

# EFFECT OF CENTRALLY ADMINISTERED GLUCAGON ON BLOOD LIPIDS IN ANESTHETISED DOGS

G. C. AGARWALA, R. MISHRA, G. JAISWAL AND V. BAPAT

*Departments of Physiology and Biochemistry,  
M. L. N. Medical College, Allahabad - 211 001*

( Received on July 10, 1986 )

**Summary :** Previously we have proposed the existence of the central glucagon sensitive receptors in dogs. The present study was undertaken to explore the role of centrally administered glucagon on lipids in view of the proposed theory that the hypothalamic lipomobilizing centres are sensitive to glucose or substances that affect glucose metabolism.

Glucagon (0.01  $\mu$ g) administered through the intracerebroventricular (ICV) route in anesthetised mongrel dogs, caused hypolipidaemia ( $P>0.001$ ), hypocholestromia ( $P>0.001$ ), decreased blood free fatty acid ( $P>0.001$ ) and triglycerides ( $P>0.001$ ) levels; but increased blood high density lipoprotein ( $P>0.01$ ) level at 30 min. These effects on the central administration of glucagon, were not observed in pancreatectomised animals and spinal cord transectomised animals. Therefore, we conclude that the lipolysis on the central administration of glucagon, is caused by the endogenous glucagon secreted from the pancreas through the sympathetic fibers.

**Key words :** intracerebroventricular (ICV)      glucagon lipids      high density lipoproteins

## INTRODUCTION

The regulatory role of the central nervous system on blood glucose and lipid metabolism has been the subject of recurrent scientific interest (4,9,21). The hypothalamus has been shown to be of great importance in the central regulation of glucose and lipid metabolism (4,18,25,35). Moreover, these centers/receptors in the hypothalamus have been demonstrated to be sensitive to glucose (2,21,30), 2-deoxy glucose (12), insulin (3,12) epinephrine (31,34) and norepinephrine (7,15,31).

---

Communication and reprint request :

Dr. G.C. Agarwala, Lecturer, Deptt. of Physiology, M.L.N. Medical College, Allahabad - 211 001

We have shown (1) that the centrally administered glucagon has a biphasic action on the peripheral blood glucose level. These data suggested the existence of receptors in the dog central nervous system (CNS), sensitive to exogenous glucagon secreted from pancreas through the autonomic fibers. As it has been proposed by many workers (12,35) that the hypothalamic lipomobilizing centres are sensitive to glucose or substances that affect glucose metabolism, the present study was undertaken to explore, if any, the effects of centrally administered glucagon on the lipid metabolism and to correlate these observations with these hyperglycaemic responses obtained previously on the central administration of glucagon in dogs.

### MATERIAL AND METHODS

The study was conducted on 52 mongrel dogs of either sex, weighing between 12-14 kg. The animals were fasted for 8 hr before chloralose (80 mg/kg body weight) was injected intravenously.

The intracerebroventricular (ICV) cannula was implanted in 44 animals in the left lateral ventricle of the brain by the technique of Sloviter and Sakata (32). A successful insertion of the cannula into the brain ventricle and the evidence for any lesion in the surrounding tissues were ascertained and confirmed (1,2). The animals were ventilated with a fixed-volume respiratory-pump through an endotracheal tube. The femoral artery and vein were exposed in the right inguinal region and were cannulated with polyethylene catheters. Hemostasis was maintained by a purse suture around the catheters at their point of insertions. The physiological saline at the rate of 1.0 ml/min, was infused through the venous cannula for the period of experiment. The arterial cannula was used to collect the blood samples. This cannula was washed by heparinized saline (1:1000) every time after collecting the blood sample.

The ICV administration of glucagon was repeated in animals undergone the following surgical procedures (3) :

- (i) vagotomy — 8 animals
- (ii) spinal cord transectomy — 8 animals
- (iii) Pancreatectomy — 8 animals (26).

After surgery, a period of 2 hr was allowed to elapse to obtain a stabilized state of animals. In a separate set of experiments, the effect of intravenously (iv) administered glucagon dose was studied in 8 animals.

