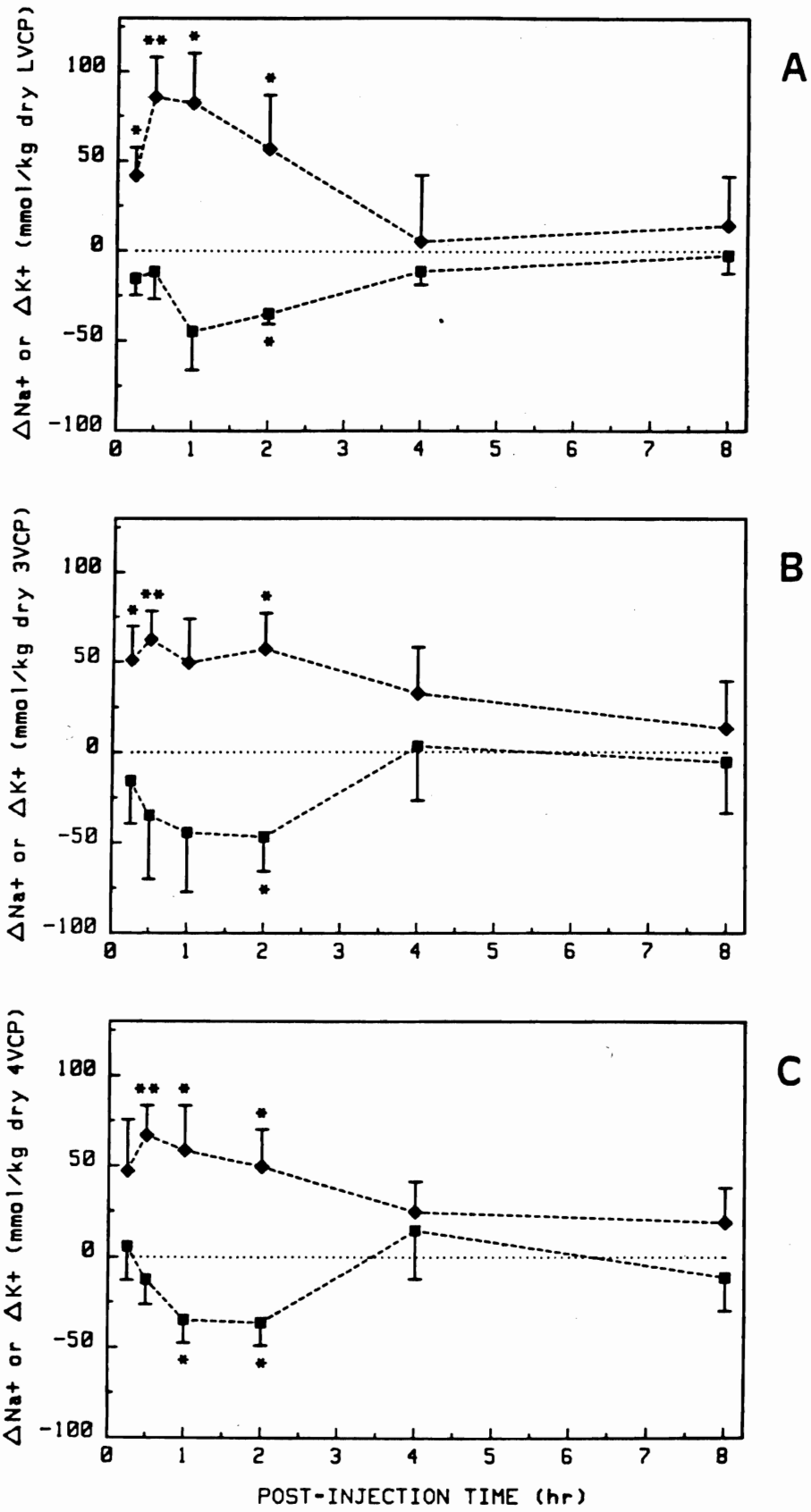
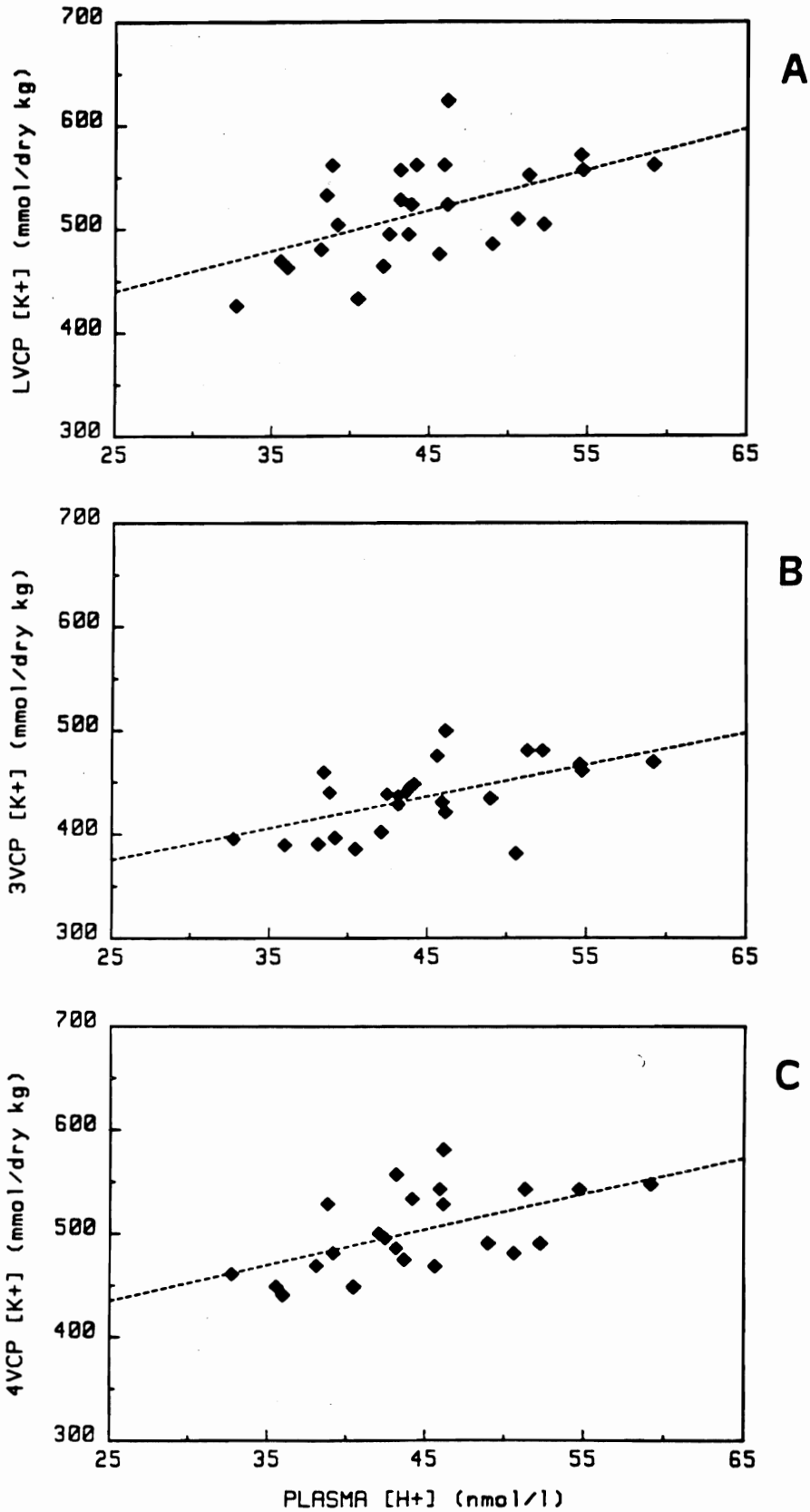

A
B
C
ΔNa+ or ΔK+ (mmol/kg dry LVCP)
ΔNa+ or ΔK+ (mmol/kg dry 3VCP)
ΔNa+ or ΔK+ (mmol/kg dry 4VCP)
100
50
0
-50
-100
0
1
2
3
4
5
6
7
8
POST-INJECTION TIME (hr)

Figure 7-8. Correlation analyses of the effect of NH_4Cl on [K] in LVCP (A), 3VCP (B) or 4VCP (C), with the effect of NH_4Cl on plasma [H].

Arterial blood, and lateral (LVCP), third (3VCP) and fourth (4VCP) ventricular choroid plexus samples were taken 0.25, 0.5, 1, 2, 4 or 8 hours after an intraperitoneal injection (4.70 mmol/kg) of NH_4Cl (treatment). The probability that the slope of each fitted line was statistically different from zero was determined with a one-tailed Student's t-test. The linear regression coefficient of determination, slope, sample population and probability, are listed respectively: (A) $r^2 = 0.288$, $m = 3.91 \pm 1.25$, $n = 26$, $p < 0.01$; (B) $r^2 = 0.330$, $m = 3.06 \pm 0.911$, $n = 25$, $p < 0.01$; (C) $r^2 = 0.314$, $m = 3.44 \pm 1.11$, $n = 23$, $p < 0.01$.


A
B
C
LVCP [K+] (mmol/dry kg)
3VCP [K+] (mmol/dry kg)
4VCP [K+] (mmol/dry kg)
700
600
500
400
300
25
35
45
55
65
PLASMA [H+] (nmol/l)

7-8A,8B,8C). LVCP and 3VCP [Na] correlated negatively with treatment increases in plasma [H] ($p < 0.05$). Changes in CP [K] and [Na] were also tested for correlation with treatment-induced changes in plasma [HCO_3] and [K]. Significant negative correlations are shown between CP [K] and plasma [HCO_3] (Figures 7-9A,9B,9C). With the exception of LVCP [Na] ($p < 0.05$), there were no significant correlations between CP [Na] and plasma [HCO_3]. Significant positive correlations were shown between CP [K] and plasma [K] (Figures 7-10A,10B,10C). There were no significant correlations between CP [Na] and plasma [K].

Figure 7-9. Correlation analyses of the effect of NH_4Cl on [K] in LVCP (A), 3VCP (B) or 4VCP (C), with the effect of NH_4Cl on plasma $[HCO_3]$.

Arterial blood, and lateral (LVCP), third (3VCP) and fourth (4VCP) ventricular choroid plexus samples were taken 0.25, 0.5, 1, 2, 4 or 8 hours after an intraperitoneal injection (4.70 mmol/kg) of NH_4Cl (treatment). The probability that the slope of each fitted line was statistically different from zero was determined with a one-tailed Student's t-test. The linear regression coefficient of determination, slope, sample population and probability are listed respectively: (A) $r^2 = 0.324$, $m = -10.6 \pm 3.14$, $n = 26$, $p < 0.01$; (B) $r^2 = 0.269$, $m = -7.28 \pm 2.50$, $n = 25$, $p < 0.01$; (C) $r^2 = 0.367$, $m = -9.44 \pm 2.70$, $n = 23$, $p < 0.01$.

