EFFECTS OF CENTRALLY ADMINISTERED INSULIN ON URINE OUTPUT* AND SODIUM EXCRETION IN DOGS

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Summary: The effects of insulin administration via intracerebroventricular (ICV), third ventricular (TV) and intracisternal (IC) routes on the urine output and sodium excretion have been studied in mongrel dogs. The central administration of insulin resulted in a significant increase in urine output and sodium excretion. This diuresis and natriuresis was not observed in animals which had undergone either spinal-cord transectomy or adrenalectomy. The insulin-sensitive receptors for this diuresis and natriuresis seem to be present in the vicinity of the fourth-ventricle of the brain.

The observations in the present study suggest that the probable efferents might be the sympathetic fibers in the spinal-cord. The centrally administered insulin inhibits the secretion of some substance from the adrenal cortex, which in turn results in the diuresis and natriuresis in animals.

Key words: central insulin urine output sodium excretion

INTRODUCTION

The concept set forth by Jungmann *et al.* (17) that a lesion in the floor of the fourth ventricle resulted in polyuria with saluresis can be useful as a first approximation towards the relationship between the central nervous system (CNS) and the kidneys. In the light of present knowledge, we know that CNS has an influence on the renal tubular functions (23, 29). In an orderly analysis of efferents, the observation that the stimulation of splanchnic nerves causes polyuria with saluresis (17) is now supported by the evidence that the kidneys are innervated with sympathetic fibers (21, 28).

James *et al.* (15) have proposed that insulin has an oliguric effect in animals. Besides, the presence of insulin in cerebrospinal fluid has been demonstrated (11, 14), many workers have shown that centrally administered insulin caused a hypoglycaemic effect in animals (4, 30).

In the present investigation, an attempt has been made to study the effect of centrally administered insulin on urine output and electrolyte excretion in contrast to its oliguric effect on intravenous administration in animals. The study has been further ventured to identify the site, the efferents and the organ influencing the urine output and electrolytes excretion.

MATERIALS AND METHODS

The study was conducted on 60 adult mongrel dogs of either sex, weighing between 10 to 15 *kg*. The animals were fasted for 18 hrs before being anaesthetised by a slow injection of

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chloralose (80 *mg/kg* body weight) in a leg vein. As this anesthesia might prevent or mask the effects of centrally administered insulin, experiments on ten animals were carried out after pentobarbitone sodium (30 *mg/kg*).

In 40 animals the ICV cannula was anchored in the left lateral ventricle of the brain by the technique of Feldberg *et al.* (7). The successful anchoring in the ventricle was indicated by the appearance at the top of the cannula of cerebrospinal fluid (CSF) which rose and fell along with the pulse and also when the dog's head was lowered and raised. The CSF from the cannula was examined microscopically for erythrocyte and epithelial cells in order to ascertain whether the cannula caused any lesions in the surrounding tissues. The experiment was discontinued whenever there was any evidence of haemorrhage. In ten of the early experiments it was confirmed that the insulin solution injected through the ICV-cannula actually reached the ventricle because, carbon-black (added to insulin solution) was found in all the ventricles of brain during autopsy.

The TV administration of insulin was done in 6 animals by the technique of Zucker *et al.* (31).

In 8 animals the IC administration of insulin was done by the technique of Chowers *et al.* (4).

The experiments were repeated on animals after the following surgical procedures:

(1) Vagotomy : in 10 animals by removing a 2.0 cm piece from both the vagi at the level of 4-6th cervical vertebrae.

(2) Spinal-cord transectomy: in 8 animals by the teachnique of Ezdinli *et al.* (6).

(3) Adrenalectomy : in 10 animals by the technique of Edwards (5).

Intravenous (I/V) administration :

In a separate set of 10 animals insulin was administered intravenously and the effects were observed in normal animals, vagotomised animals, spinal-cord transectomised animals and adrenalectomised animals.

Control experiments:

The control experiments were carried out on 10 animals by administering the inactivated insulin via the ICV route. The inactivation was done by alkalinization and then bringing up the neutral pH (4). In order to exclude the effect of the preservative substances present in the insulin solution, a 0.1 *ml* solution (1.6% glycerol, 0.25% phenol I.P. and hydrochloric acid to bring the pH up to 3.2-3.5) was administered into the lateral ventricle of the brain (3 animals). Moreover, the control experiment was carried out along with every surgical procedure.

