EFFECT OF CENTRALLY ADMINISTERED GLUCAGON ON URINE OUTPUT IN DOGS

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Summary: The effect of minimal doses of glucagon, administered by intracerebroventricular (ICV) and intracisternal(IC) routes, on urine output in mongrel dogs have been studied. The dose of 2.0 μg of glucagon was found to be the minimal dose for diuresis on peripheral administration. This dose when centrally administered, produced an oliguric effect in animals. This effect was not observed in vago-sympathectomised-spinal cord transectomised or adrenalectomised animals. It is suggested that the probable efferents might be the sympathetic fibres as they are present in vagi nerves as well in the spinal cord (26). The observations made in an attempt to find out the organ responsible for the oliguric effect, showed that some substance released from the adrenal cortex has an influence on the kidney's excretory function.

Key words: intracerebroventricular   glucagon   urine output

INTRODUCTION

The renal tubular activities are chiefly under the influence of certain hormones such as the antidiuretic hormone (4), Adrenocorticoids (8) and renin (6,18). Besides, the central nervous system (CNS) also plays an important role in modulating kidney functions (16,21). As the kidneys are innervated with adrenergic fibres (20, 22) it has been suggested that sympa-thetic stimulation reduces the glomerular filteration rate to a great extent (15, 20). Moreover, the blood osmolarity has a direct influence on kidney-excretion (12). Many workers have demonstrated that the administration of insulin in the lateral ventricle or in the fourth ventricle of the brain of animals caused not only hypoglycaemia (7,25,27,30) but also diuresis (1). In the present investigation, an attempt has been made to study the effect of centrally administered glucagon (a potent hyperglycaemic hormone, causing diuresis only when the blood glucose level rises above the renal-threshold) on urine output in dogs. In addition, efforts have also been made to identify the nervous route and the organ involved in influencing the urine output.

MATERIALS AND METHODS

The experiments were conducted on thirty three adult dogs of either sex with varying weight. The animals were anaesthetised with nembutal (Phenobarbitone sodium) 30 *mg/kg* body weight. The animals were fasted for sixteen hrs before starting the experiment and during this period water was freely available.

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Experimental Procedure : ICV cannula was inserted in 20 animals in left lateral ventricle of the brain by the technique of Feldberg *et al.* (11). The cerebrospinal fluid from the cannula was examined microscopically for erythrocytes and epithelial cells in order to ascertain that the cannula caused no lesions in the surrounding tissues. The experiment was discontinued whenever there was an evidence of haemorrhage.

IC administration was done in 5 animals by the technique of Chowers *et al.* (7).

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

1. Vagosympathectomy - in 6 animals (3 each in ICV and IC)

As the vagi nerves accompany the cervical trunk of the sympathetic nerve (26), a 2 cm piece from both the trunks was removed at the level of 4-6th cervical vertebrae.

2. Spinal cord transectomy - in 5 animals by the technique of Ezdinli *et al.* (10).
3. Spinal cord transectomy along with vagosympathectomy - in 3 animals.
4. Adrenalectomy - in 6 animals.

Intravenous administration (I/V) was done in a separate set of experiments on 6 animals after following above mentioned surgical procedures. A minimal dose of 2 μg of glucagon in 0.5 *ml.* was established by administering gradually decreasing amounts of glucagon in the vein which could set diuresis in an animal.

Both the ureters were cannulated by a polythene tube. The normal saline, at the room temperature was infused by a drip set in a vein at the rate of 2 *ml*/min as to meet the physiological need of the body. The urine samples were collected in the following sequence:

(i) After starting the saline drip, three samples at an interval of 15 min; (ii) After completing the surgical procedure, samples were collected at an interval of 15 min till three samples were equal; (iii) After administration of glucagon dose, one sample each at 15 min interval for two hr.

The volume of urine output was measured immediately.

Hormone Solution : Crystalline Porcine Glucagon was accurately weighed and dissolved in dil. HCL (pH 2.6—2.8) (Analar). This solution which served as stock solution, was suitably diluted with 1.6% glycerine so as to give a solution of 1 *ug* glucagon in 0.1 *ml.* When required, this solution was further diluted with normal saline at the time of the experiments. The stock solution was kept at 4°C. The pH of the final solution used for injection varied from 2.5 to 2.6 and the solution before injection was kept in a water bath at 37°C for 30 min.

Control Experiment : In a control experiment, the same amount of normal saline at 37°C containing the same amount of 1.6% glycerine with a pH 2.5 to 2.6 was administered in the lateral ventricle of four dogs. Urine volume in all the four dogs was observed for four hr at 15 min intervals and it was found to be quite stable.

Results are expressed as mean $\pm$ SE and their significance was tested by applying student 't' test.

