

EFFECT OF INTRACISTERAL INJECTION OF ACTH ON BLOOD GLUCOSE* AND HEPATIC GLYCOGEN IN DOGS

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Summary : The intracisternal administration of ACTH in a dose of $0.2 \mu U$ in mongrel dogs produced a significant ($P < 0.001$) rise in blood glucose (BGL) and a fall ($P < 0.01$) in hepatic glycogen concentration (HGC); in contrast its intravenous administration was devoid of this action. These changes were markedly reduced in the hepatic denervated animals and were completely abolished in animals with spinal cord transection. The above changes suggest that ACTH on the intracisternal administration causes a rise in the BGL by an action on the liver through the sympathetic fibers.

Key words : adrenocorticotropin intracisternal hyperglycaemia dog

INTRODUCTION

ACTH stimulates adrenal biosynthesis and secretion of corticosteroids from the adrenal cortex. It was also reported to have direct stimulatory action on pancreas to release insulin and produce hypoglycaemia in mice (1), rat (2) and rabbits (3).

The central role of hormones on the blood glucose is not yet clear but centrally administered insulin (4) produced hypoglycaemia whereas adrenaline (5) and glucagon (6) produced hyperglycaemia in the dogs. The present study was undertaken to investigate the effect of intracisternally administered ACTH on the blood glucose and possibly to find out its mechanism.

MATERIAL AND METHODS

The study was conducted on 66 mongrel male dogs (8-10 kg). The animals were fasted for 8 hrs before anaesthetising with pentobarbital (40 mg/kg) given intravenously.

The intracisternal cannula (18 gauge hypodermic needle) was inserted in 50 anaesthetised dogs in the cisterna magna by the technique of Sproull (18). A successful insertion of the cannula into the cisterna magna was indicated by the sudden gush of cerebrospinal fluid (CSF) through it. A sample of the CSF (5-6 drops) was collected before the cannula was sealed with plasticine. Immediately the CSF was examined microscopically for

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erythrocyte in order to ascertain whether the cannula led to any lesion in the surrounding tissues. The experiment was discontinued whenever there was any evidence of haemorrhage.

ACTH was given intracisternally in anaesthetised animals after (a) bilateral vagotomy (1) in 6 animals, (b) spinal cord transection (7) in 6 animals, (c) bilateral adrenalectomy (4) in 6 animals and (d) hepatic denervation (5) in 8 animals. In all the experiments, normal saline at the room temperature was infused intravenously in animals at the rate of 1.0 ml/min so as to meet the physiological needs. A period of 1 hr was allowed to elapse between the surgical preparation and the ACTH administration.

To rule out the effects of anaesthesia on the BGL, ACTH was administered intracisternally in 8 unanaesthetised dogs. In these animals procain (2% solution 5-8 ml) was infiltrated into the skin over lying the atlanto-occipital membrane. After 10-20 min, the animal was made to sit on its belly with head-down position and the cannula was pushed into the cisterna magna in a single attempt. Immediately after examining the CSF for erythrocytes, ACTH was administered through the cannula.

In 8 anaesthetised animals ACTH was given intravenously and the BGL was estimated for 5 hrs.

In 8 control experiments inactivated ACTH (0.2 μ U) solution was injected into the cisterna magna and its effects on the BGL and the HGC were observed. ACTH was inactivated according to Farrell *et al.* (8). Moreover, the control experiments were carried out with every surgical procedure.

ACTH solution and its dose: Porcine corticotropin (FCL Ltd. Denmark) was dissolved in 5 ml normal saline to give 4 U/ml and was stored at 2°C. This solution which served as stock solution, was further diluted with normal saline at the time of the experiment. The pH of the final solution used for injection varied from 5.6 to 5.7. The solution was prewarmed in a water bath at 37°C for 30 min before use. The dose of 0.2 μ U ACTH in 0.25 ml was found to be the minimal effective dose on intracisternal administration and was used in all subsequent experiments. This dose of ACTH was injected into the cisterna magna and the CSF was drawn back into the syringe and reinjected to facilitate mixing.