Insulin solution and its dose:

Insulin I.P. (Boots Company India Ltd., 40 *U/ml*) was used. 0.1 *ml* (4.0 *U*) of this preparation was diluted with normal saline to give 0.1 *U/ml*. When required, it was diluted further by normal saline. The dose 0.025 *U* in 0.1 *ml* was used in all experiments. After the dilution the solution was kept in water bath at 37°C. for 30 min before being injected.

The insulin solution instilled into the lateral ventricle so as to confirm that the insulin solution injected through the ICV-cannula actually reached the ventricle, was prepared by mixing 4 parts of (0.025 *U*) insulin solution with one part of a suspension of carbon black in normal saline.

Experimental procedure, sample collection and estimation:

After anesthetising the animal, normal saline was infused at the rate of 2.0 *ml*/min so as to meet the physiological needs of the body. The right femoral artery was connected to a mercury monometer so as to record the mean blood pressure. After the planned surgical procedure the animal was allowed time (one to two hr) to overcome the surgical shock. Both the ureters were cannulated and the urine samples were collected in polythene tubes after every 10 or 15 min for a period of 2 hrs (after the administration of the insulin solution).

The volume of urine output was measured immediately but the estimation of sodium and potassuim was done after the experiment was over by the flame-photometer. The sodium and potassium estimation was done in each sample of urine collected for 2 hrs.

The results expressed are the means $\pm$SE after applying the Student 't' test.

RESULTS

The preliminary experiments were set up to study and compare the effects of I/V and the central administration (ICV) of insulin on urine output and electrolyte excretion. The I/V administration of insulin caused an insignificant ($P>0.05$) fall in urine output from 1.8 ± 0.4 to 1.0 ± 0.4 *ml* after 10 min from the dose administration. Urinary sodium concentration decreased insignificantly ($P>0.05$) from 124.0 ± 4.0 to 120.0 ± 7.0 mEq/L but there was no change in urinary potassium concentration (from 106.2 ± 8.0 to 105.8 ± 6.8 mEq/L) nor in the blood pressure (from 110.0 ± 12.2 to 112.4 ± 8.6 mm of Hg) of the animal. On the contrary the ICV administration resulted in a significant ($P<0.01$) increase in urine output from 3.0 ± 0.6 to 5.5 ± 0.3 *ml* after 45 min of administering the dose. Urinary sodium concentration increased significantly ($P<0.05$) from 120.0 ± 6.0 to 154.0 ± 11.0 mEq/L but urinary potassium concentration (from 114.6 ± 8.2 to 112.2 ± 6.8 mEq/L) and the blood pressure (from 108.0 ± 4.8 to 108.0 ± 8.0 mm of Hg) did not show any change in the mean values.

TABLE I: Mean values of urine volume (U.Vol.) and urinary sodium (U.Na) and potassium (U.K) concentration after the ICV administration of normal saline, inactive insulin (I-Sol.) and the solution of preservative substances (P-Sol.) in control animals.

0.1 *ml* fluid	*No. of* animal		*Initial value at 0 hr*	*After 45 min*	*After 90 min*
Normal saline	5	U. Vol.	3.2 ± 0.4	3.4 ± 0.4	3.2 ± 0.6 *ml*/15 min
		U. Na	124.0 ± 12.2	120.6 ± 8.8	128.2 ± 10.8 mEq/L
		U.K	118.6 ± 10.8	117.4 ± 10.2	120.8 ± 11.0 mEq/L
I-Sol.	5	U. Vol.	2.8 ± 0.6	2.8 ± 0.4	3.0 ± 0.4 ml/15 min
		U. Na	118.6 ± 10.8	117.8 ± 12.0	118.4 ± 10.4 mEq/L
		U.K	116.8 ± 12.2	118.6 ± 10.8	116.4 ± 11.6 mEq/L
P-Sol.	5	U. Vol.	3.2 ± 0.6	3.0 ± 0.4	3.0 ± 0.6 *ml*/15 min
		U. Na	123.2 ± 16.6	130.0 ± 6.4	126.6 ± 8.8 mEq/L
		U.K	120.8 ± 14.2	118.0 ± 8.6	118.8 ± 8.6 mEq/L
Normal saline in adrenalectomised animal	5	U. Vol.	1.0 ± 0.6	0.9 ± 0.6	0.8 ± 0.4 *ml*/15 min
		U. Na	98.4 ± 20.0	92.8 ± 12.8	99.6 ± 16.2 mEq/L
		U.K	100.0 ± 14.8	102.0 ± 16.4	98.8 ± 12.6 mEq/L