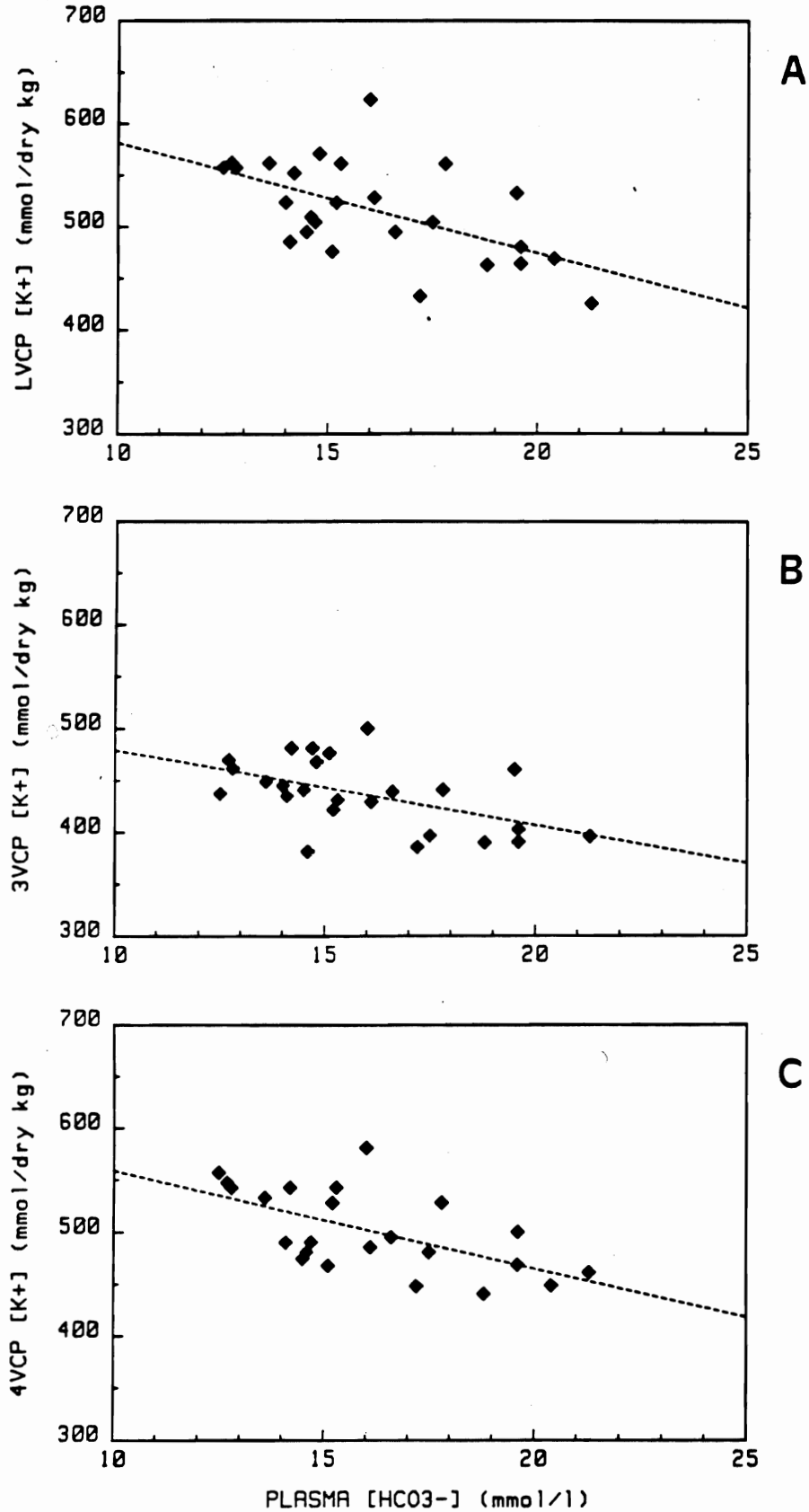
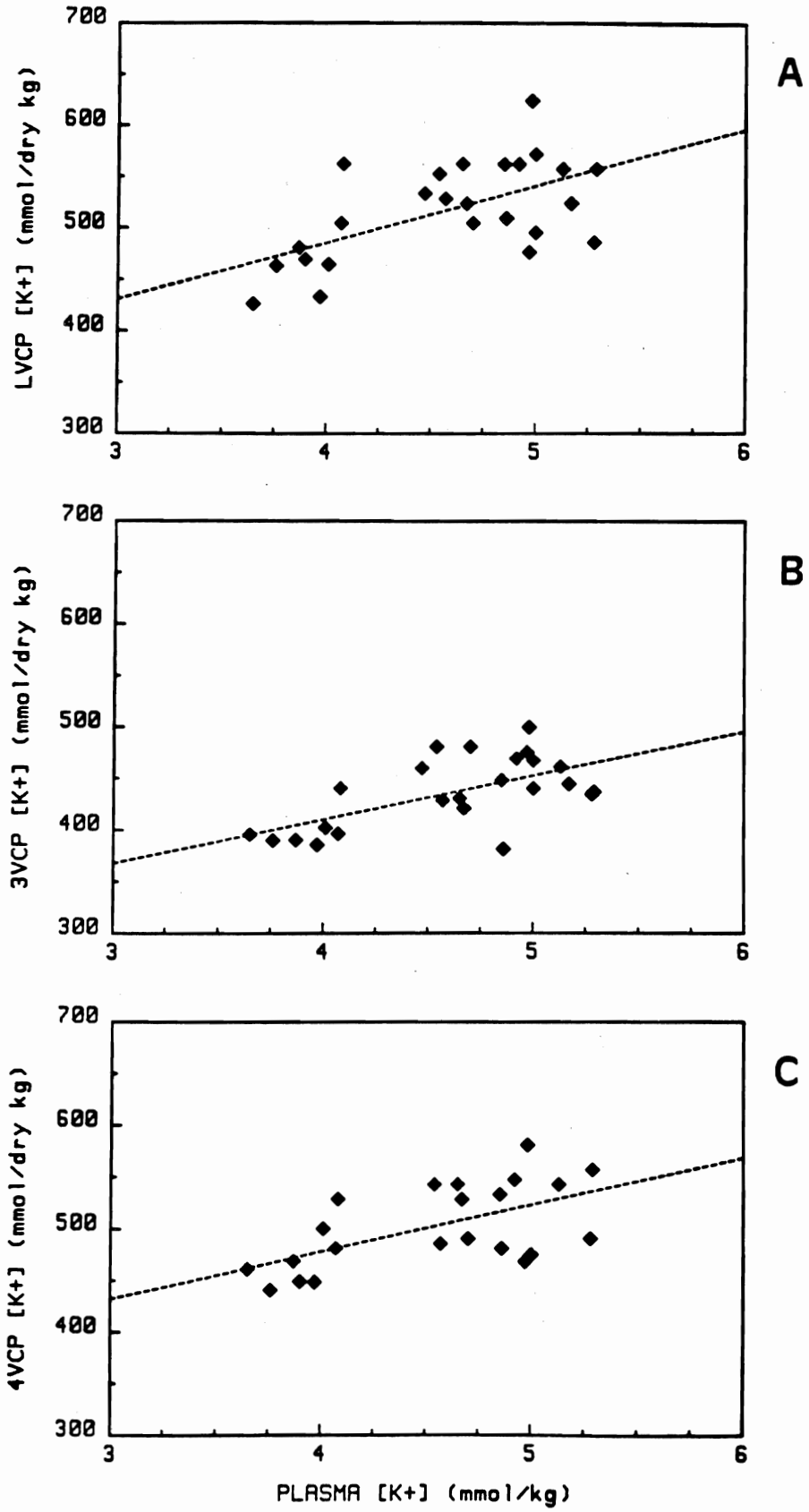

A
LVCP [K+] (mmol/dry kg)
700
600
500
400
300
10
15
20
25
B
3VCP [K+] (mmol/dry kg)
700
600
500
400
300
10
15
20
25
C
4VCP [K+] (mmol/dry kg)
700
600
500
400
300
10
15
20
25
PLASMA [HCO3-] (mmol/l)

Figure 7-10. Correlation analyses of the effect of NH_4Cl on [K] in LVCP (A), 3VCP (B) or 4VCP (C), with the effect of NH_4Cl on plasma [K].

Arterial blood, and lateral (LVCP), third (3VCP) and fourth (4VCP) ventricular choroid plexus samples were taken 0.25, 0.5, 1, 2, 4 or 8 hours after an intraperitoneal injection (4.70 mmol/kg) of NH_4Cl (treatment). The probability that the slope of each fitted line was statistically different from zero was determined with a one-tailed Student's t-test. The linear regression coefficient of determination, slope, sample population and probability are listed respectively: (A) $r^2 = 0.347$, $m = 55.1 \pm 15.7$, $n = 25$, $p < 0.001$; (B) $r^2 = 0.385$, $m = 42.6 \pm 11.5$, $n = 24$, $p < 0.001$; (C) $r^2 = 0.345$, $m = 45.5 \pm 14.0$, $n = 22$, $p < 0.01$.


A
B
C
LVCP [K+] (mmol/dry kg)
3VCP [K+] (mmol/dry kg)
4VCP [K+] (mmol/dry kg)
PLASMA [K+] (mmol/kg)
700
600
500
400
300
3
4
5
6

CHAPTER 8

COMPARISON OF THE EFFECTS OF NaCl, NH_4Cl AND OTHER SALTS ON [K] AND [Na] IN CHOROID PLEXUS; AND THE EFFECT OF ACIDOSIS ON THE UPTAKE OF ^{86}Rb INTO IN VITRO CHOROID PLEXUS: METHODS

In Vivo Experimental Procedures

Fifty-four 6-8 week-old male Sprague-Dawley rats (190-310 grams) were used in the studies described below. Prior to each experiment, the animals were housed in cages wherein they had free access to food (Purina rat chow) and water, and were regularly exposed to alternating 12-hour periods of darkness or overhead fluorescent lighting. For the experiment, rats were separated into nine groups: controls received sodium chloride; the animals in the eight treatment groups received either lactic acid (HL), sodium lactate (SL), sodium bicarbonate (SB), potassium bicarbonate (KB), ammonium bicarbonate (AB), sodium chloride (SC), potassium chloride (KC), ammonium chloride (AC) or hydrochloric acid (HC). All doses were injected intraperitoneally (4.70 mmol/kg). During the experiment, rats had continued free access to food and water in cages receiving room-light. One hour after each rat was injected, blood and choroid plexus sampling were begun. Five minutes before fluid sampling and tissue removal was begun, each animal was intraperitoneally injected with 80 mg/kg of ketamine hydrochloride. This anesthetic dose of ketamine, which

did not change the animal's rate of respiration, required 5 minutes to effect sedation. Each rat was killed by exsanguination after the blood sample was taken. The experimental procedures used in the sampling, analysis and calculation of blood pH, pCO_2 and pO_2, and cerebrospinal fluid, plasma and choroid plexus [K] and [Na] have been been described in Chapter 4. The choroid plexus samples analyzed had their residual blood removed with a brain-blood washout technique described earlier (Chapter 6).

In Vitro Experimental Procedures

Twenty-eight adult rats (150-280 grams) were separately anesthetized with an intraperitoneal injection of 100 mg/kg ketamine hydrochloride. Five minutes after the anesthetic was administered, each rat was killed by exsanguination. After the brain was quickly removed from the cranial cavity, each of the two lateral ventricular choroid plexuses (LVCP's) was separately removed and transferred into a preincubation tube containing 3 ml of simulated cerebrospinal fluid (CSF) for 20 minutes. The simulated CSF was maintained at 37°C and continuously equilibrated with humidified 95% O_2 and 5% CO_2. The gas-equilibration system is described as follows: Gas was bubbled through an aeration stone placed under 500-1000 ml of deionized water; this humidified air was equally distributed into 10 similar lengths of flexible Tygon tubing terminally connected to 10 micropipette couplers; each coupler was attached to 0.005-ml micropipettes of a length that was shortened so that the tip would remain 1 ml above the

bottom of the incubation tube and 2 ml below the surface of the incubation medium. The gas was gently (very fine bubbles), yet rapidly, bubbled into all tubes. Plexus samples were placed into gassed tubes by first removing the aeration micropipette, then placing the plexus into the medium, allowing time for the plexus to settle to the bottom of the tube, and then, replacing the aeration micropipette. Care was taken throughout the experiment to confirm that all plexuses were not agitated by the gentle currents generated by the bubbling process.

Incubation Media. A stock solution of simulated CSF, with a pH of 7.3 (Table 8-1), was divided into two aliquots. Each aliquot under the conditions of vigorous aeration and a temperature of 37°C was separately adjusted with 1 M HCl or 1 M NaOH, in order to establish one of two incubation conditions: acidosis (pH 7.00) or control (pH 7.40). Any differences in the osmolalities of the two CSF solutions, introduced by the addition of HCl or NaOH, were balanced by appropriate addition of 1 M NaCl.

An analysis of [K] and [Na] in the simulated CSF revealed that the concentrations of K and Na were the same in control and acidotic media; respectively, 2.85 and 150 mmol/l. Ionized [Ca] varied slightly with the pH of the medium; 1.15 (pH 7.0) and 1.04 mmol/l (pH 7.4).

Effect of CSF pH on the Uptake of ^{86}Rb into Lateral Ventricular Choroid Plexus. After the above 20-minute preincubation period, with identical conditions, each plexus was transferred

Table 8-1. COMPOSITION OF SIMULATED CEREBROSPINAL FLUID

INGREDIENTS	CONCENTRATION (mmol/l)
NaCl	117.00
KCl	2.96
$NaHCO_3$	18.00
$CaCl_2 \cdot 2H_2O$	1.50
$NaH_2PO_4 \cdot H_2O$	0.125
Na_2HPO_4	0.50
$MgCl_2 \cdot 6H_2O$	0.80
Urea	2.00
Lactic Acid	2.11
Citric Acid $\cdot H_2O$	0.20
Dextrose	12.10
Na_2SO_4	2.35

A solution of simulated cerebrospinal fluid (CSF) with the above ingredients has a pH of 7.3 when bubbled with humidified 95% O_2 and 5% CO_2 at 37°C. In order to achieve the desired control (pH 7.40) and acidotic (pH 7.10) media used in the experiments described in the text, two aliquots of the above solution were pH-adjusted, respectively under the same conditions, with either NaOH or HCl (1 M). The concentrations of Na in both aliquots were balanced osmotically with the addition of 1 M NaCl.

into another tube of simulated CSF containing ^{86}Rb (2 microCi/ml). After the transfer, each plexus was incubated for 0.25, 0.5, 1, 2, 4, 8, 16, or 32 minutes. After the incubation with ^{86}Rb, the plexus was quickly removed and blotted by drawing it along a standardized length of dry plate glass. Dried lateral ventricular choroid plexus samples, and their adhering aluminum foil weighing boats, were placed into 2 ml of a solution containing 15.0 mM LiCl and 0.02 N HNO_3. After 24 hours of extraction at room temperature, with occasional mixing, samples were analyzed for gamma emissions with a Beckman BioGamma II gamma radiation counter. Subsequently, all samples were analyzed for [K] and [Na] as described in Chapter 2.

Extracellular Fluid Volume. In this in vitro experiment, the extracellular fluid (ECF) volume of the lateral ventricular choroid plexus (LVCP) was determined from the steady-state volume of distribution of ^{3}H-raffinose. After 20 minutes of preincubation (above) in either control or acidotic CSF, each plexus was transferred into a corresponding ^{86}Rb-free CSF medium containing ^{3}H-raffinose, for an additional incubation time of 20 minutes. Dried choroid plexus samples on their adhering aluminum foil weighing boats, and CSF samples, were extracted overnight in 2 ml of 1 M piperidine. Liquid scintillation cocktail (see Materials) was added to each extract for LVCP and CSF ^{3}H-raffinose radioactivity analyses. A more detailed description of the procedures utilized have been described elsewhere (Johanson et al., 1974).