Blood glucose level (BGL): The samples of blood (0.5 ml each time in sodium fluoride vials) were obtained at -0 (initial), 10, 20, 30, 60, 90 and 120 min after the administration of ACTH solution in animals. In another group of 6 animals the samples of blood were obtained at 2 min interval for 10 min (so as to find out the earliest effect on the BGL) after the intracisternal administration of ACTH. The blood samples in anaesthetised animals were obtained through an indwelling catheter inserted into the abdominal aorta through the right femoral artery. After obtaining the sample of blood, an amount of 5 ml heparanized normal saline (1:200) was injected each time through the indwelling catheter. The samples of blood from the unanaesthetised animals were drawn from a vein in the fore-arm.

The blood samples were analysed for the BGL by the Folin and Wu method (10) and also by the Asatoor and King method (3). A number of blood samples were analysed by both the methods; the results obtained by the Asatoor and King method were uniformly lower by a constant amount. Hence, the results have been presented exclusively on the observations obtained by the Folin and Wu method.

Hepatic glycogen concentration (HGC): In experiments with anaesthetised animals three pieces (1.0 - 1.2 g wet tissue each) were removed from left lobe of the liver at -0 (initial), 30 and 120 min after the administration of ACTH. The pieces of liver were dropped into liquid nitrogen immediately and the frozen samples were analysed for the hepatic glycogen by the method of Edwards (4).

RESULTS

In order to assess and compare the central role of ACTH on the BGL and the HGC with that of its peripheral effects, ACTH was administered through the intravenous and the intracisternal routes in two separate groups of anaesthetised animals (Fig. 1). The

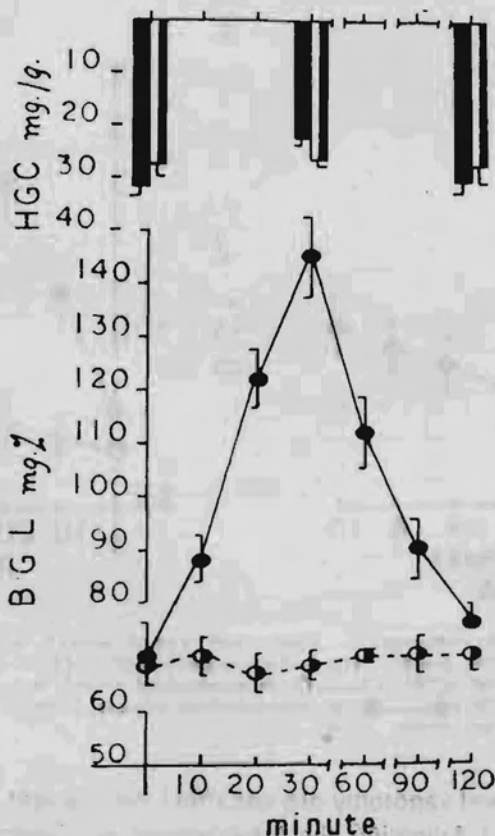


Fig. 1 : Comparison of the changes in blood glucose level (BGL) and hepatic glycogen concentration (HGC) in response to intravenous (○—○) and intracisternal (●—●) administration of ACTH (0.2 μ U) in normal animals. (Number of animals in each group=8). The results shown are means \pm SE.

intravenous administration of ACTH in animals caused no change in the BGL and the HGC. But the intracisternally administered ACTH caused a significant ($P < 0.001$) rise in the BGL of 75.6 ± 8.2 mg% by 30 min and the HGC was reduced ($P < 0.01$) by 9.8 ± 1.6 mg/g.

Changes in first 10 min: In experiments where blood samples were collected every 2 min for the first 10 min after the intracisternal administration of ACTH, the BGL rose ($P > 0.05$) for 6.1 ± 9.0 mg% at the very 2nd min. Moreover, the subsequent rise of blood glucose was gradual and remained insignificant ($P > 0.05$ even at the 10th min. (Fig. 2A).

