dition of each drug. The recovery time constant of the amplitude of after-hyperpolarization after sodium injections, however, was reversibly increased during this period in the presence of ouabain (Figure 1); the recovery time constants measured from 6 cells were 188 ± 20 sec. On the other hand, it was reversibly decreased during this period in the presence of 0.3 m*M* Ad (Figure 1); the recovery time constants measured from 6 cells were 40 ± 10 sec.

The potassium-activated hyperpolarization was demonstrated with rabbit non-myelinated nerve fibres[6]. A similar potassium-activated hyperpolarization was recorded from present preparations by use of the sucrose-gap method[7]. In the present experiment, isolated ganglia were initially perfused with the K-free Ringer's solutionfor 60 min. When the perfusate was changed to the Ringer's solution (containing 2 m*M* KCl), the potassium-activated hyperpolarization of ganglion cells, which reached a maximum value (2–4 mV) within approximately 3 min which was sustained thereafter, could be consistently recorded (Figure 2). The maximum amplitude of the potassium-activated hyperpolarization of a preparation was fairly constant between 60 and 180 min in the K-free Ringer's solution, provided each application of the Ringer's solution for approximately 3 min was repeated at an interval of 15 min. These potassium-activated hyperpolarizations were completely and reversibly abolished in the presence of 0.005 m*M* ouabain[7].

The potassium-activated hyperpolarizations initiated before, during and after an application of 0.3 m*M* Ad were recorded and compared with each other, in order to examine the effect of Ad and its reversibility. The potassium-activated hyperpolarization produced in the presence of Ad was markedly augmented compared with that recorded in the absence of Ad (Figure 2). Such an augmentation of the potassium-activated hyperpolarization was observed consistently after Ad was applied for more than 10–20 min. The increases in the maximum amplitude observed from 8 preparations were 46 ± 13%.

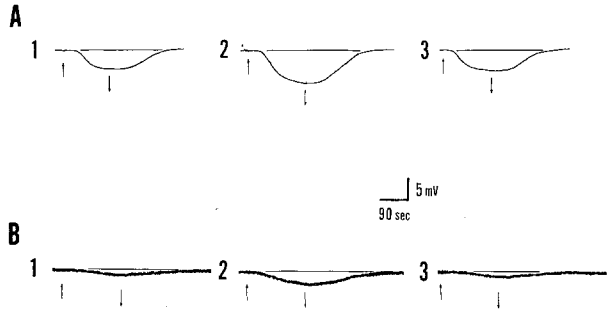


Fig. 2. Potassium-activated hyperpolarizations of bullfrog's sympathetic ganglion cells, being recorded by the sucrose-gap method (A) and an intracellular microelectrode (B). Records were taken before (1), 30 min after an addition of 0.3 m*M* Ad to the perfusate and 60 min after withdrawal of Ad (3). Note reversible increase in the amplitude of potassium-activated hyperpolarizations under the effect of Ad.

The potassium-activated hyperpolarization of a single ganglion cell, which reached a maximum value (2–3 mV) within approximately 3 min, was also recorded by an intracellular microelectrode. Its maximum amplitude was fairly constant between 60 and 180 min in the K-free Ringer's solution, provided each application of the Ringer's solution for approximately 3 min was repeated at an interval of 15 min. When 0.3 m*M* Ad was added to the K-free Ringer's solution, the resting membrane potential of a cell showed no, or small, depolarization (a few milivolts) and the membrane resistance (input resistance) showed no detectable changes. The potassium-activated hyperpolarizations was augmented in the presence of Ad (Figure 2); the increases in the maximum amplitude observed from 8 preparations were 32 ± 8%.

The recovery time constant of positive after-hyperpolarization of action potentials was assumed to characterize the rate of sodium removal, namely the rate of sodium pump from a cell[5]. This assumption was supported by the present result that the recovery time constant was markedly increased by the action of ouabain in a small concentration. On the other hand, the present experiment demonstrated that the recovery time constant was markedly decreased in the presence of Ad. Thus, the rate of sodium pump appeared to be increased in the presence of Ad. The present experiment also demonstrated that the potassium-activated hyperpolarization was augmented in the presence of Ad. If one assumes that the potassium-activated hyperpolarization is generated by an electrogenic sodium pump[6], the electrogenic sodium pump appeared to be accelerated in the presence of Ad. In conclusion, the present experiment results altogether suggested that Ad was able to accelerate the sodium pump, possibly the electrogenic sodium pump. This supports our hypothetical concept that the neurotransmitter, such as Ad, is able to regulate the membrane potential by controlling the active ionic transport.