In order to study the role of efferents in the transmission of impulses from CNS to various organs, the effects of insulin administration by I/V and ICV routes were studied. The I/V administration of insulin in vagotomised or spinal-cord transectomised animals did cause similar effects to those obtained in normal animals. The ICV administration of insulin caused a significant ($P < 0.01$) increase in urine output from 3.2±0.4 to 5.2±0.3 *ml* at 45 min after the administration of the dose. Urinary sodium concentration increased significantly ($P < 0.01$) from 116.0±3.0 to 146.5±7.0 mEq/L. There was no change in urinary potassium concentration (from 110.0±6.6 to 108.6±5.8 mEq/L) and blood pressure (from 112.8±8.0 to 116.0±4.4 mm of Hg). The ICV administration of insulin in spinal-cord transectomised animals did not cause a change in urine output and urinary sodium concentration (Table II).

In an attempt to locate the site of action of the centrally administered insulin, three possible routes were considered. The observations obtained when IC route of administration was adopted, were found to be the most significant ($P < 0.001$): there was a marked increase in urine output from 3.2±0.6 to 7.2±0.4 *ml* at 30 min after the dose administration. Urinary sodium concentration increased significantly ($P < 0.001$) from 111.5±2.0 to 167.0±10.0 mEq/L. There was no change in urinary potassium concentration (from 110.0±6.0 to 112.0±4.0 mEq/L) and blood pressure (from 109.0±6.8 to 110.0±5.6 mm of Hg). The results obtained by administering the insulin solution via ICV or TV routes were similar and were lower than those obtained via IC route (Table II).

TABLE II: Mean values of urine volume (U.Vol.), urinary sodium (U.Na) and potassium (U.K) concentration and mean blood pressure (B.P.) after the administration of insulin via the I/V and central routes in animals.

Route	*Surgical procedure*	*No. of animal*		*Initial value*	*Maximum value*	*Time in min*
				U. Vol. ml/15 min B.P. mm of Hg	*U. Na & U.K mEq/L*	
I/V	Normal animals	5	U. Vol.	1.8 ± 0.4	1.0 ± 0.4*	10
			U. Na	124.0 ± 4.0	120.0 ± 7.0*	
			U.K	106.2 ± 8.0	105.8 ± 6.8	
			B.P.	110.0 ±12.2	112.4 ± 8.6	
	Adrenalectomised animals	3	U. Vol.	1.0 ± 0.6	0.6 ± 0.2 *	10
			U. Na	120.6 ± 6.0	124.2 ± 6.0*	
			U.K	112.2 ± 8.0	111.8 ± 6.8	
			B.P.	90.0 ± 8.6	86.6 ± 10.0	
ICV	Normal animals	8	U. Vol.	3.0 ± 0.6	5.5 ± 0.3***	45
			U. Na	120.0 ± 6.0	154.0 ±11.0**	
			U.K	114.6 ± 8.2	112.2 ± 6.8	
			B.P.	108.0 ± 4.8	108.0 ± 8.0	
	Vagotomised animals	6	U. Vol.	3.2 ± 0.4	5.2 ± 0.3***	45
			U. Na	116.0 ± 3.0	146.5 ± 7.0***	
			U.K	110.0 ± 6.6	108.6 ± 5.8	
			B.P.	112.8 ± 8.0	116.0 ± 4.4	
	Spinal-cord-transectomised animals	6	U. Vol.	2.1 ± 0.8	2.2 ± 0.2*	45
			U. Na	110.0 ± 4.0	108.5 ± 7.0*	
			U.K	116.6 ± 8.8	112.4 ± 8.6	
			B.P.	84.0±14.6	80.6 ± 16.0	
	Adrenalectomised animals	8	U. Vol.	1.6 ± 0.8	1.4 ± 0.8*	45
			U. Na	103.0 ± 4.5	104.0 ± 6.0*	
			U.K	106.0 ± 6.8	104.6 ± 8.4	
			B.P.	82.0 ±16.4	86.2 ± 10.8	
TV	Normal animals	6	U. Vol.	2.0 ± 0.4	4.5 ± 0.5**	45
			U. Na	117.0 ± 7.0	152.0 ± 5.0***	
			U.K	103.4 ± 8.2	104.0 ± 7.4	
			B.P.	108.0 ±10.6	110.4 ± 12.0	
IC	Normal animals	8	U. Vol.	3.2 ± 0.6	7.2 ± 0.4****	30
			U. Na	111.5 ± 2.0	16 7.0 ±10.0****	
			U.K	110.0 ± 6.0	112.0 ± 4.0	
			B.P.	109.0 ± 6.8	110.0 ± 5.6	