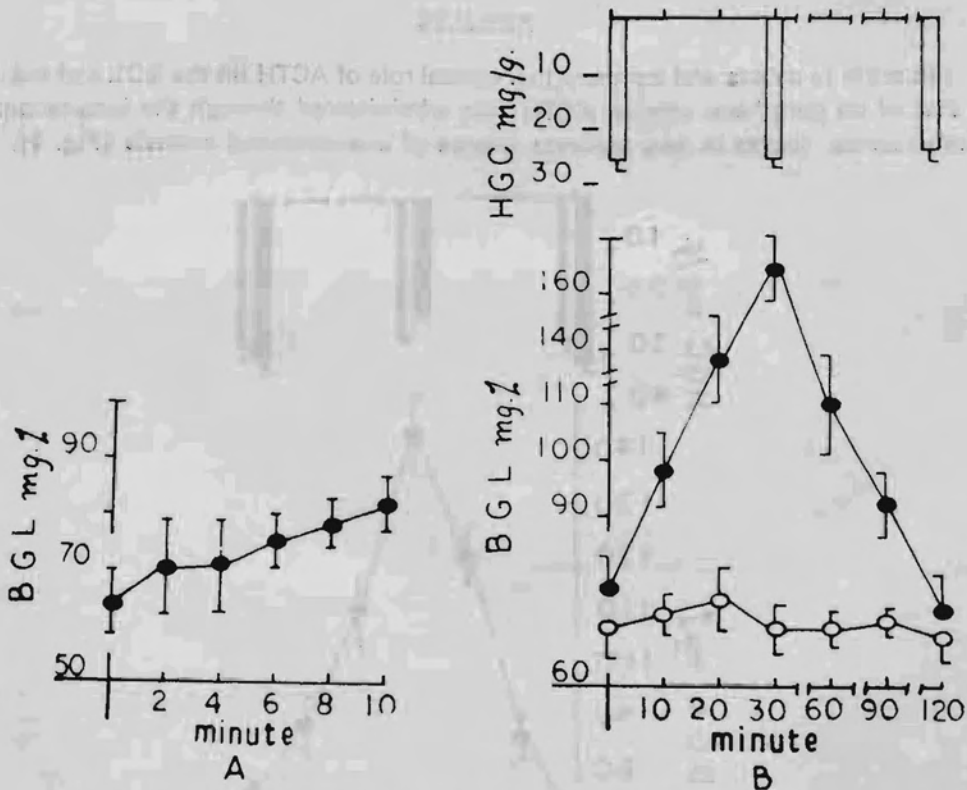


Fig. 2 : (A) - Hyperglycaemic response in anesthetised normal animals within 10 min to intracisternal administration of ACTH. (n=6). (B) - The changes in BGL and HGC in response to intracisternal administration of inactivated ACTH (O—O) in anesthetised animals and changes in BGL to intracisternal administration of ACTH (●—●) in unanesthetised animals. (Number of animals in each group=8). The results shown are means \pm SE.

Vagotomy: Bilateral vagotomy did not affect the changes in the BGL ($P < 0.001$) and the HGC ($P < 0.01$) following the intracisternal administration of ACTH, observed in the normal animals (Fig. 3A).

Spinal cord transection: The intracisternally administered ACTH in the spinal cord transectomised animals did not cause any change in the BGL and the HGC (Fig. 4A).