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## The Renal Concentrating Ability of Newly Born Brattleboro Rats (Hereditary Diabetes Insipidus)

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*Summary.* In Brattleboro rats, there was no difference in urine osmolality between animals with and without diabetes insipidus after water deprivation up to age 14 days, and it appeared at age 18 days due to increase of osmolality in non-diabetic individuals.

Vasopressin (VP) regulates body water content by acting on water reabsorption in the distal segment of the nephron. In young rats, this activity is less pronounced and newly born rats excrete a urine of low osmolality even during dehydration[1–3]. The content of VP in the neurohypophysis of the newly born is lower than in adult animals, but is still sufficient to play a role in osmoregulation[4–6]. The response of the kidneys depends

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partly on the level of circulating hormone, partly on the ability of the distal tubule to respond. VP can not be detected in the plasma of normally hydrated newly born rats up to the age of 3–4 weeks[7]. Antidiuretic activity (ADA) appears in the plasma at age 10 days after a load of hypertonic NaCl[8,9] or at age 23 days after 6–12 h of dehydration[10]. Exogenous VP does not decrease urine flow at age 2 days, and until the age 23 days, the response is not comparable to that in adult animals[1,2,11].

VALTIN and SCHROEDER[12] described the Brattleboro strain of rats which have a genetically determined diabetes insipidus (DI) due to inadequate synthesis of VP in the hypothalamus. Every litter contains homo- and heterozygous individuals differing in terms of the presence of manifest DI. The question was put whether the concentrating ability would be different in young and adult animals of this strain, and at what age homo- and heterozygous animals would differ in this respect.

*Material and methods.* The experiment was carried out on 72 young and 31 adult rats of the Brattleboro strain of both sexes. The young were exposed to 6 h of water deprivation at ages 10, 14, 18 and 22 days. A separate group of adult rats was also deprived of water for 6 h. In the young, the urinary bladder was emptied by perineal stimulation[13]. 10- and 14-day-old animals were kept without mother at 30 °C, the older at 25 °C. In the adults, the urine was collected in metabolic cages between the 4th and 6th h of water deprivation. Urine osmolality was determined by means of a Knauer osmometer. The 24 h water consumption was measured at the age of 27 days, and in the adults also by an automatic 'drinkometer'[14].

*Results and discussion.* After 6 h of water deprivation, the values of urine osmolality showed a typical Gaussian distribution in 10- and 14-day-old rats, whereas in 18- and 22-day-old animals there were two distinct populations (Figure 1). It can be assumed that the group with lower osmolality (I) consists of diabetic animals, while the group with higher osmolality (II) are non-diabetic animals. Mean values of group I were 579.4 ± 58.3 mosmol/l and 401.6 ± 19.2 mosmol/l at ages 18 and 22 days. In group II the corresponding means were 1004.4 ± 70.2 mosmol/l and 1055.6 ± 78.7 mosmol/l for the same age groups. The differences between group I and II were highly significant at both age levels ($p < 0.001$). Separate groups always included the same animals and

