

EVIDENCE FOR THE PRESENCE OF OSMORECEPTORS IN MEDULLA OF THE DOG

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Summary : Hypertonic solutions of different substances were injected into the vertebral artery of dogs anesthetized with chloralose, preventing their access to the hypothalamic osmoreceptors by ligating the basilar artery and both the external carotid arteries. The hypertonic solution of sodium chloride produced graded inhibition of water diuresis and a concomitant rise in plasma antidiuretic hormone (ADH) level; hypertonic solution of glucose produced lesser effect. Hypertonic urea solution, on the other hand, did not alter the course of water diuresis. It was concluded that osmoreceptors are also present in the medulla which sense the changes in blood osmolarity and accordingly modify the ADH release.

Key words : osmoreceptors

ADH

INTRODUCTION

Serum osmolarity plays a major physiological role in maintenance of water balance in the body. Increase in plasma osmolarity induced by hypertonic solutions or dehydration has been shown to release anti-diuretic hormone (ADH) in rabbits (12), rats (6, 9) dogs (14, 16), goats (1, 7) monkeys (15) and man (11). In dogs anti-diuresis resulting from injections of hypertonic solutions into the internal carotid artery led Verney (14) to postulate the existence of osmoreceptors in the arterial bed of internal carotid artery. The osmoreceptors were later found to be localized in supraoptic region of the hypothalamus (8).

Injections of hypertonic solutions into the vertebral artery increase the electrical activity in region of obex in the medulla of dogs, indicating presence of osmosensitive cells in the lower brain stem (4). The relevance of these sites in release of ADH has not been investigated. The present study was undertaken to investigate the role of "osmosensitive cells" of medulla oblongata in ADH release.

MATERIAL AND METHOD

The study was performed in 54 mongrel dogs (10–15 kg) of either sex. The animals were anesthetized with 10 ml/kg of warm 1% α -chloralose in 0.9% saline infused intravenously. Femoral artery was used for recording blood pressure on a kymograph with the help of a mercury manometer and femoral vein was used for continuous intravenous infusion. For recording of urine output both the ureters were cannulated and the output fed to a drop recording assembly. For injecting hypertonic solutions, the right vertebral artery was exposed and cannulated in the portion between its origin and entry into the vertebral canal. The right jugular vein was cannulated to collect blood for ADH estimation. In order to prevent entry of hypertonic solutions to the hypothalamic osmoreceptors, the basilar artery was ligated at the junction of pons and medulla as described below. The method has been described for cats (10) ; it was modified by us for dogs.

The ventral surface of occipital bone was exposed. The tympanic bullae were palpated and a hole (0.5 cm diameter) was drilled in the basilar part of the occipital bone in between the bullae. The hole was enlarged to approximately 1 to 1.5 cm diameter. The dura-mater was incised and the basilar artery was clearly visualized and ligated. To prevent the bypass of solutions via the spinocerebral branch of occipital artery and external carotid artery to the internal carotid artery, both external carotid arteries were ligated near their origin. These procedures precluded the entry of hypertonic solution to the hypothalamic osmoreceptors. The preparations were stabilized for 2 hr before use. At the end of experiment 2 ml of India ink was injected in vertebral artery to test for leakage of the dye to the hypothalamus. The experiments in which the ink was traceable in the hypothalamus at autopsy were excluded from the results.

For production of water diuresis and maintenance of anesthesia, a solution containing 1.8% dextrose, 0.14% sodium chloride and 0.1% α -chloralose was infused intravenously at a rate of 2.5 to 4.0 ml/min until a total volume of 500 ml/kg was delivered. Thereafter, the rate of infusion was adjusted to exceed slightly the rate of urine flow to maintain a constant urine output.

Hypertonic solutions (2 ml) of sodium chloride, sucrose, glucose and urea were injected into the vertebral artery over a period of 10 sec. The variations in volume and speed of injections, if any, are specified in Table I. The ADH from jugular venous blood samples was extracted on a column of XE-64 resin and assayed in rat diuresis model as described earlier (2, 3).

RESULTS

1. Effect of hypertonic sodium chloride solution on water diuresis :

The 2 ml of 0.85 M sodium chloride solution injected into vertebral artery over a period of 10 sec produced inhibition of diuresis within 10 min, the peak anti-diuresis (42%) was observed within 30 min and the complete recovery was obtained within 60 min. The plasma ADH level was also high in the blood collected at the peak effect. The effect of different volumes and strength of sodium chloride solutions injected over a variable period of time on urine output and ADH release are summarized in Table 1. The anti-diuresis was more, if strength of the solution was raised (67% by 1.0 M and 80% by 1.2 M) or volume of injection was increased (82% by 5 ml of 0.85 M) while other variables were kept constant. On the other hand, increase in the time taken for injection (15 sec) reduced the anti-diuretic response (38%) provided the strength (1.2 M) and volume (2 ml) were the same. The ADH level in all these experimental variations showed

TABLE 1 : Effect of hypertonic solutions injected into the vertebral artery on water diuresis and blood ADH level in dogs.

