EFFECT OF CENTRALLY ADMINISTERED INSULIN ON BLOOD GLUCOSE LEVELS IN DOGS

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Summary : The effects of minimal doses of insulin administered by intracerebroventricular (ICV) and intracisternal (IC) routes in mongrel dogs, on peripheral blood glucose level (BGL) have been studied. The dose of 0.1 U of insulin was found to be the minimal dose. This dose produced an immediate short lived hyperglycaemia followed by a marked and sustained hypoglycaemia. Both the effects were not observed in spinal cord transected-vagosympathectomised animals. The immediate hyperglycaemic effect was not observed in adrenalectomised animals whereas the subsequent hypoglycaemic effect did not appear only when the liver was removed. In an attempt to identify the precise site of action in the central nervous system (CNS), the cerebellomedullary angles were found to be the most sensitive sites for the action of locally applied insulin. It is suggested that insulin on central administration causes a rise in the BGL by an action on the adrenal glands and subsequently causes a marked fall in BGL by an action on the liver through some nerve fibers.

Key words : intracerebroventricular (ICV) intracisternal (IC) insulin glucose

INTRODUCTION

The control of blood glucose level (BGL) in mammals is mainly due to insulin and glucagon (27). A direct feedback mechanism is supposed to be responsible for the control of these hormones (26). The CNS has been the target for the exploration of possible connections with the BGL for a long time. Bernard (1) and Feldberg (7) have suggested areas in some parts of CNS as having influence on BGL whereas Woods *et al.* (27) have suggested that every nucleus of the brain has been implicated in metabolism at one time or another. The presence of insulin in cerebrospinal fluid (CSF) has opened to find out its role in CNS (11,16). Although it is not yet clear whether insulin in CSF has any function, the role of intracisternally administered insulin has been found to be hypoglycaemic (4, 15, 17). Some workers have suggested that the insulin caused a decrease in BGL by increasing the utilization of glucose by the nervous tissue while others differ to it. In the present investigation, on peripheral BGL have been studied. In addition, an attempt has been made to identify the precise site of action in the CNS.

MATERIALS AND METHODS

The study was conducted on 44 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 chloralose (80 *mg/kg* body weight) in a leg vein.

ICV cannula was inserted in 30 animals in left lateral ventricle of the brain by the technique of Feldberg *et al*(8). The cerebrospinal fluid from the cannula was examined microscopically

for erythrocytes and epithelial cells in ordr 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 6 animals by the technique of Chowers *et al* (4).

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

1. Vagosympathectomy—in 5 animals

The vagi nerves in dogs are joined by the cervical-trunk of the sympathetic and the two nerves continue along the dorsal aspect of the common carotid artery in a common sheath (23). At the level of 4-6th cervical vertebrae, a 2.0 cm piece from both the nerves was removed.

2. Adrenalectomy—in 5 animals
3. Pancreatectomy—in 5 animals
4. Hepatectomy—in 5 animals
5. Spinal-cord transection—in 10 animals by the technique of Ezdinli *et al* (6).
   (a) Vagi intact—5 animals
   (b) Vagosympathectomy—5 animals
6. Local application—in 3 animals was done on the cerebellomedullary angles, exposed by the technique of Feldberg (7).

Hormone solntion :

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 0.2 *U/ml*. After the dilution the solution was kept in a water bath at 37°C for 30 min before being injected.

Design of experiments :

Central administration: A minimal effective dose of 0.1 *U* of insulin in 0.5 *ml* was established by administering gradually decreasing amounts of insulin in the lateral ventricle and estimating BGL. This dose was used in all subsequent experiments.

Intravenous (I/V) administration : In a separate set of experiments, effects of I/V administration of the minimal dose of insulin (0.1 *U*) on BGL was studied in (a) animals with vagi intact, (b) animals with vagosympathetomy, (c) animals with spinal-cord transectomy and (d) animals with spinal-cord transectomy along with vagosympathectomy.

The blood samples (0.5 *ml*) were collected in following sequence :

1. After inserting the venous cannula.
2. A. After completing the surgical procedure.
   B. Half-an-hour after the surgical procedure, when the BGL had established, and immediately before the administration of the insulin dose.
3. Immediately after administration of the insulin dose.
4. One sample each at 10 or 15 min intervals for two hrs, from the time the insulin dose was administered.

After withdrawing the blood samples each time, 20 *ml* of normal saline was administered intravenously so as to meet the physiological needs of the animal body.

Control experiment :

In 5 animals, the control experiment was carried out by ICV administration of the inactivated insulin. The inactivation was done by alkalinization and then bringing up the neutral pH (4). In order to examine the effect from the preservative substances present in the insulin vial, 0.5 *ml* solution (1.6% Glycerol, 0.25% Phenol I.P. and Hydrochloride acid to adjust the pH 3-3.5) was administered into the lateral ventricle of brain of 2 animals. Moreover, with every surgical procedure the control experiment was carried out. The blood samples were taken and BGL was estimated.

The blood glucose estimation was done by the Folin and Wu method (9) after collecting the blood samples in fluoride tubes.