Calculations

^{86}Rb Volume of Distribution. The volume of distribution of ^{86}Rb was determined on a dry tissue-weight basis; i.e., activity of ^{86}Rb per gram of dry LVCP, divided by the activity of ^{86}Rb per ml of incubation fluid.

Materials

All of the chemicals used were of analytical grade: lactic acid, sodium lactate (Baker, Phillipsburg, NJ), sodium bicarbonate, potassium bicarbonate, ammonium bicarbonate, sodium chloride, potassium chloride, ammonium chloride and hydrochloric acid (Baker), calcium chloride dihydrate, monobasic sodium phosphate, dibasic sodium phosphate, magnesium chloride hexahydrate, urea, citric acid monohydrate, anhydrous dextrose (Baker) and anhydrous sodium sulfate, with exceptions noted, were obtained from Mallinckrodt, Inc. (Paris, KY). All solutions were prepared with deionized water. ^{3}H-raffinose (7.8 mCi/mmol) and ^{86}Rb (1.0 mCi/mg) were acquired from New England Nuclear (Boston, MA). Sterile 80 x 12.5 mm polypropylene tubes with screw caps, by Nunc InterMed, were obtained through Cole Scientific (Calabasas, CA). Disposable 0.005-ml micropipettes were acquired from Fischer Scientific Corporation (Pittsburg, PA). The liquid scintillation cocktail used contained toluene (2600 ml), triton-X 100 (1300 ml), PPO (21.9 gram) and POPOP (0.200 gram). All of the other materials used have been described in Chapters 2, 4 and 6.

Instrumentation

Plasma osmolality was determined with an Advanced DigiMatic model 3DII Osmometer by Advanced Instruments, Inc. (Needham Heights, MA.). ^{3}H-raffinose was obtained from New England Nuclear (Boston, MA). Fluid and tissue ^{3}H-raffinose and ^{86}Rb analyses were, respectively, analyzed with a Beckman LS 7500 liquid-scintillation counter and a Beckman BioGamma gamma emission counter (Irvine, CA). All of the other instruments used have been described in Chapters 2, 4 and 6.

CHAPTER 9

COMPARISON OF THE EFFECTS OF NaCl, NH_4Cl AND OTHER SALTS ON [K] AND [Na] IN CHOROID PLEXUS; AND THE EFFECT OF ACIDOSIS ON THE UPTAKE OF ^{86}Rb INTO *IN VITRO* CHOROID PLEXUS: RESULTS

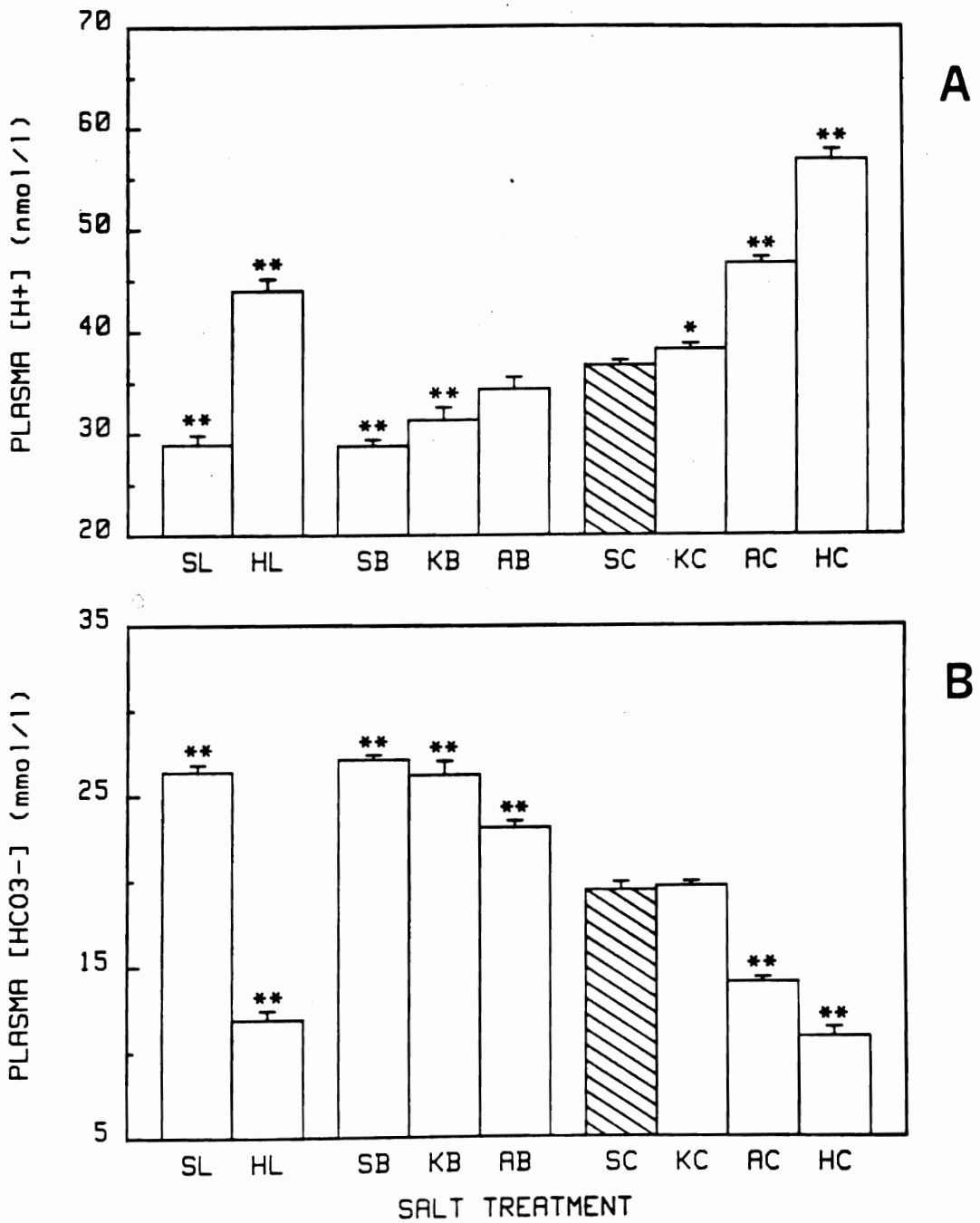
In Vivo Salt Treatments

The following lactate, bicarbonate and chloride salts were given by intraperitoneal injection: sodium lactate (SL), lactic acid (HL), sodium bicarbonate (SB), potassium bicarbonate (KB), ammonium bicarbonate (AB), sodium chloride (SC), potassium chloride (KC), ammonium chloride (AC) or hydrochloric acid (HC). The effects of these agents on the acid-base chemistry of the blood and on the sodium and potassium ion concentrations of cerebrospinal fluid, plasma, and lateral (LVCP) and third (3VCP) ventricular choroid plexus tissues, are all compared below; the results shown are the drug-induced effects after a 60-minute exposure period. Unless otherwise stated, all effects of treatment were statistically compared to the SC (control) group with a one-way analysis of variance followed with the Hartley-Newman-Keuls sequential multiple range comparison.

Acid-Base and Gas Analyses of Arterial Blood. The three acidifying salts, HL, AC and HC, induced the greatest increases in plasma [H] (Figure 9-1A); these increases were, respectively, 7, 12 and 20 nM. The neutral (not acidifying or alkalinizing) agent,

Figure 9-1. Effects of eight separate salt treatments, compared to control, on [H] (A) and [HCO_3] (B) in arterial plasma.

Arterial blood samples were taken 60 minutes after an intraperitoneal injection (4.70 mmol/kg) of either sodium lactate (SL), lactic acid (HL), sodium bicarbonate (SB), potassium bicarbonate (KB), ammonium bicarbonate (AB), sodium chloride (SC), potassium chloride (KC), ammonium chloride (AC) or hydrochloric acid (HC). Values shown are means $\pm$ SEM of data from five to six adult male rats. The significance of the induced differences from control (SC) was determined with the Hartley-Neuman-Keuls multiple range comparison: * indicates $p < 0.05$; ** indicates $p < 0.01$.


A
PLASMA [H+] (nmol/l)
70
60
50
40
30
20
**
**
**
**
*
**
**
SL
HL
SB
KB
AB
SC
KC
AC
HC
B
PLASMA [HCO3-] (mmol/l)
35
25
15
5
**
**
**
**
**
**
**
SL
HL
SB
KB
AB
SC
KC
AC
HC
SALT TREATMENT

KC, also caused a significant, but smaller increase in [H]. Of the alkalinizing salts, SL, SB and KB all significantly reduced plasma [H], by 8, 8 and 5 nM, respectively; AB had no effect on [H]. Plasma bicarbonate was maximally increased by about 7 mM, by SL, SB and KB; AB also induced a significant increase (Figure 9-1B). Plasma bicarbonate was maximally decreased about by 8 mM by HL and HC; AC also caused a significant decrease; KC had no effect. Blood pH, pCO_2, pO_2 and hematocrit (HCT) values are shown in Table 9-1. Blood pCO_2 was not significantly altered by any of the groups from a baseline value of about 29 torr, with the exception that HL induced a reduction of about 7 torr ($p < 0.01$). In general, changes in pCO_2 followed the increases and decreases in pH. Blood pO_2 remained stable in all groups, except with HL and HC; in these two cases, pO_2 was elevated about 14 torr. None of the nine salts examined significantly affected plasma osmolality which averaged 284 mOsm/kg. The HCT averaged about 39%, except for the HL and HC groups in which it was respectively increased to 52 and 44% ($p < 0.01$).

Cerebrospinal Fluid and Plasma [K] and [Na]. Treatment effects on cerebrospinal fluid (CSF) [K] are shown in Figure 9-2A; the two acidifying agents, HL and HC, and AB, all reduced CSF [K] by about 0.14 mmol/l. CSF [K] was not significantly altered by the other salts. CSF [Na] tended to be lower in the HL, AB, AC and HC groups (Figure 9-2B); the other salts did not induce a significant effect. The effects of the various salts on plasma

Table 9-1. EFFECTS OF EIGHT SEPARATE SALT TREATMENTS, COMPARED TO CONTROL, ON pH, pCO_2, pO_2 AND HCT IN ARTERIAL BLOOD

SALT	pH	pCO_2	pO_2	HCT
SL	7.54 ± .014**	30.9 ± 0.99	74.9 ± 1.52	37.9 ± .88
HL	7.36 ± .012**	21.7 ± 0.52**	88.4 ± 2.54**	51.5 ± 1.4**
SB	7.54 ± .010**	32.4 ± 0.44	73.6 ± 1.61	37.6 ± .60
KB	7.51 ± .018**	33.6 ± 2.13*	73.5 ± 2.24	39.8 ± 1.1
AB	7.47 ± .015	32.4 ± 0.78	75.1 ± 1.22	38.2 ± .60
SC	7.44 ± .006	29.2 ± 1.09	76.4 ± 0.90	37.2 ± 1.2
KC	7.41 ± .012*	30.7 ± 0.62	70.6 ± 1.16	39.3 ± .80
AC	7.32 ± .014**	28.0 ± 0.69	78.6 ± 1.27	39.5 ± .76
HC	7.25 ± .008**	25.8 ± 1.22	92.2 ± 1.80**	43.5 ± .72**

Blood gases are listed in torr units. HCT refers to the hematocrit. Arterial blood samples were taken 60 minutes after an intraperitoneal injection (4.70 mmol/kg) of either sodium lactate (SL), lactic acid (HL), sodium bicarbonate (SB), potassium bicarbonate (KB), ammonium bicarbonate (AB), sodium chloride (SC), potassium chloride (KC), ammonium chloride (AC) or hydrochloric acid (HC). Values shown are means ± SEM of data from five to six adult male rats. The significance of the induced differences was compared to control (SC) with the Hartley-Neuman-Keuls multiple range comparison; statistical siginificance is shown: * indicates $p < 0.05$; ** indicates $p < 0.01$.

Figure 9-2. Effects of eight separate salt treatments, compared to control, on [K] (A) and [Na] (B) in cerebrospinal fluid.

Cerebrospinal fluid (CSF) was sampled 60 minutes after an intraperitoneal injection (4.70 mmol/kg) of either sodium lactate (SL), lactic acid (HL), sodium bicarbonate (SB), potassium bicarbonate (KB), ammonium bicarbonate (AB), sodium chloride (SC), potassium chloride (KC), ammonium chloride (AC) or hydrochloric acid (HC). Values shown are means $\pm$ SEM of data from four to six adult male rats. The significance of the induced differences from control (SC) was determined with the Hartley-Neuman-Keuls multiple range comparison: * indicates $p < 0.05$; ** indicates $p < 0.01$.