Solution injected	Number of experiments	Time (sec) taken for injection	Urine output (ml/5 min) \pm SEM		Plasma ADH level (μ U/ml)	
			Control	At peak effect	Control	At peak effect
Sodium chloride (0.85 M, 2 ml)	8	10	5.0 \pm 0.6	2.9 \pm 0.4*	0.54 \pm 0.01	0.95 \pm 0.03*
Sodium chloride (0.85, 5 ml)	6	10	5.8 \pm 0.43	1.4 \pm 0.3***	0.45 \pm 0.04	1.2 \pm 0.06**
Sodium chloride (1.0 M, 2 ml)	6	10	5.9 \pm 0.45	1.9 \pm 0.18**	0.32 \pm 0.01	0.7 \pm 0.02**
Sodium chloride (1.2 M, 2 ml)	6	10	4.2 \pm 0.9	0.6 \pm 0.42***	0.64 \pm 0.05	2.0 \pm 0.08***
Sodium chloride (1.2 M, 2 ml)	6	15	5.4 \pm 0.7	3.6 \pm 0.3*	0.72 \pm 0.03	1.4 \pm 0.06**
Sucrose (2.0 M, 2 ml)	8	10	5.0 \pm 0.2	3.2 \pm 0.16*	0.46 \pm 0.01	0.92 \pm 0.04**
Glucose (2.0 M, 2 ml)	6	10	6.0 \pm 0.4	3.6 \pm 0.2*	0.24 \pm 0.008	0.51 \pm 0.02*
Urea (2.0 M, 2 ml)	8	10	4.7 \pm 0.4	4.1 \pm 1.6	0.68 \pm 0.03	0.76 \pm 0.05

Value differs significantly from Control :

*P < 0.05, **P < 0.01 and ***P < 0.001.

parallel changes. The time course of antidiuresis was similar in all these experiments except for minor variations. Following all these injections there was 10–20 mm Hg rise of blood pressure, which persisted for 10 min only.

2. *Effect of hypertonic sucrose, glucose and urea solutions on water diuresis :*

The 2 ml of 2.0 mol sucrose solution produced inhibition of diuresis within 10 min, the maximum effect (33%) was observed within 20 min and complete recovery was obtained within 60 min. The glucose solution produced similar but lesser effect on urine outflow. There was a concomitant rise in plasma ADH level (Table I). However, the 2 ml solutions of 2.0M urea injected in the vertebral artery and 2 ml of 1.2 M sodium chloride injected into femoral vein, failed to alter the course of water diuresis. With all the injections in vertebral artery, there was 10–20 mm Hg rise of blood pressure, which persisted for 5–10 min only.

DISCUSSION

Verney (14) observed that in the dogs intracarotid arterial injection of hyperosmolar solutions of sodium chloride, sodium sulphate and sucrose produced significant inhibition of water diuresis, whereas, equiosmolar solution of glucose was less effective and that of urea was ineffective in altering the course of water diuresis. The osmosensitive cells in the arterial bed of internal carotid artery were located in the supraventricular nucleus of the hypothalamus (8, 14). Verney (14) suggested that the cell membrane of these cells which he named "osmoreceptors", is impermeable to sodium chloride and sucrose, less permeable to glucose and freely permeable to urea. Further, he postulated that those ions which did not cross the cell membrane freely created a hyperosmolar environment outside these cells and set up a stimulus, causing ADH release from neurohypophysis.

In the present investigation, three varying concentrations of sodium chloride injected in equal volumes into the vertebral artery produced concentration-related inhibition of diuresis and a concomitant rise in plasma ADH titre (see, Table I). Further, antidiuresis was volume dependent for equi-osmolar solution and also dependent on injection time when volume and osmolarity of solutions were the same. Thus, it appears that it is the total concentration of the impermeable ions present at a time around the osmosensitive cells which governs the magnitude of anti-diuretic response. This suggestion gets further support from the observation that hyperosmolar sodium chloride solution injected into femoral vein failed to alter the course of water diuresis, probably as the solution reaching the medulla become diluted on route. Hypertonic solutions of sucrose and glucose also inhibited water diuresis although to a lesser degree but the course of antidiuresis was similar to that produced by sodium chloride. Hypertonic solution of urea failed to alter the course of water diuresis. Since urea freely enters the cells it is

unable to create a hyperosmolar extracellular environment. Thus, the effect of impermeable (sodium chloride), semipermeable (glucose and sucrose) and freely permeable (urea) agents on the osmosensitive cells of medulla is similar to the effect of these substances on hypothalamic osmoreceptors as judged by inhibition of diuresis and ADH release. The rise in blood pressure occurred with all the hyperosmolar solutions but was not responsible for ADH release as rise in blood pressure has been reported to inhibit ADH release (13). Therefore, it can be suggested that in dogs the osmosensitive cells are also present in the arterial bed of vertebral artery which respond to hyperosmolar solutions in a similar manner as described for hypothalamic osmoreceptors by Verney (14). Ligation of basilar artery and both the external carotid arteries prevented the direct and back flow of hypertonic solutions to the hypothalamic osmoreceptors. Hence it can be said that the osmoreceptors of the vertebral arterial bed situated in the medulla are also capable of modulating the ADH release in response to increase in plasma osmolarity and probably they participate in physiological regulation of ADH release in response to changes in osmolarity.

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