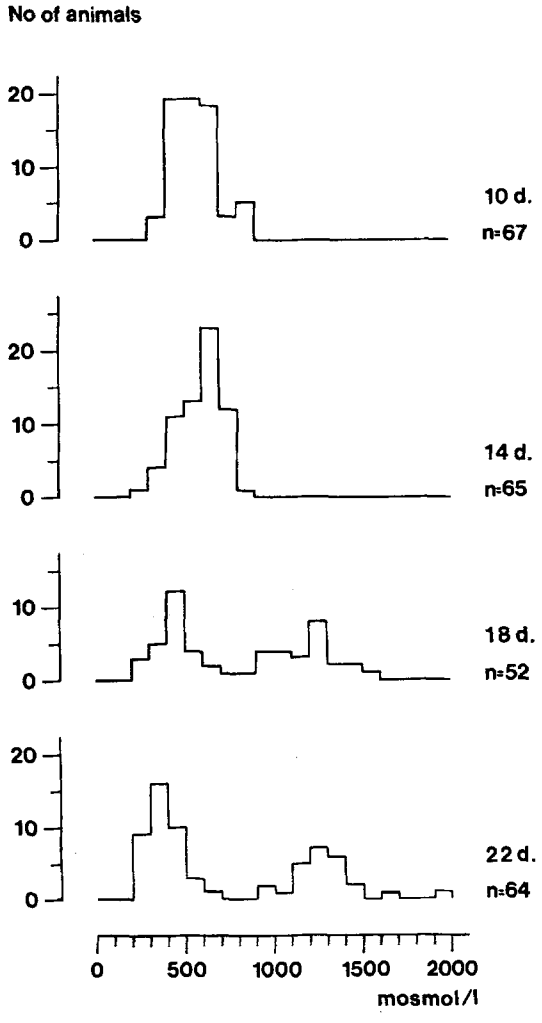


Fig. 1. Histogram representing frequency distribution of values of urine osmotic pressure after 6 h of water deprivation in rats aged 10, 14, 18 and 22 days. Abscissa: urine osmotic pressure in mosmol/l. Ordinate: number of rats in separate class intervals. $n$ = number of animals in which osmolality has been estimated.

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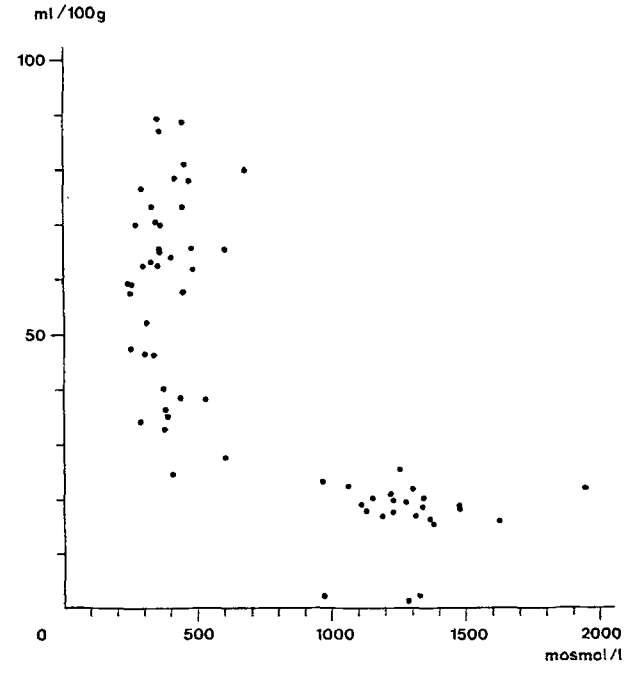


Fig. 2. Urine osmolality after 6 h of water deprivation measured in rats aged 22 days, and 24 h water intake in the same animals at age 27 days. Absissa: urine osmotic pressure in mosmol/l. Ordinate: water intake in ml/100 g b.wt./24 h.

therefore it was possible to distinguish retrospectively groups I and II in 10- and 14-day-old animals as well. At ages 10 and 14 days group I had a mean urine osmolality of 591.0 ± 17.4 mosmol/l and 610.9 ± 23.1 mosmol/l, group II 562.4 ± 26.4 mosmol/l and 583.9 ± 21.3 mosmol/l respectively. Hence the difference in concentrating ability becomes manifest in DI and non-DI rats at ages 18 days, and in adult-hood it further increases (584.0 ± 22.4 mosmol/l in homozygous and 2068.0 ± 134.4 mosmol/l in heterozygous).

Measurement of water intake (Figure 2) showed that, in individuals with a high urinary osmotic pressure, the water intake did not exceed 25 ml/100 g b.wt./24 h. Animals with low osmolality had a high water intake. In DI rats, there is a very tight distribution of values of urinary osmotic pressure with a large scatter in values of water intake, whereas in non-DI rats the reverse is true. A similar non-linear relationship between water intake and urinary osmotic pressure was also observed in DI rats by VALTIN and SCHROEDER[12].