*Control experiments:* In a group of 8 animals, the control experiments were set by administering (0.2 ml) normal saline containing all other constituents of glucagon solution

(except crystalline glucagon) (pH 2.9 - 3.0) into the lateral ventricle of the brain and the blood samples were collected at 15 *min* intervals for 4 hr. The results were found appreciably constant throughout the period. Besides, with every surgical preparation the control experiments were carried out.

*Glucagon solution and its dose* : The glucagon solution was prepared and the minimal effective dose 0.01  $\mu$ g in 0.2 ml (1) was used in all the experiments (peripheral and central).

*Collection and analysis*: The arterial blood samples (1.0 ml each) were collected at -0 (before the administration of dose), 2 (in ICV - experiments only), 15, 30, 45, 60, 75, 90, 105 and 120 *min* after the administration of the glucagon dose in animals. These samples were processed immediately for the biochemical analysis by the following techniques: Blood glucose by Somogyi - Nelson method (29); blood total protein by Folin - Lowry method (29); blood total lipid (13); blood free fatty acid (11); blood triglycerides (5); and blood total cholesterol and high density lipoproteins (10).

## RESULTS

Intravenous administration of 0.01  $\mu$ g glucagon in the anaesthetised animals caused an insignificant ( $P < 0.05$ ) rise in the blood glucose level (BGL) for  $3.8 \pm 2.2$  mg% at 15 *min*, but no change was observed in blood total protein (TP), blood total lipid (TL), blood total cholesterol (Chole.), blood triglycerides (TG), blood free fatty acid (FFA), and blood high density lipoproteins (HDL) levels (Table I).

TABLE I: Mean values of blood glucose (BGL), total proteins (TP), total lipids (TL), cholesterol (Chole.), triglycerides (TG), free fatty acids (FFA), and high density lipoproteins (HDL) following the intravenous administration of glucagon (0.01  $\mu$ g) in normal anaesthetised animals (number : 8);

	Blood level at min				
	-0	15	30	60	120
1. BGL mg%	82.00 $\pm$ 3.00	85.80 $\pm$ 2.20	82.80 $\pm$ 1.70	82.20 $\pm$ 1.90	81.80 $\pm$ 1.60
2. TP g%	12.01 $\pm$ 0.21	12.02 $\pm$ 0.19	12.02 $\pm$ 0.16	12.01 $\pm$ 0.16	12.01 $\pm$ 0.20
3. TL g%	1.26 $\pm$ 0.13	1.26 $\pm$ 0.12	1.26 $\pm$ 0.11	1.27 $\pm$ 0.14	1.26 $\pm$ 0.11
4. Chole. mg%	148.76 $\pm$ 3.20	150.00 $\pm$ 2.70	150.06 $\pm$ 3.66	150.26 $\pm$ 4.11	150.00 $\pm$ 3.01
5. TG mg%	130.21 $\pm$ 3.30	130.33 $\pm$ 4.21	130.67 $\pm$ 2.98	132.00 $\pm$ 2.54	131.07 $\pm$ 3.22
6. FFA mg%	92.48 $\pm$ 4.16	92.67 $\pm$ 3.44	92.43 $\pm$ 4.22	92.86 $\pm$ 2.45	92.08 $\pm$ 3.21
7. HDL mg%	187.28 $\pm$ 5.02	186.00 $\pm$ 3.67	186.01 $\pm$ 4.32	186.00 $\pm$ 4.32	186.00 $\pm$ 4.00

Intracerebroventricular (ICV) administration of glucagon ( $0.01 \mu\text{g}$ ) resulted (i) in anesthetised animals :

The BGL decreased immediately (transitory effect at 2 min) from  $88.26 \pm 2.61$  to  $80.25 \pm 2.10 \text{ mg}\%$  (for  $8.01 \pm 2.10 \text{ mg}\%$ ) ( $P > 0.05$ ); thereafter the mean BGL rose steadily from  $88.26 \pm 2.61$  to  $121.80 \pm 3.00 \text{ mg}\%$  (for  $33.54 \pm 3.00 \text{ mg}\%$ ) ( $P > 0.01$ ) at 60 min which returned to its resting level by 90 min.