*P > 0.05 **P < 0.05 ***P < 0.01 ****P < 0.001

The I/V administration of insulin in adrenalectomised animals resulted in an insignificant ($P > 0.05$) decrease in urine output from 1.0 ± 0.6 to 0.6 ± 0.2 *ml* after 10 min. Urinary sodium concentration increased insignificantly ($P > 0.05$) from 120.6 ± 6.0 to 124.2 ± 6.0 mEq/L. Similarly, there was no change in urinary potassium concentration (from 112.2 ± 8.0 to 111.8 ± 6.8 mEq/L) and blood pressure (from 90.0 ± 8.6 to 86.6 ± 10.0 mm of Hg). On the contrary, the central administration of insulin in adrenalectomised animals did not cause a change in urine output, urinary sodium and potassium concentration (Table II).

The administration of normal saline, inactive insulin and insulin preservative substances via ICV route in different group of control animals showed no special change in urine output and urinary sodium concentration (Table I). Moreover, the urine output and urinary electrolyte excretion were found to be stable for 4 hrs.

A change in the anesthetic substance did not show any alteration in the results obtained from either of them.

DISCUSSION

The centrally administered insulin caused a significant diuresis and natriuresis in contrast with its oliguric effect on intravenous administration (15). These effects were observed irrespective to its hypoglycaemic effect on central administration of insulin in animals (1). The response to centrally administered insulin is mediated through some central structures as is evident from the two absolutely opposite effects (oliguria in I/V; diuresis in ICV) as well that there was no effect of centrally administered insulin in spinal-cord transectomised animals. Moreover, Chowers *et al.* (4) have reported that insulin does not cross the CSF-blood barrier.

Since there is no difference in the diuresis and natriuresis on central administration of insulin in vagotomised animals, it can safely be suggested that the sympathetic fibers in the spinal-cord act as efferents on renal functions (9,17,23).

Two possible mechanisms for the diuresis and natriuresis under the influence of CNS. have been cited so far:

(i) An alternation in the renal blood flow due to neural stimulation either directly (12) or via the renin secretion (9,27) causes a change in glomerular filtration rate and thereby a change in urine output and sodium excretion. The glomerular filtration rate is directly proportional to the renal blood flow (9,10).

(ii) **A hormonal mechanism:**

A. A decrement in antidiuretic hormone secretion either due to low-osmolarity stimuli (22) or metabolic acidosis (2) or due to catecholamines from the adrenal medulla (8).

B. Adrenocorticoids (other than aldosterone) having a diuretic effect on renal tubules (8,20).

C. A decrement in aldosterone (a potent antidiuretic agent) secreted from the adrenal cortex (9,13,24).

The observations in the present study do not support the proposition that the alternation in renal blood flow causes a change in urine output because no change in blood pressure has been observed. Moreover, if the diuresis is due to an increased renal blood flow, it would have appeared even in adrenalectomised animals.

Although the results in the present study tend to support the role of a hormonal mechanism, the diuresis and natriuresis cannot be due to a centrally secreted substance because centrally administered insulin could not cause the diuresis and/or natriuresis in spinal-cord transectomised animals nor could it in adrenalectomised animals. Similarly, the role of adrenocorticoids (other than aldosterone) can also be safely excluded because their secretion is chiefly dependent on centrally secreted adrenocorticotropic hormone. The role of nervous influence on adrenocorticoid secretion is still controvertial and the influence of these adrenocorticoid (16,19) and antidiuretic hormone (9,26) on urinary sodium excretion is negligible.

Our observations suggest that the diuresis and natriuresis on central administration of insulin is possibly due to a decrement in aldosterone secretion, to a great extent if not totally but inasmuch as aldosterone secretion depends upon the renin-angiotensin-aldosterone system (3,27). Moreover, the release of renin is a direct activity of the renal tubules (27,18). The present observations that there is no change in urine output and urinary sodium concentration on central administration of insulin in spinal-cord transectomised and in adrenalectomised animals supports the conclusions of many workers (9,12,13,25,29).

The attempt to locate precisely the site of action of centrally administered insulin has not yielded conclusive results but the results obtained from IC administration as compared to those obtained by ICV and TV routes, support the sites proposed by Jungman *et al.* (17) and Wise *et al.* (29) viz. the insulin-sensitive receptors are located in or near the vicinity of the fourth ventricle of the brain.

The results in the present study also suggest that the central administration of insulin, in contrast to its oliguric effect on I/V administration, causes an inhibition of the renin-angiotensin-aldosterone system via the sympathetic fibers in the spinal-cord. This is responsible for the diuresis and the natriuresis in the animals.

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