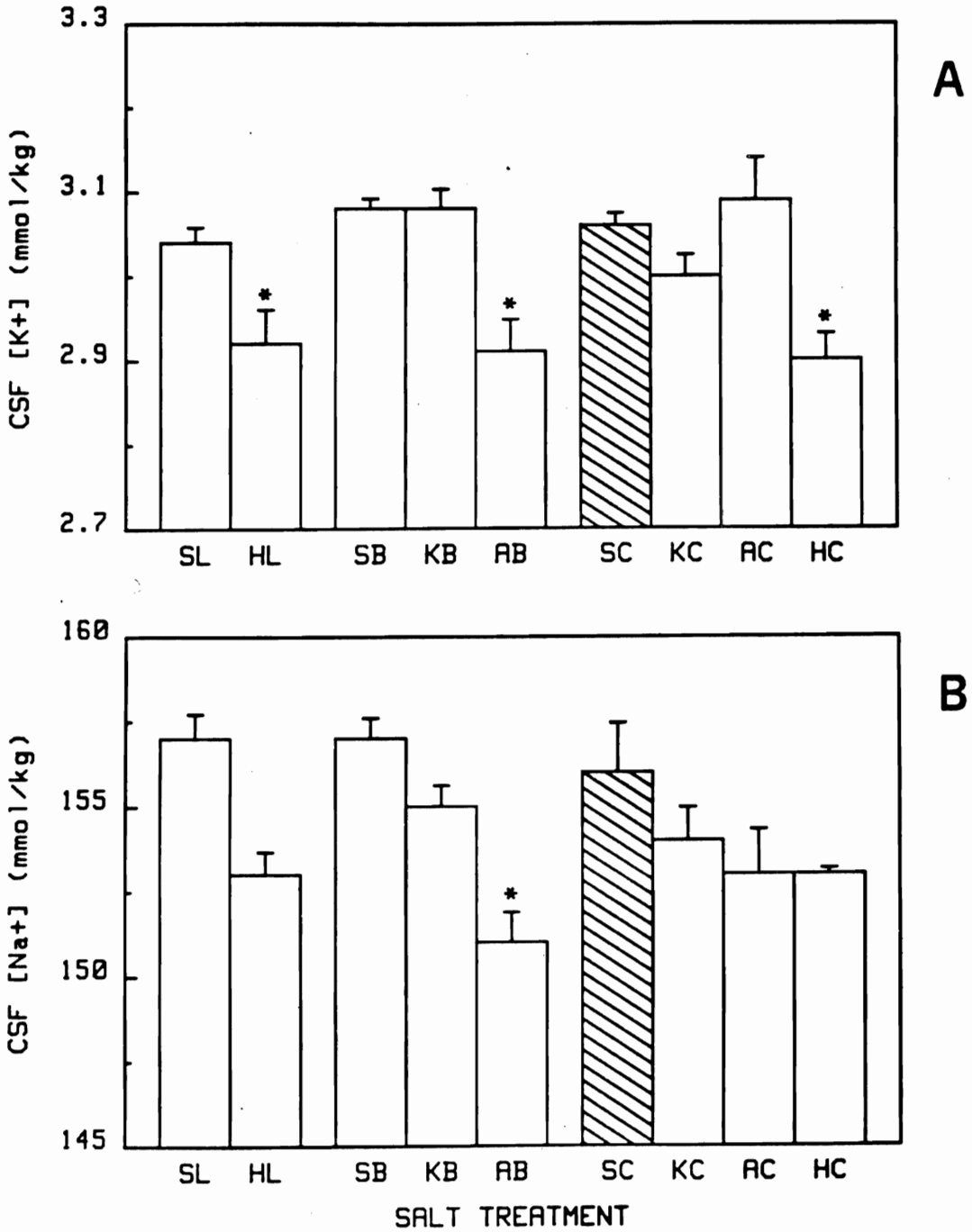
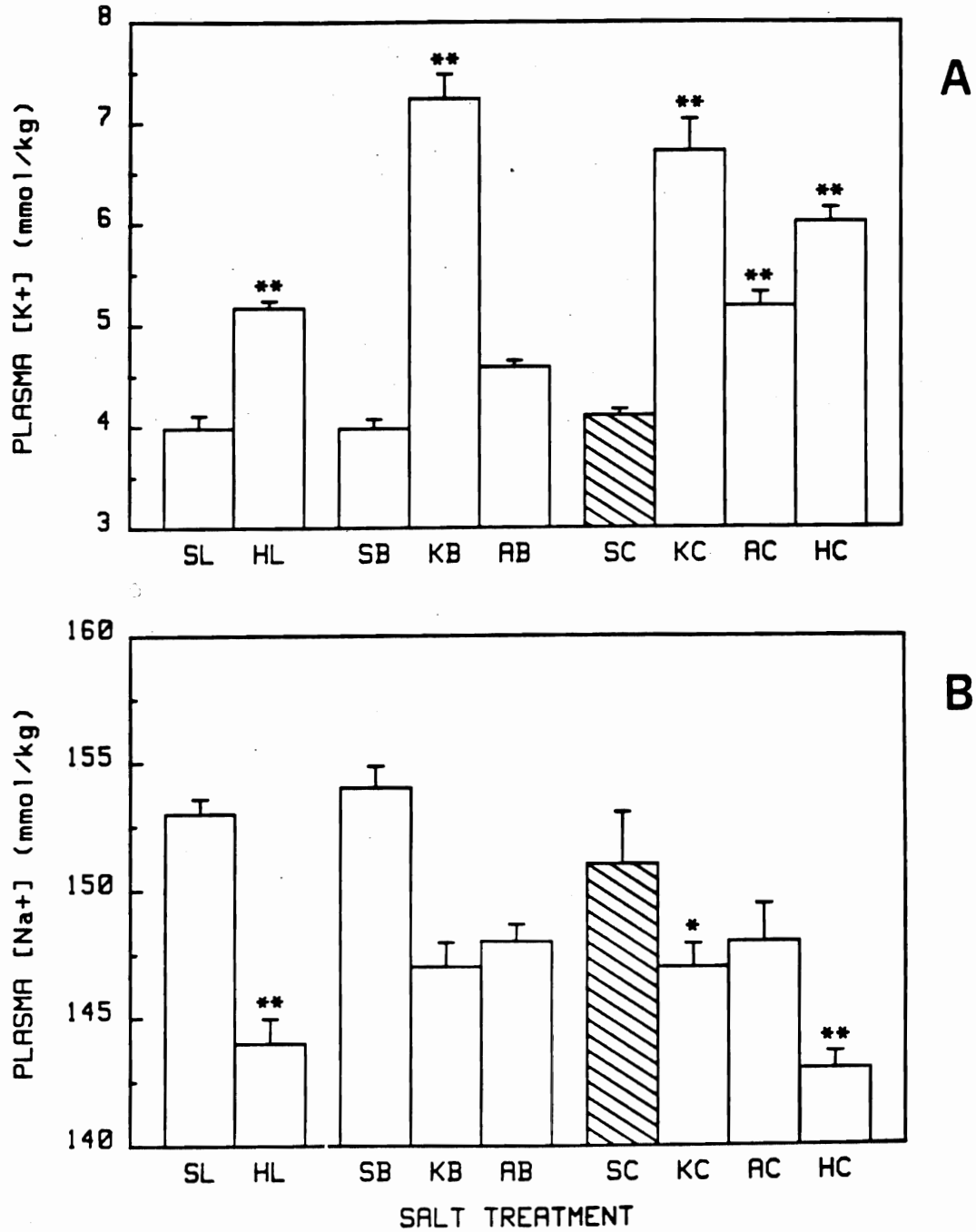

A
3.3
3.1
2.9
2.7
CSF [K+] (mmol/kg)
SL HL SB KB AB SC KC AC HC
B
160
155
150
145
CSF [Na+] (mmol/kg)
SL HL SB KB AB SC KC AC HC
SALT TREATMENT

Figure 9-3. Effects of eight separate salt treatments, compared to control, on [K] (A) and [Na] (B) in arterial plasma.

Arterial blood samples were taken 60 minutes after an intraperitoneal injection (4.70 mmol/kg) of either sodium lactate (SL), lactic acid (HL), sodium bicarbonate (SB), potassium bicarbonate (KB), ammonium bicarbonate (AB), sodium chloride (SC), potassium chloride (KC), ammonium chloride (AC) or hydrochloric acid (HC). Values shown are means $\pm$ SEM of data from five to six adult male rats. The significance of the induced differences from control (SC) were determined with the Hartley-Neuman-Keuls multiple range comparison: statistical * Indicates $p < 0.05$; ** indicates $p < 0.01$.


A
PLASMA [K+] (mmol/kg)
8
7
6
5
4
3
**
**
**
**
**
SL HL SB KB AB SC KC AC HC
B
PLASMA [Na+] (mmol/kg)
160
155
150
145
140
**
*
**
SL HL SB KB AB SC KC AC HC
SALT TREATMENT

[K] and [Na] are shown in Figure 9-3. As seen in panel A, plasma [K] was increased by HL, KB, KC, AC and HC by 1.1, 3.1, 2.6, 1.1 and 1.9 mmol/kg water, respectively. The greatest increases were caused by KB and KC, not by HL, AC or HC; in comparison, SL and SB had no significant effect. Plasma [Na] was significantly reduced about 8 mmol/l by both HL and HC; other salts showed slight or no significant changes from control.

Choroid Plexus [K] and [Na]. The treatment-induced alterations of [K] and [Na] in the LVCP are shown in Figure 9-4. LVCP [K] was substantially increased by HL, AC and HC, respectively 95, 76 and 110 mmol/dry kg (Figure 9-4A). AB also caused a significant increase in plexus [K] (also, Table 9-2). The alkalinizing salts tended to reduce plexus [K] or to have no effect. LVCP [Na] was significantly decreased about 60-80 mmol/dry kg by HL and HC (Figure 9-4B). The other salts did not significantly change LVCP [Na], although SB tended to raise plexus [Na]. Fewer treatment-induced alterations were seen with 3VCP (Figure 9-5). HC significantly increased 3VCP [K] by 110 mmol/dry kg (Figure 9-5A). 3VCP [Na] was reduced about 30 mmol/dry kg by HL and HC (Figure 9-5B) — about one-half the decrease seen with the LVCP. SB raised plexus [Na] about 50 mmol/dry kg. It is apparent in Figures 9-4 and 9-5 that the 3VCP has approximately 80 mmol/dry kg less [K] than the LVCP in each corresponding salt group; additionally, 3VCP has approximately 45 mmol/dry kg more [Na] than does LVCP in each corresponding salt group.

Figure 9-4. Effects of eight separate salt treatments, compared to control, on [K] (A) and [Na] (B) in LVCP.

Lateral ventricular choroid plexus (LVCP) samples were taken 60 minutes after an intraperitoneal injection (4.70 mmol/kg) of either sodium lactate (SL), lactic acid (HL), sodium bicarbonate (SB), potassium bicarbonate (KB), ammonium bicarbonate (AB), sodium chloride (SC), potassium chloride (KC), ammonium chloride (AC) or hydrochloric acid (HC). Values shown are means $\pm$ SEM of data from five to six adult male rats. The significance of the induced differences from control (SC) was determined with the Hartley-Neuman-Keuls multiple range comparison: * indicates $p < 0.05$; ** indicates $p < 0.01$.

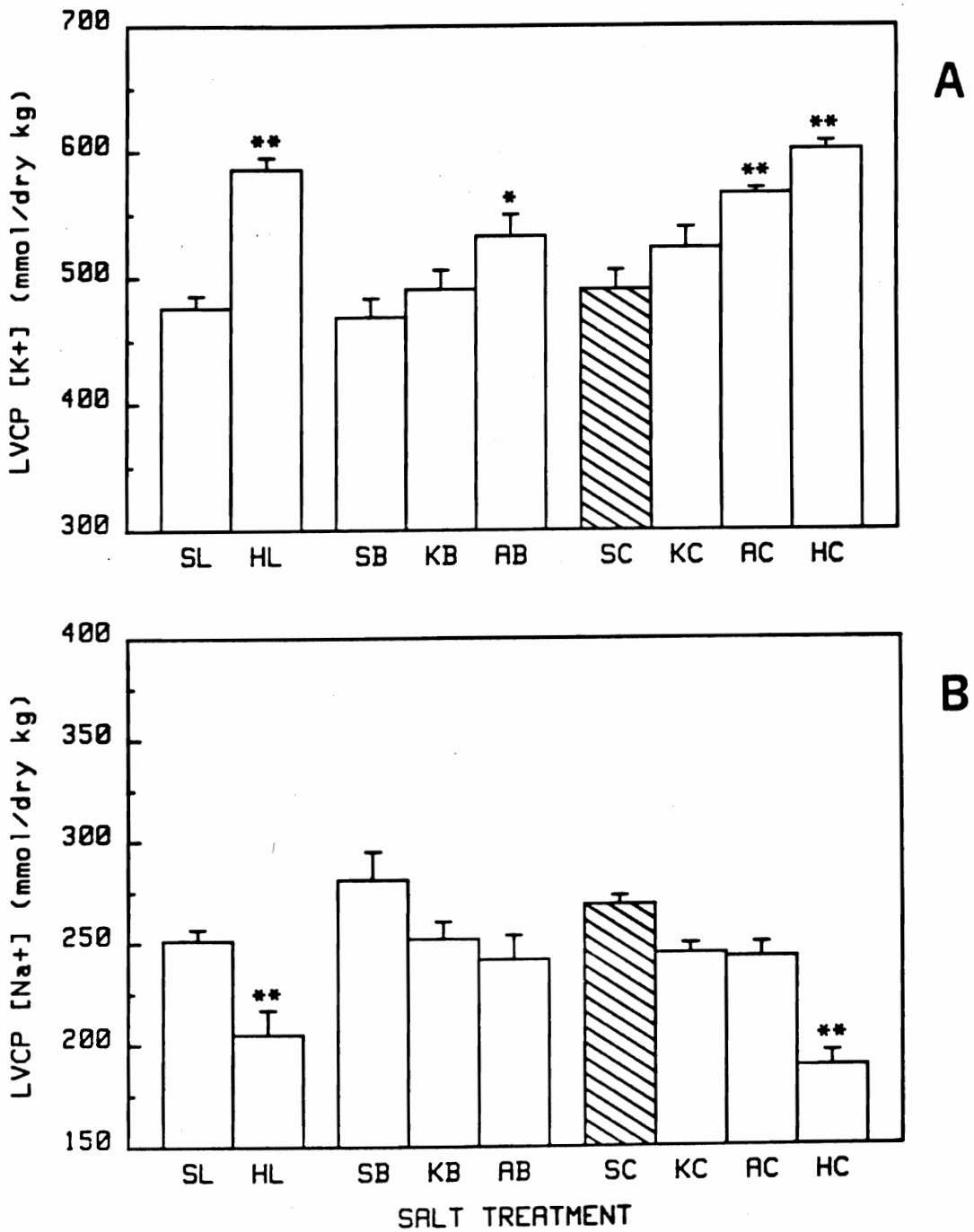

A
LVCP [K+] (mmol/dry kg)
700
600
500
400
300
**
*
**
**
SL
HL
SB
KB
AB
SC
KC
AC
HC
B
LVCP [Na+] (mmol/dry kg)
400
350
300
250
200
150
**
**
SL
HL
SB
KB
AB
SC
KC
AC
HC
SALT TREATMENT