The TP and TL decreased steadily for  $4.06 \pm 0.5 \text{ g}\%$  ( $P > 0.01$ ) and  $0.39 \pm 0.09 \text{ mg}\%$  ( $P > 0.001$ ) respectively at 30 min and returned to their resting levels by 75-90 min (Fig. 1).

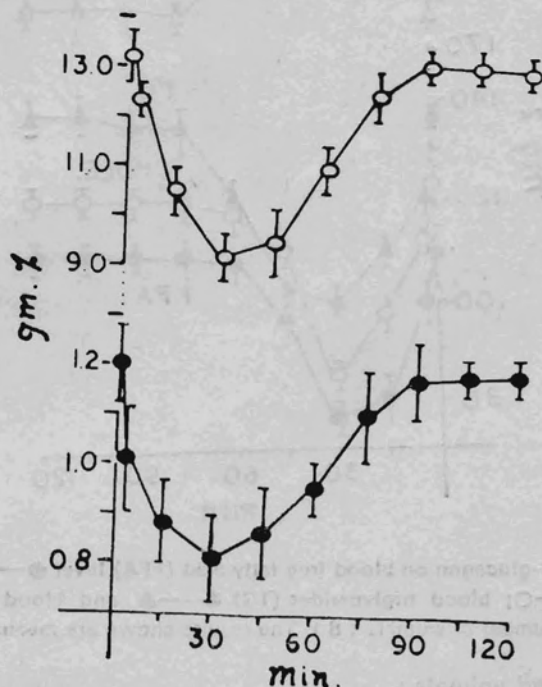


Fig. 1 : Effect of the ICV-glucagon on blood total lipids level ●—● and blood total protein level ○—○ ( number of animals : 8 ) The results shown are means  $\pm$  S. E.

The Chole. decreased steadily for  $38.61 \pm 2.40 \text{ mg}\%$  ( $P > 0.001$ ); the TG decreased steadily for  $36.73 \pm 3.66 \text{ mg}\%$  ( $P > 0.001$ ); the FFA decreased steadily for  $32.41 \pm 4.57 \text{ mg}\%$  ( $P > 0.001$ ); but the HDL rose steadily for  $31.00 \pm 4.01 \text{ mg}\%$  ( $P > 0.01$ ). The maximum change in blood Chole., TG, FFA and HDL level were observed at 30 min after the ICV administration of the glucagon dose, which returned to their resting levels by 60-75 min (Fig. 2).



## (ii) in vagotomised animals :

The ICV administration of glucagon produced an identical changes in BGL (hyperglycaemic phase), TP, TL, Chole., TG, FFA and HDL to those which were observed in normal anaesthetised animals (i). The transitory hypoglycaemic phase (at 2 min) was absent.

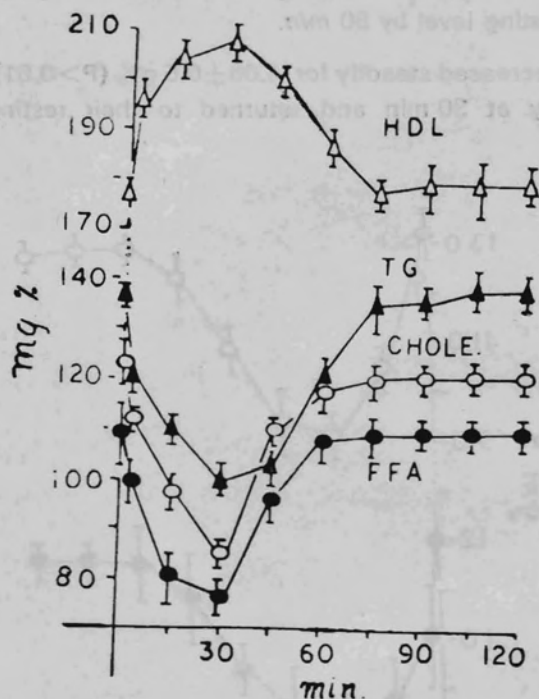


Fig. 2 : Effect of the IVC-glucagon on blood free fatty acid (FFA) level ●—●; blood total cholesterol (Chole.) level ○—○; blood triglycerides (TG) ▲—▲ and blood high density lipoproteins (HDL) △—△ . (Number of animals : 8 ). The results shown are means  $\pm$  S.E.