Results are expressed as mean ±SE; their significance was tested by applying Student 't' test.

RESULTS

The initial experiments consisted of a comparison of the effects of ICV and IC administration as well as the local application of insulin on BGL. The results are shown in Fig. 1. ICV administration resulted in a nonsignificant ($P>0.05$) immediate increase in BGL from 130.0±3.3 to 143.5±1.7 followed by a significant ($P<0.05$) marked fall from 130.0±3.3 to 100.0±2.0 *mg%*. IC administration caused a nonsignificant ($P>0.05$) immediate rise in BGL from 128.5±3.5 to 141.5±3.5 followed by a significant ($P<0.05$) fall from 128.5±3.5 to 101.5±2.2 *mg%*.

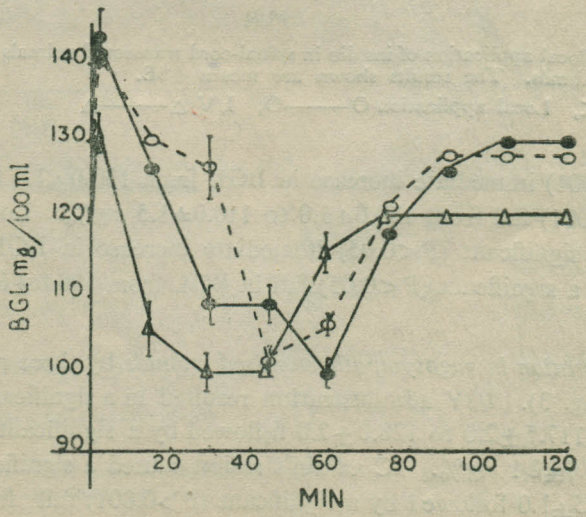

Fig. 1 : Effect of ICV and IC administration and local application of insulin on BGL. The results shown are means ±SE.

ICV●———●, IC O— — — —O, Local application. △———△

Local application resulted in a nonsignificant ($P>0.05$) immediate increase from 120.0±1.6 to 131.0±2.5 followed by a significant ($P<0.01$) fall from 120.0±1.6 to 100.0±2.8 *mg%*.

ICV and local application in spinal-cord transected animals: In order to study the role of the spinal-cord in transmission of impulses from CNS to various organs, the effect of ICV administration and local-application was studied in spinal-cord transected animals and the results are shown in Fig. 2. ICV administration in spinal-cord transected animals resulted

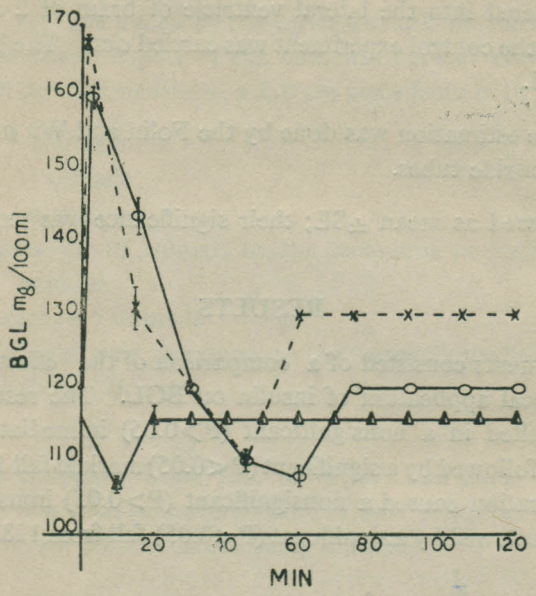

Fig. 2 : Effect of ICV and local application of insulin in spinal-cord transected animals and I/V administration in vagi intact animals. The results shown are means ±SE.
ICV X— — —X, Local application O———O, I/V △———△

in a significant ($P>0.001$) immediate increase in BGL from 130.0±1.8 to 167.5±1.1 followed by a significant ($P>0.001$) fall from 130.0±1.8 to 110.0±1.5 *mg%*. Local application in such animals too caused a significant ($P<0.05$) immediate increase in BGL from 120.0±0.6 to 160.0±1.5 followed by a significant ($P<0.05$) fall in BGL from 120.0±0.6 to 108.0±1.5 *mg%*.

Central administration in vagosympathectomised animals by three routes showed the same pattern of changes (Fig. 3). ICV administration resulted in a significant ($P<0.05$) immediate increase in BGL from 117.5±2.0 to 128.0±2.0 followed by a significant ($P<0.05$) fall in BGL from 117.5±2.0 to 107.0±2.1 *mg%*. IC administration caused a significant ($P>0.01$) increase from 90.0±1.2 to 100.5±1.0 followed by a significant ($P>0.001$) fall from 90.0±1.2 to 68.0±0.7 *mg%*. Local application resulted in a nonsignificant ($P>0.05$) increase in BGL from 135.0±1.6 to 150.0±2.0 which was followed by a significant ($P<0.05$) fall from 135.0±1.6 to 100.0±2.5 *mg%*.

Effect of ICV and local application in spinal-cord transected-vagosympathectomised animals are shown in Fig. 3. Both the immediate hyperglycaemic and the subsequent hypoglycaemic effects did not appear when insulin was administered by these two routes.