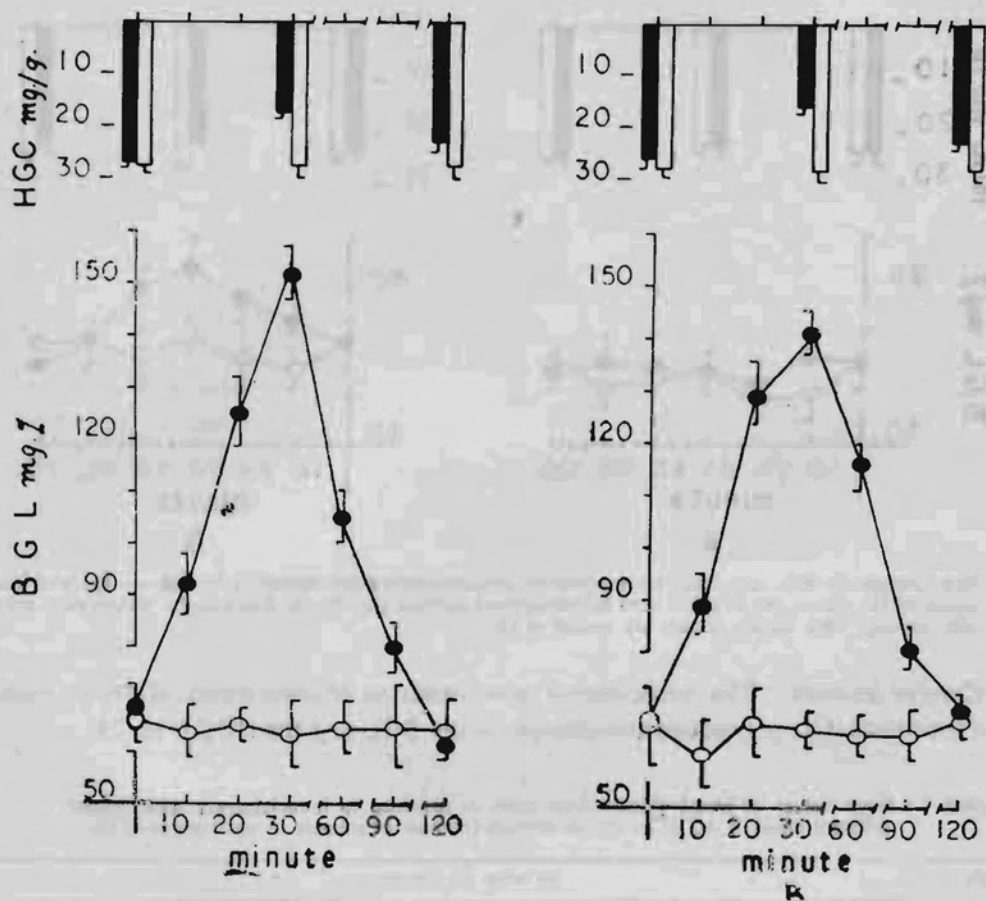


Fig. 3 : The changes in BGL and HGC in response to intracisternal administration of ACTH (●—●) and inactivated ACTH (○—○) in vagotomised animals (A) and adrenalectomised animals (B). (n=6 in each group). The results shown are means \pm SE.

Adrenalectomy and hepatic denervation: The intracisternal administration of ACTH in the adrenalectomised animals caused a significant ($P < 0.001$) rise in the BGL of 74.6 ± 4.1 mg% and reduction of the HGC ($P < 0.01$) by 10.5 ± 1.0 mg/g at 30 min. (Fig. 3B). In the hepatic denervated animals the intracisternally administered ACTH caused an insignificant ($P > 0.05$) rise in the BGL of 15.4 ± 6.4 mg% and a fall ($P > 0.05$) in the HGC by 1.8 ± 1.0 mg/g (Fig. 4B).

Spinal cord transection: The intracisternally administered ACTH in the spinal cord transectomised animals did not cause any change in the BGL and the HGC (Fig. 4A).