## (iii) in pancreatectomised animals :

The ICV administration of glucagon did not produce any change in BGL, TP, TL, Chole., TG, FFA and HDL for 4 hr.

## (iv) in spinal cord transectomised animals :

The ICV administration of glucagon caused a transitory (at 2 min) decreased in BGL from  $72.51 \pm 2.67$  to  $63.70 \pm 1.8$  mg% (for  $8.81 \pm 1.8$  mg%) ( $P > 0.05$ ) which returned to its resting level by 15 min. No change was observed in blood TP, TL, Chole., TG, FFA and HDL for 4 hr.

## DISCUSSION

We have previously suggested from experiments performed on animals that there are glucagon sensitive receptors in the dog central nervous system, which influence the secretion of glucagon from the pancreas through the autonomic fibers (1). The results obtained in this study, present further evidences for the existence of such receptors by demonstrating that the glucagon secreted from the pancreas under the influence of centrally administered glucagon also affects the lipid metabolism.

There can be little doubt that the effects obtained, resulted from the glucagon stimulation of structures located along the ventricular system and not from leakage of the glucagon into the blood, because glucagon was administered in a dose too small to have any effect on blood lipids when injected directly into the systemic circulation. Moreover, the centrally administered glucagon did not produce any change in blood lipids when administered in animals undergone either pancreatectomy or spinal cord transection.

It is not possible to determine from the present study, the site at which the glucagon sensitive receptors are located. It might be located either within the ependymal lining of the ventricles or in the close vicinity to the ventricular system although it is impossible to predict to what distance the glucagon spread from the ventricle in effective concentration. Because, in the present study, the glucagon was injected into the lateral ventricle (animal in its normal sitting position) and peripheral effects appeared at 2 min, it would presumably be delivered along with the bulk flow of cerebrospinal fluid to the third ventricle, where hypothalamus is exposed to this injected dose. The hypothalamus has been shown to be of great importance in the central regulation of all autonomic functions. There are many evidences that hypothalamus does stimulate the secretion of endogenous glucagon from the pancreas through the sympathetic fibers when either stimulated electrically (4,18,25) or chemically (12,30,31). Our results do suggest that the hypothalamus participates in the secretion of glucagon from pancreas as, the glucagon administered through the ICV route in the spinal cord transectomised animals, could not elicit any change in blood lipids level and that the hypothalamus is the seat for all the peripheral sympathetic activities (8,19).

The resulted hypolipidaemia, on the central administration of glucagon, in the present study, does not follow the observations of others (12,31,34) who had proposed an increased blood FFA level with or without affecting BGL on electrical stimulation of the hypothalamus. We did observe a significant hyperglycaemia on the ICV administration of

glucagon but except HDL which increased significantly, the blood Chole., TG, and FFA decreased significantly (Fig. 2). Moreover, these centrally oriented changes in Chole., TG, FFA and HDL were not observed in pancreatectomised and spinal cord transectomised animals, which substantiates our suggestions that the observed results on BGL, Chole. TG, FFA and HDL on the central administration of glucagon are due to the secretion of the endogenous glucagon secreted from the pancreas via the sympathetic fibers (16,27). The part/s of hypothalamus, if responsible, is exclusively influencing the secretion of endogenous glucagon (18,31,34) and is not mediating any effect on the lipid metabolism directly.

The significant decrease in in blood total protein (TP) and lipid (TL) levels on the central administration of glucagon can safely be attributed to the endogenous glucagon's proteolytic (17,23,33) and lipolytic (6,24) effects on gluconeogenesis (22,28) and the synthesis of blood HDL in liver (8,19). Recent data from this laboratory does indicate that the intravenous administration of glucagon in biological concentration (1.0  $\mu\text{g}/\text{kg}$  body wt) induces hyperglycaemia accompanied with a significantly increased HDL in dogs (unpublished reports).