Table 9-2. EFFECTS OF EIGHT SALTS, ON THE INDUCED CHANGES FROM CONTROL, ON [K] AND [Na] IN LVCP AND 3VCP

SALT	LVCP [K]	LVCP [Na]	3VCP [K]	3VCP [Na]
SL	- 14	- 18	- 34	0.0
HL	95**	- 64**	58	- 36
SB	- 22	11	- 39	49
KB	0.0	- 18	- 17	- 35
AB	43*	- 28	18	1
KC	33	- 25	15	- 5
AC	76**	- 27	37	- 5
HC	11*	- 80	110**	- 30

[K] and [Na] are in mmol/kg dry tissue units. Lateral (LVCP) and third (3VCP) ventricular choroid plexus samples were taken 60 minutes after an intraperitoneal injection (4.70 mmol/kg) of either sodium lactate (SL), lactic acid (HL), sodium bicarbonate (SB), potassium bicarbonate (KB), ammonium bicarbonate (AB), potassium chloride (KC), ammonium chloride (AC) or hydrochloric acid (HC). The values shown are the means $\pm$ SEM of the differences from control of each of the eight salt treatments (see Figures 9-4 and 9-5). The significance of the induced differences from control is that depicted in Figures 9-4 and 9-5: * indicates $p < 0.05$; ** indicates $p < 0.01$.

Figure 9-5. Effects of eight separate salt treatments, compared to control, on [K] (A) and [Na] (B) in 3VCP.

Third ventricular choroid plexus (3VCP) samples were taken 60 minutes after an intraperitoneal injection (4.70 mmol/kg) of either sodium lactate (SL), lactic acid (HL), sodium bicarbonate (SB), potassium bicarbonate (KB), ammonium bicarbonate (AB), sodium chloride (SC), potassium chloride (KC), ammonium chloride (AC) or hydrochloric acid (HC). Values shown are means $\pm$ SEM of data from five to six adult male rats. The significance of the induced differences from control (SC) was determined with the Hartley-Neuman-Keuls multiple range comparison: * indicates $p < 0.05$; ** indicates $p < 0.01$.

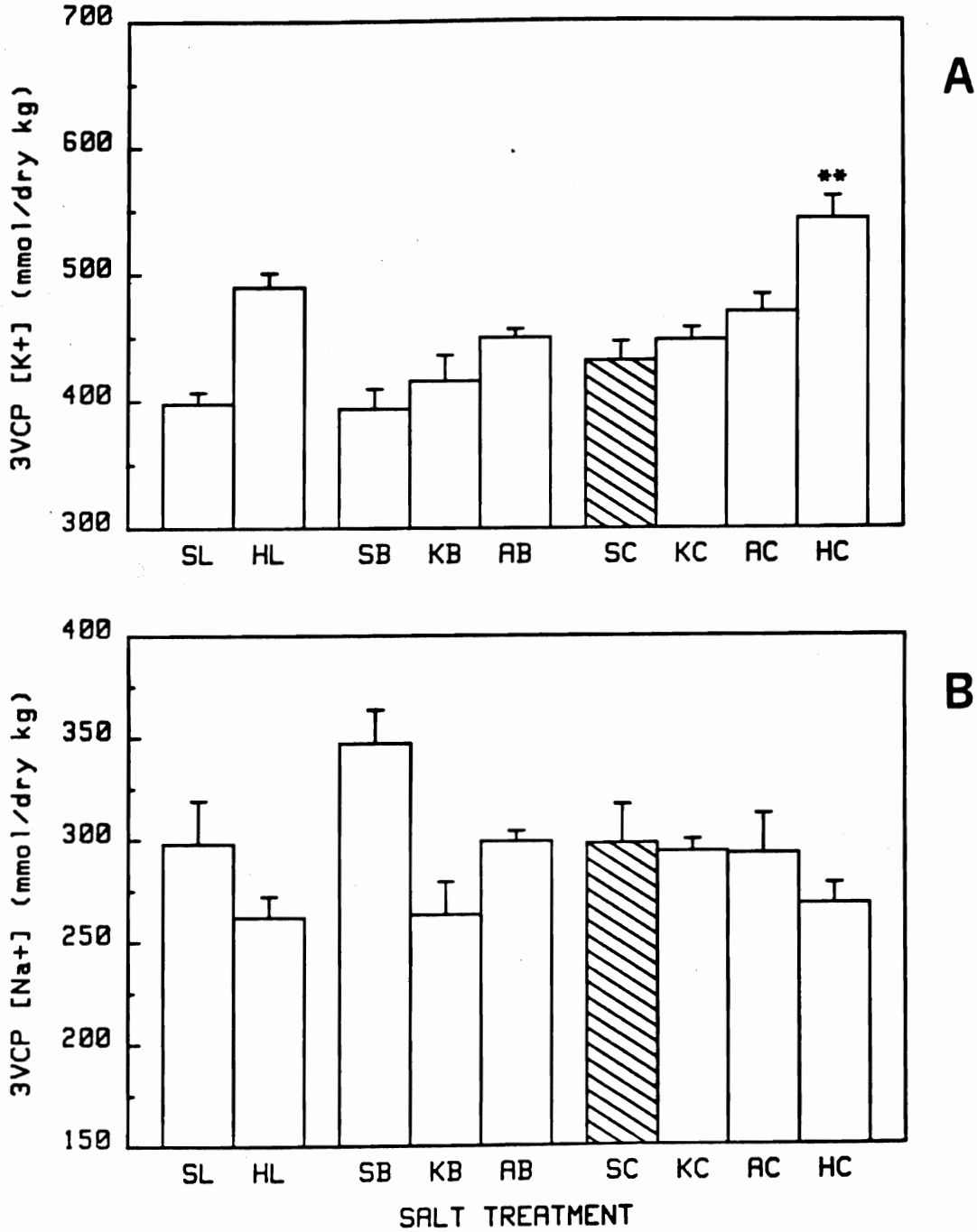

A
3VCP [K+] (mmol/dry kg)
700
600
500
400
300
**
SL
HL
SB
KB
AB
SC
KC
AC
HC
B
3VCP [Na+] (mmol/dry kg)
400
350
300
250
200
150
SL
HL
SB
KB
AB
SC
KC
AC
HC
SALT TREATMENT

Figure 9-6. Correlation analyses of the effects of nine salts on [K] (A) or [Na] (B) in LVCP, with the effect of all nine salts on plasma [H].

Lateral ventricular choroid plexus (LVCP) and arterial blood samples were obtained 60 minutes after an intraperitoneal injection (4.70 mmol/kg) of either sodium lactate (SL), lactic acid (HL), sodium bicarbonate (SB), potassium bicarbonate (KB), ammonium bicarbonate (AB), sodium chloride (SC), potassium chloride (KC), ammonium chloride (AC) or hydrochloric acid (HC). Treatment data are represented by symbols corresponding to the respective salts: filled square (HC), filled diamond (AC), unfilled square (KC), unfilled diamond (SC), filled inverted triangle (AB), unfilled triangle (KB), unfilled inverted triangle (SB), filled circle (HL) and unfilled circle (SL). The probability of the slope of each line, fitted to the data of the nine salt treatments, being statistically different from zero was determined with a one-tailed Student's t-test. The linear regression coefficient of determination, slope, sample population and probability are listed respectively: (A) $r^2 = 0.649$, $m = 4.77 \pm 0.517$, $n = 48$, $p < 0.001$; (B) $r^2 = 0.442$, $m = -2.37 \pm 0.401$, $n = 46$, $p < 0.001$.

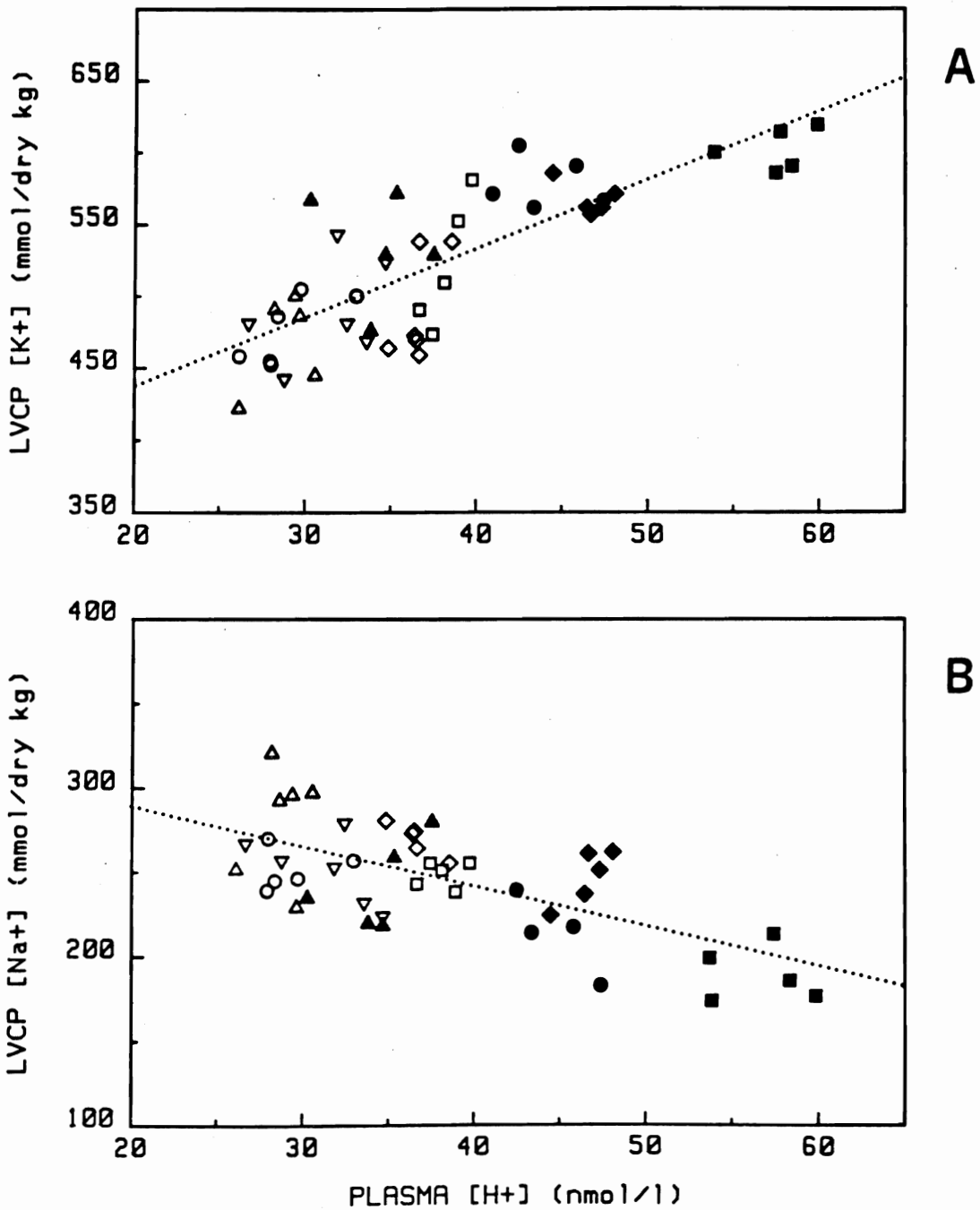
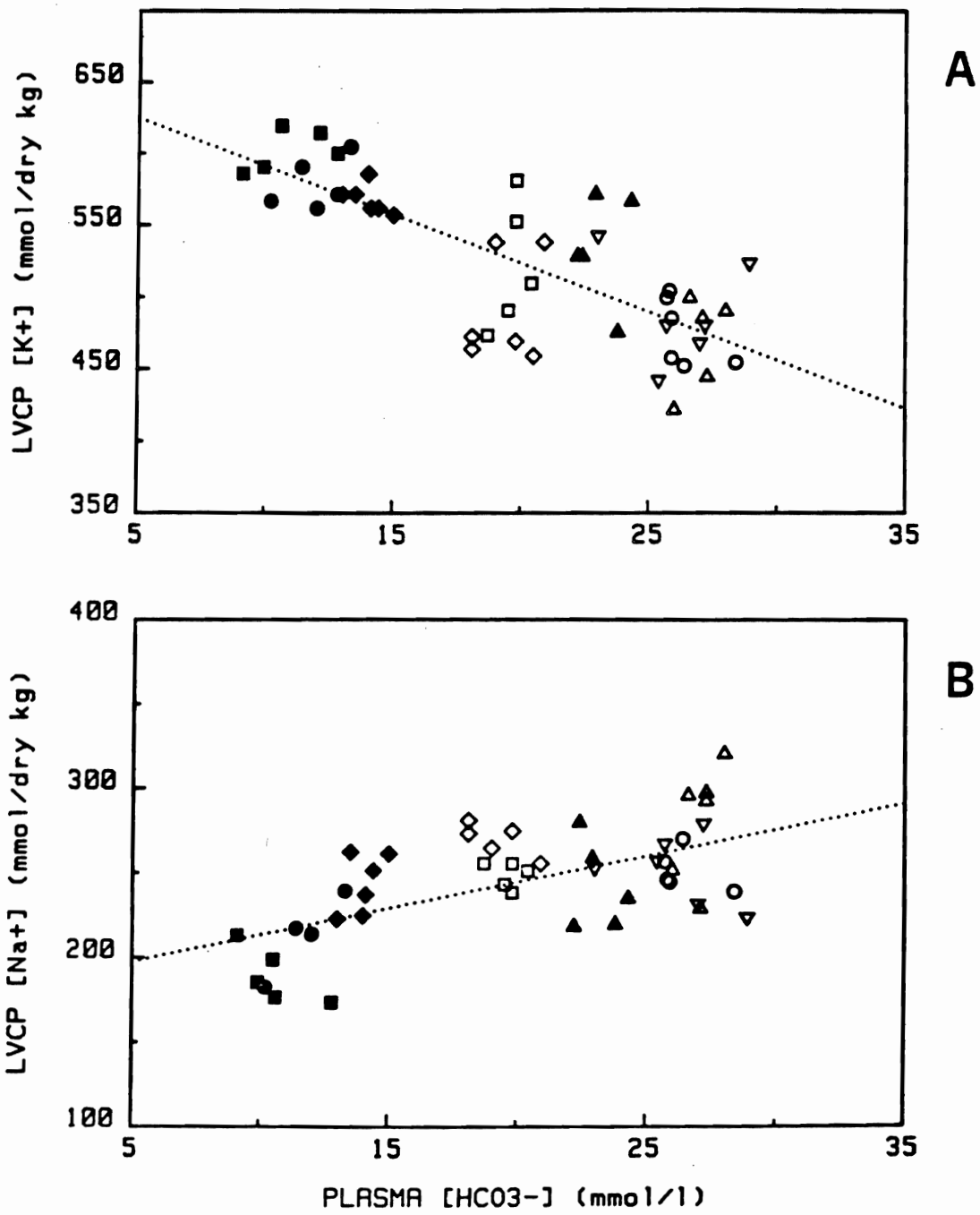