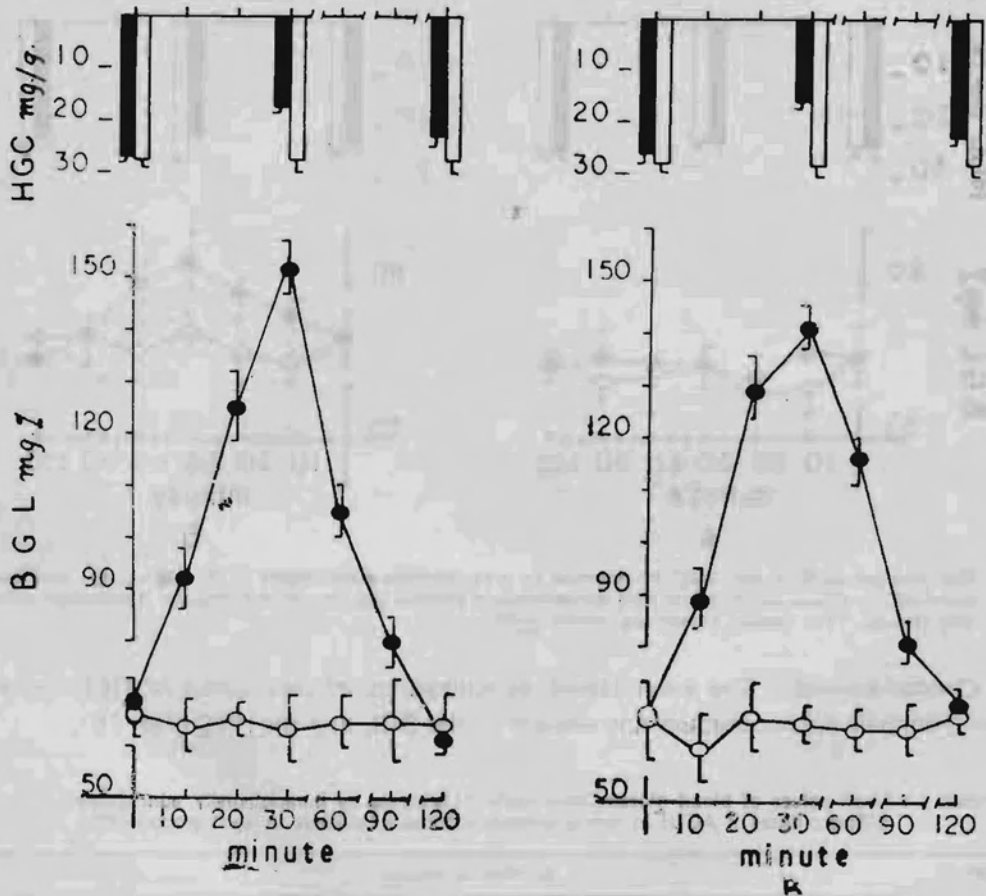


Fig. 3 : The changes in BGL and HGC in response to intracisternal administration of ACTH (●—●) and inactivated ACTH (○—○) in vagotomised animals (A) and adrenalectomised animals (B). (n=6 in each group). The results shown are means \pm SE.

Adrenalectomy and hepatic denervation: The intracisternal administration of ACTH in the adrenalectomised animals caused a significant ($P < 0.001$) rise in the BGL of 74.6 ± 4.1 mg% and reduction of the HGC ($P < 0.01$) by 10.5 ± 1.0 mg/g at 30 min. (Fig. 3B). In the hepatic denervated animals the intracisternally administered ACTH caused an insignificant ($P > 0.05$) rise in the BGL of 15.4 ± 6.4 mg% and a fall ($P > 0.05$) in the HGC by 1.8 ± 1.0 mg/g (Fig. 4B).

Unanesthetised animals: In unanesthetised animals the intracisternal administration of ACTH caused a significant ($P < 0.001$) rise in the BGL of 87.6 ± 6.2 mg% at 30 min. (Fig. 2B).

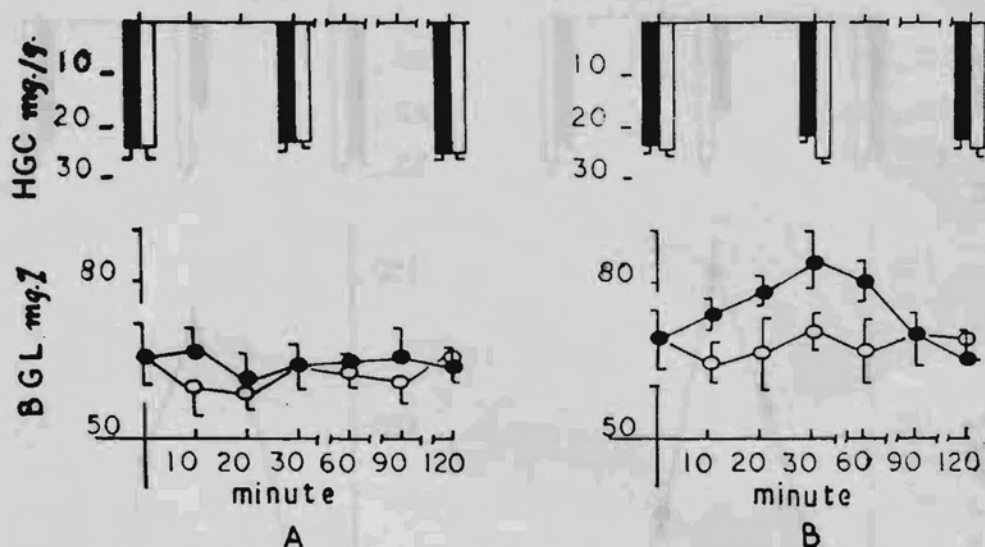


Fig. 4 : The changes in BGL and HGC in response to intracisternally administered ACTH (●—●) and inactivated ACTH (○—○) in spinal cord transectomised animals (A) ($n=6$) and hepatic denervated animals (B) ($n=8$). The results shown are means \pm SE.