Our suggestion for the increased secretion of endogenous glucagon on the ICV administration of glucagon, is further strengthened by the results obtained on blood FFA level. The proposed hypothalamic lipomobilizing centers under the influence of glucose or substances that affect glucose metabolism (12) resulted in increased FFA without affecting BGL whereas, in the present study, the FFA level has decreased significantly ( $P < 0.001$ ) which is quite in agreement with many workers (22,36,37) who had reported a decreased FFA level in glucagon induced hyperglycaemic animals, suggesting (22) oxidation of FFA in the liver.

However, further experiments are needed to determine the precise localization of the glucagon sensitive receptors, it is of interest to know that the centrally administered glucagon did influence the carbohydrate, lipid and protein metabolism. Moreover the endogenous glucagon has a definite role in inducing a rise in blood HDL which is proposed to be a protective factor in coronary heart diseases (14) and to be a part of the longevity syndrome in families (20).

#### ACKNOWLEDGEMENTS

The authors thank the Director, Bombay Trust Hospital, Bombay for the financial assistance and to Eli Lilly & Co. for kindly supplying glucagon.

#### REFERENCES

1. Agarwala, G.C. and S.K. Bapat. Effect of centrally administered glucagon and blood glucose levels in dogs. *Ind. J. Med. Res.*, **66** : 323-330, 1977.

2. Agarwala, G.C., V. Bapat, K.N. Singh and R.K. Mittal. Effect of centrally administered glucose on blood glucose level in dogs. *Ind. J. Med. Res.*, **65** : 720-728, 1977
3. Agarwala, G.C., R.K. Mittal, S.K. Bapat and U.R. Bhardwaj. Effect on centrally administered insulin on blood glucose level in dogs. *Ind. J. Physiol. Pharmac.*, **21**:11-18, 1977.
4. Anand, B.K. and S. Dua. Blood sugar changes induced by electrical stimulation of the hypothalamus in cat. *Ind. J. Med. Res.*, **43** : 123-127, 1955.
5. Amenta, J.S. A rapid chemical method for quantification of lipids separated by thin-layer chromatography. *J. Lipid Res.*, **5** : 270-274, 1964.
6. Aydin, A. and J.E. Sokal. Uptake of plasma free fatty acids by the isolated rat liver : effect of glucagon. *Am. J. Physiol.*, **205** : 667-670, 1963.
7. Barbosa, M.C. and R.H. Migliorini. Free fatty acid mobilization in rats following intracerebroventricular norepinephrine. *Am. J. Physiol.*, **242** : E248-E252, 1982.
8. Bell, G.H., Emslie-smith and C.R. Paterson. Text book of physiology and biochemistry. 9th ed. Livingstone, The ELBS & Churchill. p. 184 & 189, 1979.
9. Brodie, B.B., R.P. Maickel and D.N. Stern. Automatic nervous system and adipose tissue. In 'Handbook of physiology.' Adipose tissue. Washington, D.C., *Am. Physiol. Soc.*, p 583-600, 1965.
10. Carr, J.J. and I.J. Dreker. Simplified rapid technique for extraction and determination of serum cholesterol with saponification. *Clin. Chem.*, **2** : 353-357, 1956.
11. Chakravorty, M.M., D. Bhattacharya and M.K. Kundu. Simple photometric method for the micro-determination of fatty acids in lipids. *J. Am. Oil Chem. Soc.*, **46** : 473-477, 1969.
12. Coimbra, C.C., J.L. Gross and R.H. Migliorini. Intraventricular 2-deoxyglucose, glucose, insulin and free fatty acid mobilization. *Am. J. Physiol.*, **236** : E317-E327, 1979.
13. Colowick, S.P. and N.O. Kaplan. Methods in enzymology. vol. III. 3rd ed. Academic press Inc. New York. p. 306, 1957.
14. Cordon, T., W.P. Castelli, M.C. Hjortland, W.B. Kannel and T.R. Daiber. High density lipoprotein as a protective factor against coronary heart disease. *Am. J. Med.* **62** : 707-712, 1977.
15. DeJong, A., J.H. Strubbe and A.B. Steffens. Hypothalamic influence on insulin and glucagon release in the rat. *Am. J. Physiol.*, **233** : E380-E388, 1977.
16. Esterhuizen, A.C. and S.L. Howell. Ultrastructure of the A— cells of cat islets of Langerhans following sympathetic stimulation of glucagon secretion. *J Cell Biol.*, **46** : 593-598, 1970.
17. Foa, P.P. Glucagon. *Ergeb. Physiol.*, **60** : 141-219, 1968.
18. Frohman, L.A. and L.L. Bernardis. Effect of hypothalamic stimulation on plasma glucose, insulin and glucagon levels. *Am. J. Physiol.*, **221** : 1596-1603, 1971.
19. Ganong, W.F. Review of medical physiology. 10th ed. California, LANGE Medical Publications. p. 238 & 279, 1981.
20. Glueck J., R.W. Fallet, F. Millet and P.M. Steiner. Familial hyperalphalipoproteinaemia. *Arch. Intern. Med.*, **135** ; 1025-1026, 1975.
21. Goldfien, A., K.S. Gullixson and G. Hargrove. Evidence for centers in the central nervous system that regulate fat mobilization in dogs. *J. Lipid Res.*, **7** : 357-367, 1966.
22. Gorman, C.K., J.M. Salter and J.C. Penhos. Effect of glucagon on lipids and glucose in normal and eviscerated rats and on isolated perfused rat liver. *Metabolism*, **16** : 1140-1157, 1967.
23. Grande, F. Effect of glucagon on plasma free fatty acids and blood sugar in birds. *Proc. Soc. Exptl. Biol.*, **128** : 532-534, 1968.
24. Guyton, A.C. Text book of medical physiology. 6th ed. Tokyo, W.B. Saunders Co., p967, 1981.