RESULTS

The initial experiments consisted of a comparison of the effects of I/V and ICV administrations of glucagon on urine output. The results are shown in Fig. 1. I/V administration resulted in a significant ($P < 0.05$) increase in urine output from 2.8 ± 0.2 to 3.2 ± 0.2. ICV administration caused a significant ($P < 0.01$) decrease in urine output from 3.6 ± 0.4 to 1.0 ± 0.3. Similar results on urine output were obtained when glucagon was administered through I/V route in vagosympathectomised or spinal cord transectomised or spinal cord tansectomy with vagosympathectomised or adrenalectomised animals.

In order to study the role of the spinal cord and vagosympathetic nerves in transmission of impulses from CNS to various organs, the effect of ICV administration of glucagon was studied in vagosympathectomised, spinal cord transectomised and spinal cord transectomy with vagosympathectomised animals (Fig. 1 & 2). ICV administration in vagosympathectomised animals
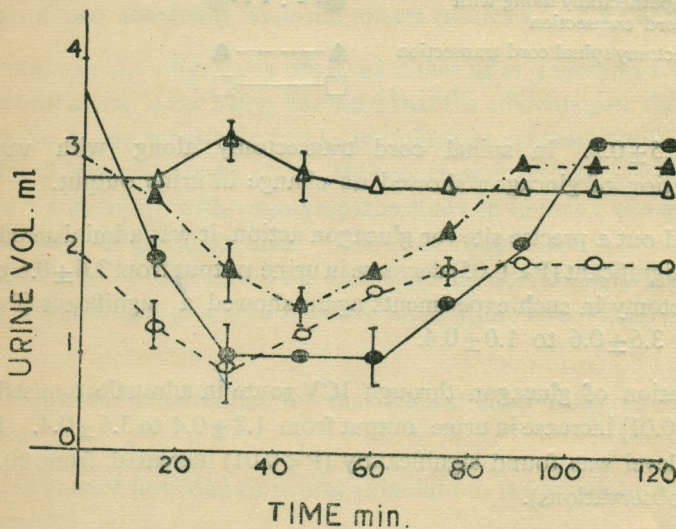

Fig. 1:   Effect of I/V and ICV administration of gulcagon on urine output. The results shown are means $\pm$ SE.

I/V   △————————△
ICV-Normal   ●————————●
ICV-Vagosympathectomy   ●- - - - - - ●
ICV-Spinal cord transectomy   ▲—.—.—▲

resulted in a significant (P<0.05) decrease in urine output from 2.0±0.4 to 0.9±0.4, while in spinal cord transectomised animals it caused a significant (P<0.05) decrease in urine output
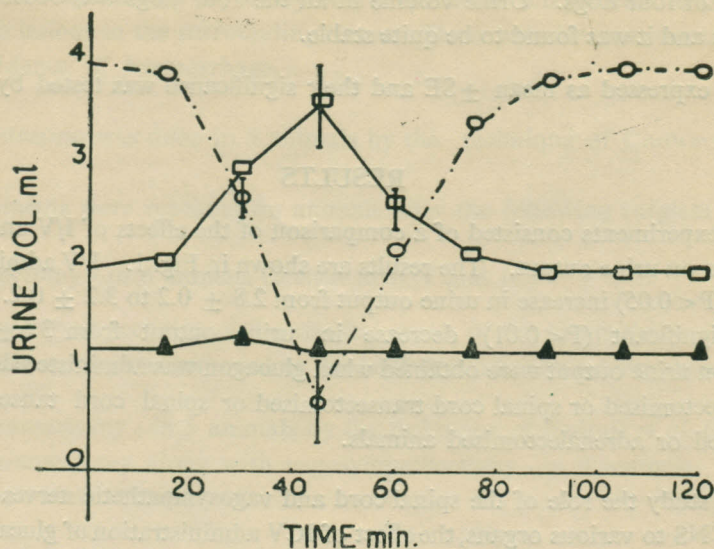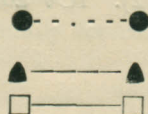

Fig. 2:   Effect of ICV and IC administration of glucagon on urine output . The results shown are means ±SE.

ICV-Vegosympathectomy along with
      spinal cord transection   ●- - - - -●

ICV-Adrenalectomy spinal cord transection   ▲———▲

IC-   Normal   □———□

from 3.0±0.2 to 1.5±0.2. In spinal cord transectomy along with vagosympathectomy the ICV administration of glucagon showed no change in urine output.

In order to find out a precise site for glucagon action, it was administered through IC route, which resulted in a significant (P<0.05) decrease in urine output from 3.6±0.6 to 0.7±0.4 (Fig.2.). The vagosympathectomy in such experiments again showed a   significant (P<0.05) decrease in urine output from 3.6±0.6 to 1.0±0.4.

The administration of glucagon through ICV route in adrenalectomised animals, resulted in a significant (P<0.01) increase in urine output from 1.8±0.4 to 3.6±0.4. In this experiment the blood glucose level was found significantly (P<0.01) increased from 90.6±4.5 to 160.1± 2.0 (unpublished observations).