A
650
550
450
350
LVCP [K+] (mmol/dry kg)
20
30
40
50
60
B
400
300
200
100
LVCP [Na+] (mmol/dry kg)
20
30
40
50
60
PLASMA [H+] (nmol/l)

Figure 9-7. Correlation analyses of the effects of nine salts on [K] (A) or [Na] (B) in LVCP, with the effect of all nine salts on plasma [HCO_3].

Lateral ventricular choroid plexus (LVCP) and arterial blood samples were taken 60 minutes after an intraperitoneal injection (4.70 mmol/kg) of either sodium lactate (SL), lactic acid (HL), sodium bicarbonate (SB), potassium bicarbonate (KB), ammonium bicarbonate (AB), sodium chloride (SC), potassium chloride (KC), ammonium chloride (AC) or hydrochloric acid (HC). Treatment data are represented by symbols corresponding to the respective salts: filled square (HC), filled diamond (AC), unfilled square (KC), unfilled diamond (SC), filled inverted triangle (AB), unfilled triangle (KB), unfilled inverted triangle (SB), filled circle (HL) and unfilled circle (SL). The probability of the slope of each line, fitted to the data of the nine salt treatments, being statistically different from zero was determined with a one-tailed Student's t-test. The linear regression coefficient of determination, slope, sample population and probability are listed respectively: (A) $r^2 = 0.583$, $m = -6.74 \pm 0.830$, $n = 49$, $p < 0.001$; (B) $r^2 = 0.366$, $m = 3.12 \pm 0.613$, $n = 47$, $p < 0.001$.


A
LVCP [K+] (mmol/dry kg)
650
550
450
350
5
15
25
35
B
LVCP [Na+] (mmol/dry kg)
400
300
200
100
5
15
25
35
PLASMA [HCO3-] (mmol/l)

Since LVCP yielded greater responses than 3VCP to the salt treatments, only LVCP [K] and [Na] are correlated with plasma [H] (Figure 9-6), plasma [HCO_3] (Figure 9-7) and plasma [K] (Figure 9-8). By linear regression analysis, the best correlations and significantly different slopes were shown between LVCP [K], and plasma [H] or plasma [HCO_3]; not between LVCP [K] and plasma [K]. Similarly, with LVCP [Na], the best correlations and sigificantly different slopes were shown with plasma [H] or plasma [HCO_3-]; again, not with plasma [K]. The results of the individual data, from each respective salt group, have been portrayed with one of nine symbol fonts. It can be seen from these data that the acidic salts (HC, HL and AC) caused the greatest increases in [K] and decreases in [Na] in the LVCP; KC and SC, the agents which did not affect plasma pH, did not alter the control levels of [K] and [Na] in the LVCP; while, the alkalinizing salts, AB, KB, SB and SL, caused the greatest decreases in [K] and increases in [Na] in LVCP.

In Vitro ^{86}Rb Uptake

Lateral Ventricular Choroid Plexus Rubidium. An analysis of the initial uptake of ^{86}Rb into LVCP incubated in control or acidotic media revealed that the slopes of the lines fitted to the initial uptake data under these two conditions were not significantly different (Figure 9-9A,9B); thus, there did not appear to be an acidosis-induced increase in the active transport of ^{86}Rb into LVCP (see Discussion). The steady-state uptake of

Figure 9-8. Correlation analyses of the effects of nine salts on [K] (A) or [Na] (B) in LVCP, with the effect of all nine salts on plasma [K].

Lateral ventricular choroid plexus (LVCP) and arterial blood samples were taken 60 minutes after an intraperitoneal injection (4.70 mmol/kg) of either sodium lactate (SL), lactic acid (HL), sodium bicarbonate (SB), potassium bicarbonate (KB), ammonium bicarbonate (AB), sodium chloride (SC), potassium chloride (KC), ammonium chloride (AC) or hydrochloric acid (HC). Treatment data are represented by symbols corresponding to the respective salts: filled square (HC), filled diamond (AC), unfilled square (KC), unfilled diamond (SC), filled inverted triangle (AB), unfilled triangle (KB), unfilled inverted triangle (SB), filled circle (HL) and unfilled circle (SL). The probability of the slope of each line, fitted to the data of the nine salt treatments, being statistically different from zero was determined with a one-tailed Student's t-test. The linear regression coefficient of determination, slope, sample population and probability are listed respectively: (A) $r^2 = 0.051$, $m = 9.95 \pm 6.38$, $n = 47$, $p < 0.10$; (B) $r^2 = 0.116$, $m = -9.01 \pm 3.80$, $n = 45$, $p < 0.05$.

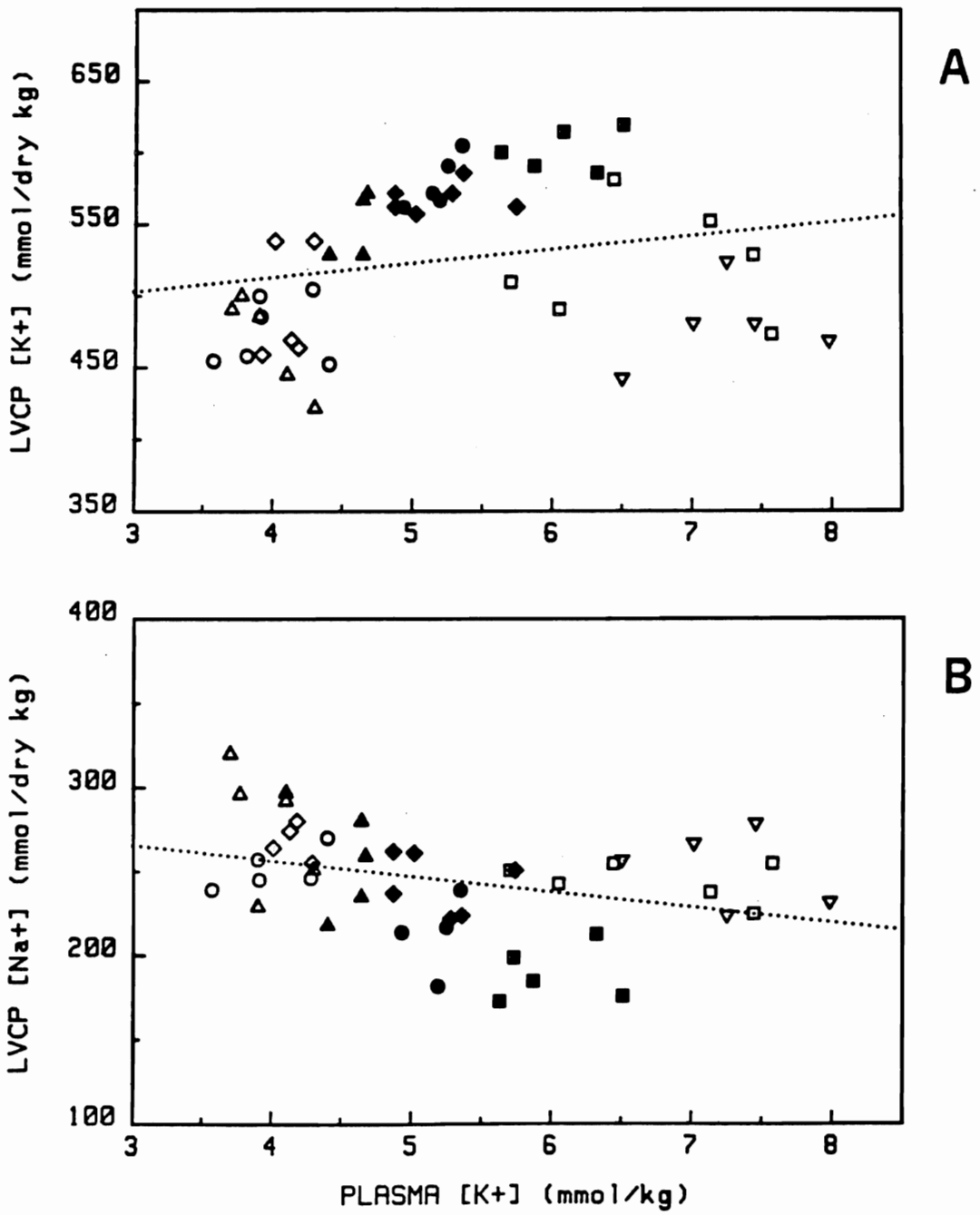
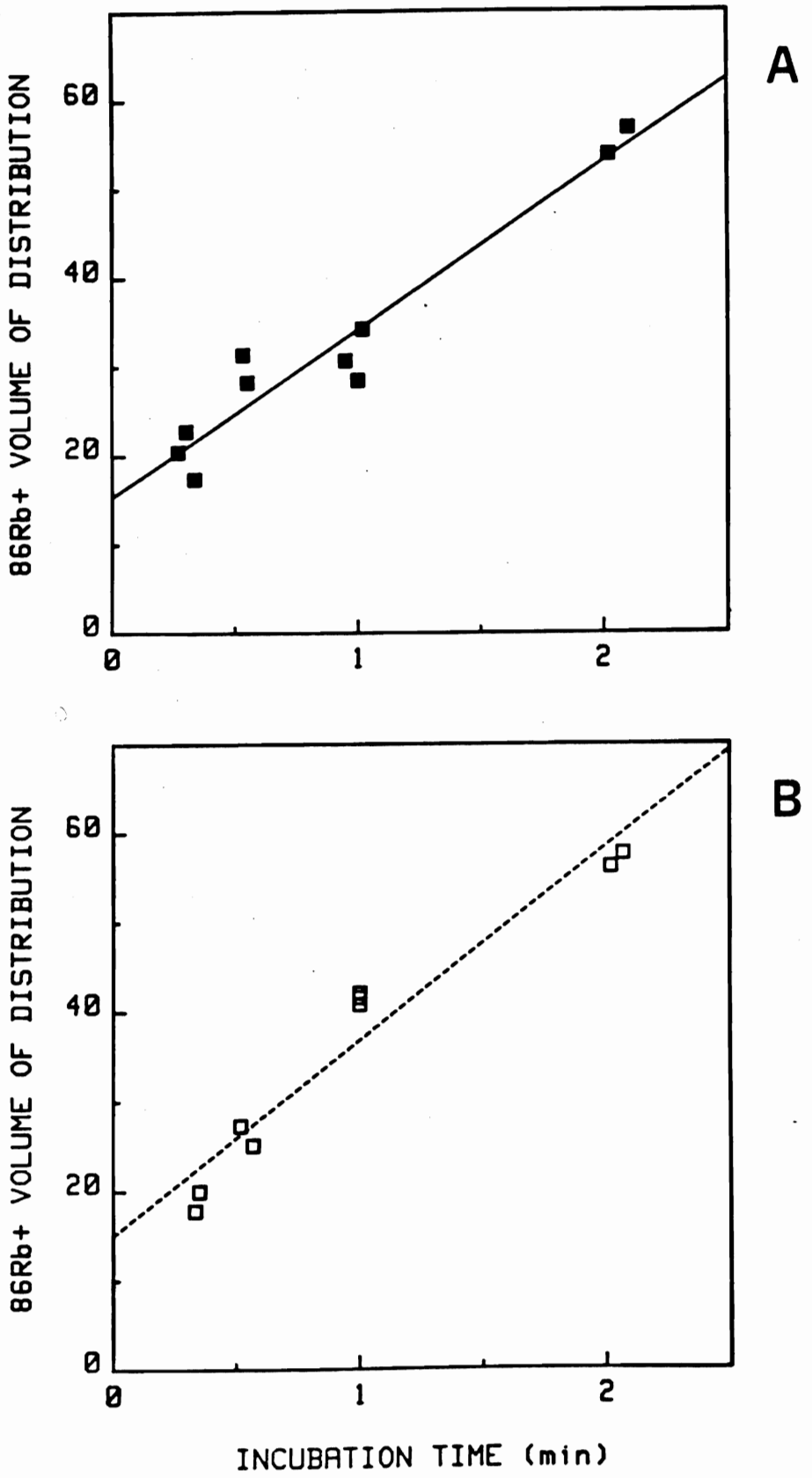

A
LVCP [K+] (mmol/dry kg)
650
550
450
350
3
4
5
6
7
8
B
LVCP [Na+] (mmol/dry kg)
400
300
200
100
3
4
5
6
7
8
PLASMA [K+] (mmol/kg)

Figure 9-9. Effect of cerebrospinal fluid pH on the initial uptake of ^{86}Rb into the *in vitro* LVCP.