Control animals: The intracisternal administration of inactivated ACTH in control group of 8 animals did not produce any change in the BGL and the HGC (Fig. 2B).

TABLE I : Mean values of blood glucose level mg% in response to intracisternally administered different doses of ACTH in normal animals (number of animals in each group = 5).

Dose of ACTH	At time in minute						
	—0	10	20	30	60	90	120
1.0 μ U	82.0 ± 4.8	96.6 ± 5.1	130.2 ± 4.8	168.1 ± 6.2	142.3 ± 3.0	108.3 ± 3.6	88.8 ± 5.1
0.8 μ U	76.4 ± 6.1	92.7 ± 4.2	116.0 ± 6.7	157.2 ± 4.6	128.6 ± 3.0	96.8 ± 3.2	72.2 ± 3.8
0.4 μ U	68.8 ± 4.0	99.2 ± 6.6	130.0 ± 8.8	170.0 ± 6.8	138.6 ± 4.1	102.0 ± 4.3	70.0 ± 3.0
0.2 μ U	70.0 ± 3.8	89.8 ± 4.8	107.6 ± 5.8	128.6 ± 3.1	101.9 ± 6.6	83.7 ± 3.7	74.3 ± 2.6
0.1 μ U	76.8 ± 4.2	79.0 ± 3.2	78.1 ± 4.0	76.1 ± 6.4	74.3 ± 2.0	73.6 ± 3.6	71.8 ± 6.1

DISCUSSION

The observations in the present study show that hyperglycaemia occurs after the intracisternal administration of ACTH and that it is due to mobilization of hepatic glycogen. This could be a central effect of ACTH mediated through the sympathetic fibres to the liver.

Unlike the proposed hypoglycaemic effect (6,11,14) of systemically administered ACTH, the intracisternally administered ACTH in the present study produced hyperglycaemia in normal animals. The hyperglycaemic response following the intracisternal administration of ACTH is considered central because (a) administration of the same dose of ACTH (0.2 μ U) through the intravenous route did not cause any change in the BGL and the HGC as well (b) since the hyperglycaemia and the hepatic glycogenolytic response were not observed on the intracisternal administration of ACTH in animals with spinal cord transection. Moreover, the bilateral vagotomy in animals did not affect these responses.

The hyperglycaemic effect in response to the intracisternal administration of ACTH in the present study can not be a result of the proposed hepatic glycogenolysis due to catecholamines (5), glucocorticoids (16) and/or glucagon (15) secreted under the influence of sympathetic fibres. The adrenalectomy in the present observations could not affect the hyperglycaemic and the hepatic glycogenolytic responses to the intracisternal administration of ACTH in animals. Furthermore, no significant changes in the BGL and the HGC were observed when ACTH was administered intracisternally in the hepatic denervated animals where pancreas remained intact.

The hyperglycaemic response to intracisternal administration of ACTH in normal animals can be attributed to the integrity of sympathetic fibres to the liver. There are evidences (5,17) that the stimulation of sympathetic fibres to liver activates the enzymes phosphorylase and glucose-6-phosphatase. The observations, insignificant change in the BGL in hepatic denervated animals and no change in the BGL to intracisternally administered ACTH in the spinal cord transectomised animals, are in agreement with the suggestion of other workers (17,19). Accordingly, the stimulation of sympathetic fibres produces a rapid and substantial changes in the hepatic activities of glycogenolytic enzymes. The small insignificant rise in the BGL and decrease in the HGC observed in the hepatic denervated animals in response to the intracisternal administration of ACTH can not be explained at present. It can be due to a few intact sympathetic fibres to the liver (4) and/or due to a release of catecholamine from the postganglionic sympathetic nerve endings (12).

Although the structure (s) sensitive to the intracisternally administered ACTH for producing the hyperglycaemic effect in animals is yet to be identified, Farrell (9) has suggested

an area in the brain-stem influenced by the trophic hormone. However, it is of interest that the observations in the present study indicate that the hepatic glycogenolytic mechanism does respond to the intracisternally administered ACTH resulting hyperglycaemic effect in animals.

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