The ICV administration of preservative substances of glucagon in normal saline, in a set of control animals showed n o special change in urine output irrespective of different surgical procedures.

The time for peak effect in central administration varied from 30 to 45 min whereas in I/V it was 20-30 min.

DISCUSSION

The present study was directed towards finding out the effect of centrally administered glucagon on urine output with the intention of exploring any nervous tract that might be influencing the kidney functions.

Unlike the well-accepted diuretic action (osmotic diuresis due to rise in blood glucose level) of glucagon the central administration by the different routes studied has the peculiarity of a significant oliguric effect which had no relation with the blood glucose level. That this type of response to glucagon administration was mediated through some central structure is evidenced by two observations :

1. I/V administration of the same dose (2.0 μg) of glucagon caused a significant diuresis on account of increase in blood glucose level. The results were the same irrespective of whether the animal was vagosympathectomised or spinal cord transectomised or spinal cord transectomy with vagosympathectomised or adrenalectomised.

2. The oliguric effect did not appear when glucagon was administered by the ICV route in spinal cord transectomy with vagosympathectomised animals.

Moreover, the possibility of leakage of centrally administered glucagon can be excluded safely by the appearance of two absolutely opposite effects (diuresis in I/V; oliguria in ICV).

Since no variation in the oliguria is observed either after vagosympathectomy or after the spinal cord transection alone, it can safely be stated that the efferents pass through the spinal cord as well as through the vagosympathetic trunks inasmuch as sympathetic fibres are present both in the spinal cord and the vagi nerves (26). This conclusion seems to be supported by the fact that in spinal cord transectomy with vagosympathectomy in animals, the effect did not appear after ICV administration of glucagon dose. The exact route through which the oliguric effect is brought about could not be ascertained but the IC administration helped to limit the extent of the drug diffusion, suggesting a glucagon sensitive area, in the near vicinity, as having a role on kidney function.

There are two different possibilities for the oliguric effect resulting from the stimulation of central structures:

1. Alteration in renal blood flow due to neural stimulation, for which two different possibilities have been suggested :-
   (A) As the glomerular arterioles are innervated by adrenergic fibres, the stimulation of the sympathetic centre can influence the renal blood flow (3,5,14, 20,22).

   (B) The neural stimuli have an influence on release of renin from the juxtaglomerular apparatus (2,6,28) and in turn it has an effect on renal blood flow (3,13).

The glomerular filtration rate is directly proportional to renal blood flow (14). Our observations do not support that the oliguric effect on central administration of glucagon, is due to a change in renal blood flow otherwise it must have appeared in adrenalectomised animals too.

2. Adrenal gland plays an important role in electrolyte as well as water balance in the body. This role is due to its hormone secretions such as :-

(A) Epinephrine from the adrenal medulla, which causes an oliguric effect by virtue of its hemodynamic action (3,14).

(B) Adrenocorticoids (other than aldosterone) from the adrenal cortex causing a change in urinary volume (8,14).

(C) Aldosterone from the adrenal cortex having the property of directly affecting the electrolyte reabsorption and indirectly affecting the water retention in the renal tubules (15).

The control existing on the aldosterone secretion from the glomerulosa cells of the adrenal cortex has been attributed to two possible factors:

(i) The renin-angiotensin-aldosterone system (3,24,29) where the renin through angiotensin has a potent effect on aldosterone secretion.

(ii) The existence of a humoral mechanism governing the secretion of aldosterone (3,17).

Moreover, although there is no definite evidence of a role for the nerve fibres in adrenal cortical activity, the cortex is found to be innervated by sympathetic fibres (3,5,9,19,23). The role of these sympathetic fibers in adrenal cortex has yet to be established.

The observations in the present investigation favour the possibility of a role for the adrenal cortex. Medullary action can be precluded safely because of the absence of a change in the animals's blood-pressure when glucagon is centrally administered. The suggested humoral mechanism controlling the aldosterone secretion may also be over-ruled because the oliguria does not appear in vagosympathectomised animals having spinal cord transection. The role of adrenocorticoids (other than the aldosterone) can not, however, be excluded as the rise in blood glucose level persists even in adrenalectomised animals. Further, we can not differentiate between the two mechanisms for aldosterone secretion i.e. either through a direct stimulation of the sympathetic fibres present in the adrenal cortex or via the renin-angiotensin-aldosterone system as the sympathetic fibres are involved in both the mechanisms. Aldosterone, secreted via either of the mechanisms, has an oliguric influence, thereby increasing the extracellular fluid volume. The diuresis in the adrenalectomised animals, on central administration of glucagon is attributed to the rise in blood glucose level which was followed by glucosuria.

Although further investigations are required, the observations in the present study make it amply clear that the CNS plays a role in maintaining the body fluid volume through the adrenal cortex influencing the renal functions.

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