Hence, the concentrating ability of homozygous Brattleboro rats aged 10, 14 and 18 days does not differ from that of adult ones. The difference between homo- and heterozygous animals appeared at the 18th day of life, when the concentrating ability of the heterozygous increased significantly in comparison with corresponding homozygous rats. The data obtained thus provide direct evidence that the presence of endogenous VP plays no role in the concentrating ability of suckling rats as suggested from earlier indirect observations[1-6].

## Use of Indomethacin to Reverse Neonatal Hypotension[1]

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*Summary.* Treatment with indomethacin elevated systemic arterial pressure and pulmonary vascular resistance in hypotensive newborn goats. Indomethacin may be of value in restoration of systemic arterial pressure in stress-induced hypotension.

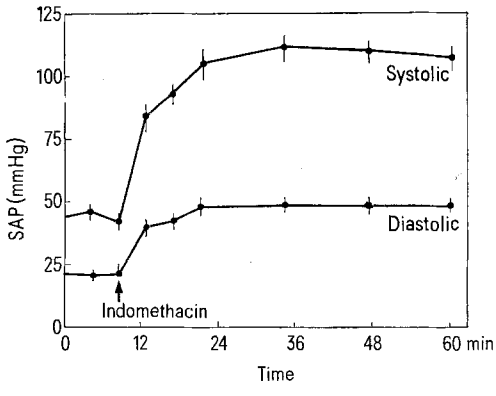
Systemic hypotension in the newborn may be the result of many factors including hypoxia[3], asphyxia[4], respiratory distress[4], hyaline membrane disease[4,5] and hemorrhage[6]. Hypotension, in part, may be the result of prostaglandin release. These experiments were undertaken to evaluate effects of indomethacin, a prostaglandin synthetase inhibitor[7], on the systemic circulation of hypotensive newborn goats.

Hypotension (mean pressure less than 32 mm Hg in the descending aorta) was produced in eleven newborn goats (1–7 days of age, wt. 3.0 ± 0.2 kg[8]) by periods of hypoxia, asphyxia, acidemia, prolonged anesthesia and surgery, or fluid loss. Arterial pH, $Po_2$ and $Pco_2$ were corrected (pH 7.39 ± 0.01, $Pco_2$ = 35 ± 1 mm Hg and $Po_2$ = 160 ± 9 mm Hg) prior to indomethacin administration. Pulmonary vascular resistance was evaluated in 5 newborns by pump perfusing the left lung with blood from the inferior vena cava and measuring pulmonary arterial and left atrial pressures.

Treatment of hypotensive animals with indomethacin (2.4 mg/kg i.a.) increased[9] systolic, diastolic, and pulse pressures (Figure). Mean systemic arterial pressure increased 158% (26 ± 3 to 67 ± 5 mm Hg). Heart rate and pulmonary vascular resistance increased 8% (165 ± 9 to 178 ± 10) and 37% (0.79 ± 0.14 to 1.08 ± 0.19 mm Hg · kg · min/ml), respectively. These effects of indomethacin on cardiovascular dynamics persisted for the duration of monitoring (3 h).

Indomethacin was effective in reversing systemic hypotension in all animals studied. This vasopressor response may be a consequence of the removal of a direct vasodepressor action of prostaglandins on systemic vasculature or removal of inhibition due to prostaglandins of sympathetic vasoconstrictor activity[10]. [In preliminary experiments, treatment with indomethacin increased mean systemic arterial pressure only 12% following phentolamine administration (4 mg/kg, $n$ = 3).].

These results suggest that indomethacin may be useful in reversing stress induced hypotension. Thorough consideration of effects of indomethacin on pulmonary vascular resistance is indicated before indomethacin is used in the newborn.




Effect of treatment with indomethacin (2.4 mg/kg i.a.) on systemic arterial pressure (SAP) of 11 newborn goats with stress-induced hypotension. Results are means ± SE.

[1] Supported in part by Nos. NIH-HL-10834-06, NIH-T01-HL05979-02S1 and Florida Heart No. 74-AG-2.

[2] Acknowledgments. The authors wish to thank N. L. MURTHA, L. TUMARKIN, J. HOWARD and P. EITZMAN for their technical assistance and Merck, Sharpe and Dohme for the gift of indomethacin. This research was conducted under the guiding principles in the care and use of animals approved by the Council of the American Physiological Society and the National Institute of Health.

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