25. Helman, A.M., P. Giraud, S. Nicolaidis, C. Oliver and R. Assan. Glucagon release after stimulation of the lateral hypothalamic area in rats : Predominant  $\beta$ -adrenergic transmission and involvement of endorphin pathway. *Endocrinology*, **113** : 1-6, 1983.
26. Iversen, J. Adrenergic receptors and the secretion of glucagon and insulin from the isolated perfused canine pancreas. *J. Clin. Invest.*, **52** : 2102-2116, 1973.
27. Kaneto, A., H. Kajinuma and K. Kosaka. Effect of splanchnic nerve stimulation on glucagon and insulin output in the dog. *Endocrinology*, **96** : 143-150, 1975.
28. McCarry, J.D., P.H. Wright and D.W. Foster. Hormonal control of ketogenesis. *J. Clin. Invest.*, **55** : 1202-1209, 1975.
29. Plummer, D.T. An introduction to practical biochemistry. 2nd ed. New Delhi, TMH. p 145, 183 & 184, 1984.
30. Sakata, K., H. Shigeo and H.A. Sloviter. Effect on blood glucose concentration of changes in availability of glucose of the brain. *Am. J. Physiol.*, **204** : 1127-1132, 1963.
31. Shimazu, T. Central nervous system regulation of liver and adipose tissue metabolism. *Diabetologia*, **20** : 343-356, 1981.
32. Sloviter, H.A. and K. Sakata. Inactivity of cerebrospinal fluid in regulation of blood glucose concentration. *Am. J. Physiol.*, **204** : 153-156, 1963.
33. Sokal, J.E. Effect of glucagon on gluconeogenesis by the isolated perfused rat liver. *Endocrinology*, **78** : 538-548, 1966.
34. Steffens, A.B., G. Damsama, J. VanDerGugten and P.G. Luiten. Circulating free fatty acids, insulin and glucose during chemical stimulation of hypothalamus in rats. *Am. J. Physiol.*, **247** : E765-E771, 1984.
35. Teixeira, V.L., J. Antunes-Rodrigues and R.H. Migliorini. Evidence for centres in the CNS that selectively regulate fat mobilization in the rat. *J. Lipid Res.*, **14** : 672-677, 1973.
36. Witters, L.A. and C.S. Trasko. Regulation of hepatic free fatty acid metabolism by glucagon and insulin. *Am. J. Physiol.*, **237** : E23-E29, 1979.
37. Wolfer, R.R., M.J. Durkot and M.H. Wolfe. Investigation of Kinetics of integrated metabolic responses to adrenergic blockade in conscious dogs. *Am. J. Physiol.*, **241** : E385-E395, 1981.