Lateral ventricular choroid plexus (LVCP) samples were incubated in simulated cerebrospinal fluid (CSF) containing ^{86}Rb for 0.25, 0.5, 1 or 2 minutes after being preincubated for 20 minutes in a similar solution not containing ^{86}Rb (all CSF solutions were equilibrated with humidified 95% O_2 and 5% CO_2 at 37°C). Two CSF conditions were investigated: control pH (7.4) and acidosis pH (7.0). All values depicted represent the mean ± SEM of two to three LVCP samples. In panel A, the continuous line corresponds to the slope of the initial uptake data of ^{86}Rb under control conditions (filled squares); in panel (B), the dashed line corresponds to the slope of the initial uptake data of ^{86}Rb under the conditions of CSF acidosis (unfilled squares).


A
B
86Rb+ VOLUME OF DISTRIBUTION
0
20
40
60
0
1
2
INCUBATION TIME (min)

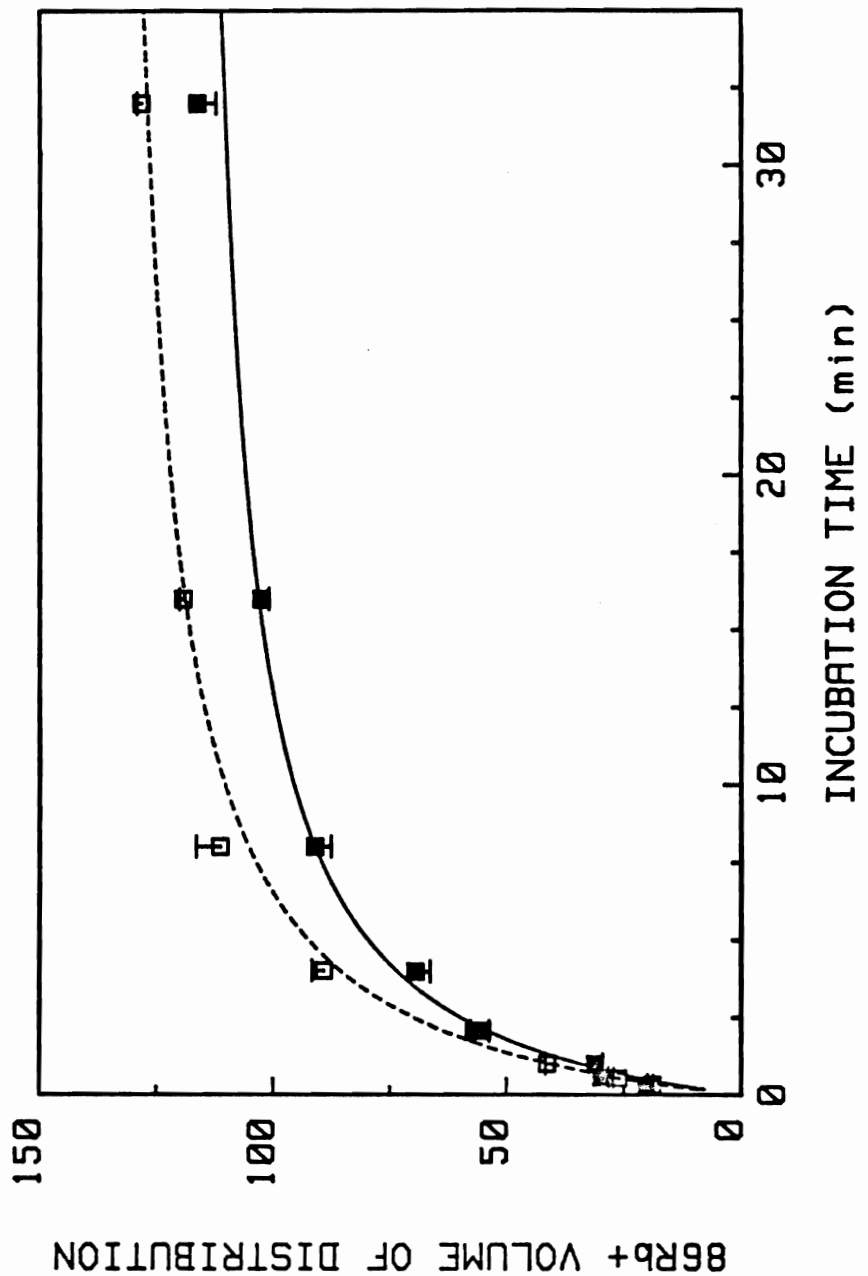
^{86}Rb into LVCP is depicted in Figure 9-10. A significant elevation in the plateau of the pH 7.0 uptake curve revealed that acidosis induced a significant increase, over control, in the volume of distribution of ^{86}Rb in LVCP; this effect is consistent with a decrease in the efflux of intracellular ^{86}Rb (see Discussion).

In Vitro Extracellular Fluid Volume

Acidosis appeared to reduce the extracellular fluid (ECF) volume of distribution (V_d) of ^{3}H-raffinose in the *in vitro* lateral ventricular choroid plexus. The control V_d (%) was 45.2 $\pm$ 1.9; while under acidotic conditions the V_d decreased to 33.9 $\pm$ 1.0. These values are statistically different ($p < 0.01$).

Figure 9-10. Effect of cerebrospinal fluid pH on the uptake of ^{86}Rb into the *in vitro* LVCP.

Lateral ventricular choroid plexus (LVCP) samples were incubated in simulated cerebrospinal fluid (CSF) containing ^{86}Rb for 0.25, 0.5, 1, 2, 4, 8, 16 or 32 minutes after being preincubated for 20 minutes in a similar solution not containing ^{86}Rb (all CSF solutions were equilibrated with humidified 95% O_2 and 5% CO_2 at 37°C). Two CSF conditions were investigated: control pH (7.4) and acidosis pH (7.0). All values depicted represent the mean $\pm$ SEM of two to three LVCP samples. Filled squares along the continuous curve correspond to control data; unfilled squares along a dashed curve correspond to acidosis data.


86Rb+ VOLUME OF DISTRIBUTION
150
100
50
0
0
10
20
30
INCUBATION TIME (min)

CHAPTER 10

DISCUSSION

Stimulus and Mechanism of the CP Response to Ammonium Chloride

Plasma Ammonia. In order to analyze the effect of plasma ammonium (NH_4) on the CP response, the concentration of plasma ammonia was analyzed for 8 hours after an injection of NaCl or NH_4Cl (Chapters 6 and 7). Several authors have demonstrated that NH_4 can substitute for extracellular K in the stimulation of Na-K ATPase in soleus muscle and choroid plexus (Akaike, 1975; Claire-Aicken and Thomas, 1977; Saito and Wright, 1982). Such direct stimulation might account for an effect of NH_4 to reduce CP [Na] but it would not account for an increase in CP [K] (a stimulation of Na-K ATPase with NH_4 would lead to an increase in intracellular NH_4, not K). Although plasma NH_4 might be effecting a reduction in CP [Na] during the first hour (see Figure 7-7), its return to the control level at 1 hour (Figure 7-1) would not explain the sustained decrease in CP [Na] at 2 hours (Figure 7-7).

A possible indirect effect of ammonia on Na-K ATPase, through an effect on the concentration of ATP, was investigated by Schenker and Mendelson (1964); however, no change in cerebral cortical ATP concentration was observed in rats injected with ammonium acetate.

Wiechetek, Breves and Holler (1979) investigated the effect of NH_4 on the activity of adenylate cyclase (AC) in various tissues. They reported that physiological concentrations of NH_4 reduced the activity (-30%) of AC in liver particles. Furthermore, NH_4 did not affect AC activity in muscle particles, but did increase AC activity (+40%) in brain particles. If increases in plasma NH_4 had an effect to stimulate AC in rat CP and thus to stimulate CP Na-K ATPase (see Pershing and Johanson, 1982), this effect would only account for the alterations seen in CP [K] and [Na] during the first hour, not the second through fourth hours.

Plasma Bicarbonate. Pershing and Johanson (1982) reported that both respiratory and metabolic acidosis induce an increase in CP [K] and a decrease in that of [Na]; since plasma [HCO_3] increases and decreases, respectively, in these types of acidosis, plasma [HCO_3] is not considered a likely inducer of the CP responses seen in NH_4Cl-induced acidosis. Thus, the high degree of correlation between CP [K] and plasma [HCO_3] (Figure 7-9) is most likely attributable to the coincidental correlation between plasma [HCO_3] and [H] (Figure 7-4).

Plasma Catecholamines. The literature is replete with evidence which demonstrates that catecholamines modulate the activity of Na-K ATPase in vitro and in vivo. A differentiation between alpha- and beta-adrenoceptor modulation needs discussion. Akaike (1981) demonstrated a central mechanism which mediates the

inhibition of rat skeletal muscle Na-K ATPase by the stimulation of alpha-adrenoceptors; this inhibition was observed in rats fed a K-deficient diet. In contrast to alpha-adrenoceptor stimulation, beta-adrenoceptor stimulation serves to increase the activity of Na-K ATPase both *in vivo* and *in vitro*. Bia and DeFronzo (1981) reviewed the action of epinephrine to stimulate Na-K ATPase. Bia and DeFronzo (1981) demonstrated that the epinephrine-stimulated uptake of K is independent of the concentration of plasma insulin and that this effect is mediated by beta-2, not by beta-1, adrenoceptors. Clausen and Flatman (1980) demonstrated that physiological concentrations of epinephrine enhance Na-K exchange, an effect inhibited by ouabain or propranolol. Lockwood and Lum (1974) proposed that beta-2 adrenoceptors subserve a hypokalemic action to protect the intact animal against hyperkalemia. Rosa, Silva and Young (1980) demonstrated that epinephrine enhances the extrarenal disposal of an acute potassium load. Vick, Todd and Luedke (1972) reported that epinephrine increases the uptake of K by liver and skeletal muscle. Wang and Clausen (1976) have used salbutamol (beta-2 adrenoceptor agonist) to alleviate hyperkalemia and paralysis precipitated by exercise or administration of potassium chloride. In addition, Wang and Clausen (1976) have demonstrated that salbutamol and adrenaline stimulate ^{42}K influx and ^{22}Na efflux in isolated rat soleus muscle. Thus, a plausible beta-adrenoceptor mediated mechanism exists through which catecholamines might stimulate *CP* Na-K ATPase.

The stimulus and sources of catecholamine release during systemic acidosis have been investigated by several authors. Hypercarbic acidosis raises the titer of plasma catecholamines in dogs; the stimulus of this increase has been attributed to increases in blood CO_2 and/or decreases in plasma pH (Morris and Millar, 1962); probable sources have been attributed to the acidosis-induced release of catecholamines from adrenal glands (Morris and Millar, 1962), and/or the acidosis-induced inhibition of catecholamine reuptake by sympathetic nerve endings (Morris and Millar, 1962; Vanhoutte et al., 1981). Pershing and Johanson (1982) suggested that an acidosis-stimulated release of catecholamines could enhance the activity of CP Na-K ATPase, and thus Na-K exchange.

In 1-wk rats there is a paucity of innervation to the CP's (Lindvall et al., 1981), and also an inability of the LVCP and 4VCP to increase their content of K in response to metabolic acidosis (Pershing and Johanson, 1982). Thus it seemed possible that an increase in the impulse traffic of the sympathetic (adrenergic) nerves to the CP, or an acidosis-induced decrease in the reuptake of catecholamines into the sympathetic nerve endings, might explain the CP responses in adults after NH_4Cl treatment. The ability of the sympathetic nervous system to stimulate CP Na-K ATPase seemed even more certain by the observation of Lindvall et al. (1982) that sympathectomy reduces the activity of Na-K ATPase in rat CP. However, sympathectomy did not alter the CP response

to NH_4Cl (Figure 5-1). If a humorally adrenergically-mediated modulation of CP Na-K ATPase occurs during NH_4Cl-induced metabolic acidosis, it was unclear if the CP adrenoceptors could be stimulated by catecholamines released from the adrenal medulla. As shown in Figure 5-2, even bilateral adrenalectomy did not block the NH_4Cl-induced CP response. Cohen, Piasecki and Jackson (1982) suggested that the adrenal medulla is a major source of catecholamines during hypoxemia; however, except for NH_4Cl-induced acidosis in bilaterally adrenalectomized rats (Chapter 5), blood pO_2 in metabolic acidosis was never decreased below control; in fact, blood pO_2 was usually increased (Tables 7-2 and 9-1). What is the likelihood that catecholamines are responsible for the NH_4Cl-induced effect on CP [K] and [Na]? It is unlikely that catecholamines are the primary stimulus because neither sympathectomy (Figure 5-1) nor adrenalectomy (Figure 5-2) prevented the induced CP response. Furthermore, neither nadolol nor propranolol pretreatment (beta-adrenoceptor blocking drugs; unpublished results) blocked the NH_4Cl-induced CP response. Poole-Wilson and Langer (1975) demonstrated that acidosis reduces the efflux of K from septal cardiac cells and leads to an increase in the cardiac content of K. Poole-Wilson and Langer (1975) could not mimic this increase in K by epinephrine administration or block this increase with propranolol. Since Nathanson (1980) demonstrated a greater beta-adrenoceptor mediated increase in adenylate cyclase activity in 4VCP, rather than in LVCP, one might

expect catecholamines to increase 4VCP [K] more than LVCP [K]; however, since NH_4Cl increased 4VCP [K] less than LVCP [K] (Figure 3-8), this is further evidence against a catecholamine-induced increase in Na-K exchange.

Plasma Potassium. Since extracellular K can stimulate Na-K ATPase (Stekhoven and Bonting, 1981), it is of interest to consider the role of plasma [K] as a possible stimulator of Na-K exchange in CP. Since CP Na-K ATPase is predominantly located on the apical membrane, a discussion of the effect of an increase in [K] in the plasma on apical Na-K exchange is probably moot, unless that increase in plasma [K] is transferred into CSF. Since no increase in CSF [K] was detected in metabolic acidosis (Chapters 3, 5, 7 and 9), and since decreases in CSF [K] were in fact observed (Table 5-3 and Figure 9-2), it is considered unlikely that elevated plasma [K] is the prime stimulus of NH_4Cl-induced increases in CP [K] (Figure 9-8).

Plasma Sodium. Since the Na-H exchanger (antiporter) requires an inwardly directed Na gradient and an outwardly directed H gradient, the observed increase in plasma [H] (Figure 7-2) and decrease in plasma [Na] (Figure 7-3) would be expected to lower the influx of Na and efflux of H across the basolateral membrane of the CP. Such effects on Na influx and H efflux would be expected to reduce CP [Na] (Figure 7-6 and 9-4) and increase CP [H]; these responses have been observed in both HCl- and NH_4Cl-induced acidosis (Murphy, unpublished data).

Plasma Hydrogen. The literature is replete with evidence which demonstrates that acidosis decreases the conductance of K in the membranes of several cell types. Biagi, Kubota, Sohtell and Giebisch (1981) demonstrated, in rabbit proximal straight tubule perfused *in vitro*, that reducing bath pH with low concentrations of bicarbonate led to a depolarization of the basolateral membrane; their results suggest an acidosis-induced decrease in the K permeability of the basolateral membrane. In the rat, metabolic acidosis caused an 80% decrease in distal tubular K secretion (Malnic, de Mello Aires and Giebisch, 1971); this effect was attributed to a decrease in intracellular pH. O'Neil (1981) discussed a model of K transport across rabbit cortical collecting tubule. Cortical collecting tubule is similar to *CP* in that one side of the epithelium has Na-K ATPase (basolateral membrane), while the apical side (lumen facing) appears to contain Na-H antiporters (O'Neil, 1981). K transport across these tubule epithelial cells is considered a two-step process whereby extracellular K is pumped into the cell by basolateral Na-K ATPase and leaves the cell (apical side) down an electrochemical gradient; if decreased luminal pH lowers the permeability of the membrane to K, this model can account for a reduction in the apical secretion of K when the pH of the lumen is decreased. Wanke, Carbone and Testa (1979) demonstrated that perfused squid giant axon behaves as if it had membrane-titratable groups which regulate the conductance of the membrane to K (pKa 6.9); this

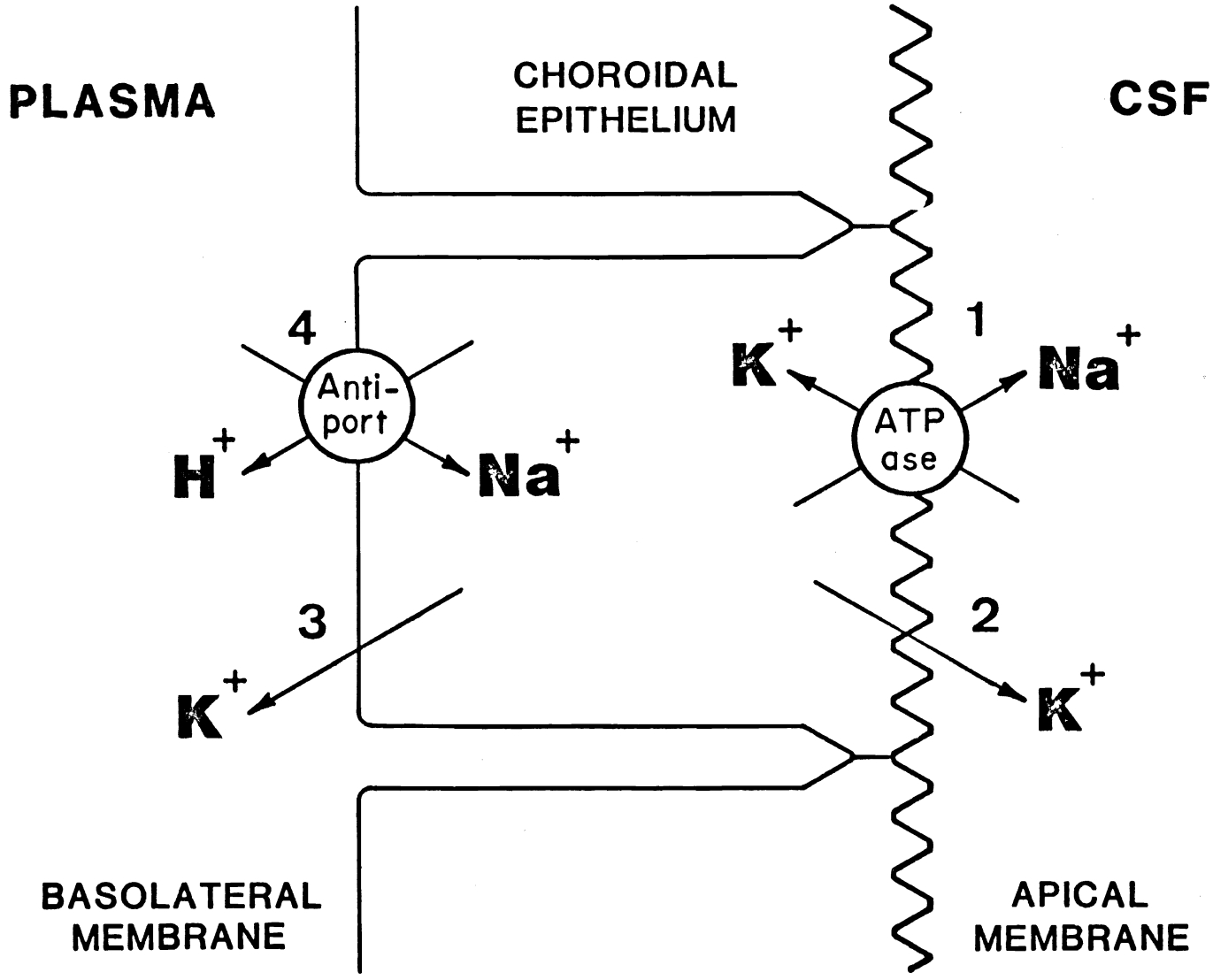
effect was only observed when protons were applied intracellularly. Stanton and Giebisch (1982) demonstrated, with microperfused rat distal tubule in vitro, that a decrease in bath pH inhibited K secretion into the lumen, while an increase in bath pH stimulated K secretion. Boudry, Stoner and Burg (1976) observed, in rabbit cortical collecting tubules perfused in vitro, that when perfusate pH was lowered from 7.4 to 6.8, K secretion into the lumen decreased by an average of 47%; net sodium absorption was also slightly decreased. Since Murphy (unpublished data) demonstrated that an intraperitoneal injection of HCl causes a decrease in plasma, CSF and CP pH, it is proposed that an increase in plasma, CSF and/or CP [H] decreases the permeability of the basolateral and/or apical membranes of the CP to K. Conceivably, such an effect on either or both membranes could lead to an increase in CP [K] (as seen in Chapters 3, 5, 7 and 9). If a reduction in the K permeability of the apical membrane were to occur, a decrease in CSF [K] would be expected. Such a decrease in CSF [K] was observed (Table 5-3, Figure 9-2). Finally, if acidosis were to induce a decrease in the efflux of K (by decreasing the permeability of the CP membranes to K), an increase in the content of Rb (a physiological analog of K) would be expected. LVCP was incubated in vitro in an acidotic medium (simulated CSF) containing ^{86}Rb. As seen in Figure 9-10, an increase in the steady-state volume of distribution of ^{86}Rb occurred without any demonstrable increase in the Na-K

exchange-mediated uptake (influx) of ^{86}Rb (Figure 9-9). Further support that acidosis does not stimulate the uptake of K (i.e., stimulate Na-K ATPase) comes from Skou (1979). He reported that a reduction in pH from 7.4 to 5.7 decreases the activity of Na-K ATPase (ox brain). Henquin (1981) demonstrated that extracellular acidosis reduced the efflux of ^{86}Rb from pancreatic islet cells. Poole-Wilson and Langer (1975) observed that acidosis reduces the efflux of K from septal cardiac cells and leads to an increase in the cardiac content of K. All of the above results, taken together, suggest that acidosis in the CP induces a decrease in the intracellular efflux of ^{86}Rb and K by decreasing the permeability of the basolateral and/or apical membranes to Rb and K. Since the *in vitro* CP is exposed primarily to the acidotic medium on the CSF-facing side, presumably the decrease in ^{86}Rb efflux occurred mainly across the apical membrane.

Conclusion. CP [K] increases and [Na] decreases in the young adult male rat treated intraperitoneally with NH_4Cl. It is concluded that an increase in plasma [H] caused by the intraperitoneal injection of NH_4Cl is the primary stimulus of the NH_4Cl-induced increase in CP [K]. It is further concluded that an increase in plasma, CSF and/or CP [H] causes a reduction in the efflux of K across the basolateral and/or apical membranes of the CP (see #2 and #3 in Figure 10-1). These reductions in K efflux lead to an increase in CP [K]. The observed decrease in CP [Na] is attributed to a reduction in basolateral Na-H exchange (see #4

Figure 10-1. Model of the choroidal epithelium of the blood-CSF barrier and its relationship to the plasma and cerebrospinal fluid.

The arrows indicate the direction of ion movements. The slope of each line indicates the gradient in effect (control conditions) for each ion as it crosses the basolateral (plasma-facing) or apical (CSF-facing) sides of the epithelium (CP). Na-K ATPase activity, primarily located on the apical side of the CP (#1), actively pumps CSF K into the cell as it extrudes intracellular Na; this activity is primarily responsible for the establishment and maintenance of the intracellular and extracellular K and Na gradients, i.e., the intracellular concentration of CP K > plasma and CSF [K]; the intracellular concentration of CP Na < plasma and CSF [Na]. Intracellular K leaves the epithelial cells down an electrochemical gradient (#2,#3); this movement is governed by the permeability of the basolateral and apical membranes to K. It is proposed that NH_4Cl-induced increases in plasma, CSF and/or CP [H], decrease the permeability of the basolateral and/or apical membranes to K efflux (#2,#3); this leads to an accumulation of K within the CP. It is further proposed that an NH_4Cl-induced decrease in plasma [Na], in addition to the increase in plasma [H], decreases CP [Na] by reducing the Na and H gradients responsible for Na influx and H efflux by the basolateral Na-H antiporter (#4).


PLASMA
CHOROIDAL EPITHELIUM
CSF
4
Anti-port
H+
Na+
1
K+
Na+
ATP ase
3
K+
2
K+
BASOLATERAL MEMBRANE
APICAL MEMBRANE

in Figure 10-1). The reduction in Na-H exchange is attributed to an increase in plasma [H] and perhaps a decrease in plasma [Na].

Finally, if systemic acidosis leads to a decrease in CSF pH and thus a release of K into the interstitial fluid of the brain (from CNS cells), a decrease in the efflux of K across the apical membrane of the CP into the CSF could minimize the build-up of [K] in the interstitial fluid.

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