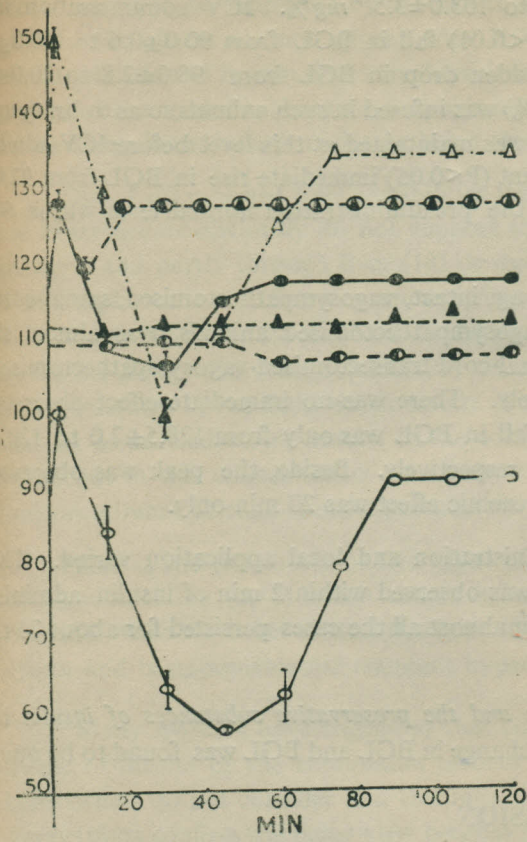

Fig 3 : Effect of ICV, IC and I/V administration and local applicaion of insulin in animals :

ICV—vagosympathectomy

ICV—spinal-cord transectomy along with vagosympathectomy

IC-vagosympathectomy

Local application-vagosympathectomy

Local application-spinal-cord transectomy along with vagosympathectomy

I/V-spinal-cord transectomy along with vagosympathectomy

The results shown are means ±SE.

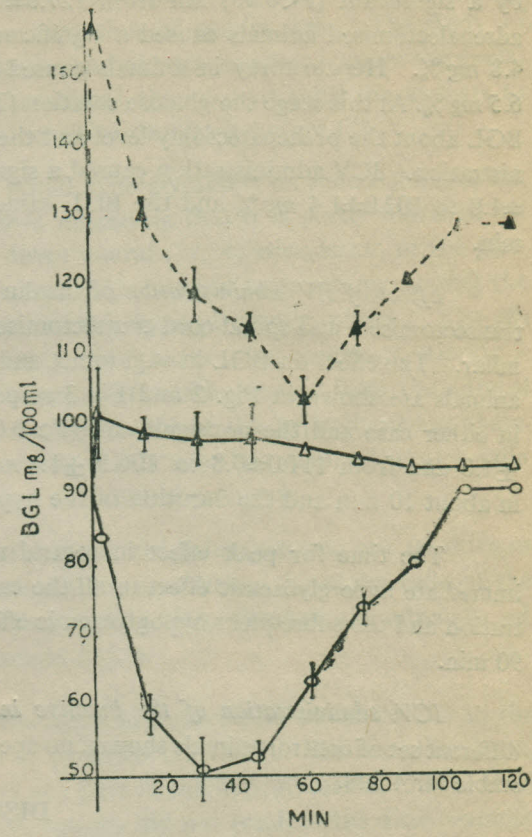

Fig 4 : Effect of ICV administration of insulin in animals after :

Pancreatectomy

Adrenalectomy

Hepatectomy

The results shown are means ±SE.

Effect of ICV administration of insulin in pancreatectomised, adrenalectomised and hepatectomised animals are shown in Fig. 4. ICV administration in pancreatectomised animals caused a significant ($P<0.05$) immediate rise in BGL from 127.5±2.1 to 157.0±3.0 followed by a significant ($P<0.05$) fall from 127.5±2.1 to 103.0±3.5 *mg%*. ICV administration in adrenalectomised animals caused a significant ($P<0.01$) fall in BGL from 90.0±0.6 to 50.0±4.8 *mg%*. Hepatectomy in animals caused a sudden drop in BGL from 98.0±2.8 to 20.0±6.5 *mg%*. At this stage the glucose solution (20 *g%*) was infused in such animals so as to bring up BGL about the prehepatectomy level and then it was maintained at this level before ICV administration. ICV administration caused a significant ($P<0.05$) immediate rise in BGL from 92.0 ±1.8 to 102.0±1.4 *mg%* and the BGL came to the preadministration normal level within 60 min.

Effect of I/V administration of insulin in vagi intact, vagosympathectomised, spinal-cord transectomised and spinal-cord transectomised-vagosympathectomised animals, was almost similar. The effect on BGL in vagi intact and spinal-cord transectomised-vagosympathectomised animals are shown in Fig. 2 and Fig 3 respectively. There was no immediate effect observed in either case and the nonsignificant ($P>0.05$) fall in BGL was only from 128.5±2.0 to 120.0 ±1.0 and from 111.0±0.8 to 106.5 ±1.4 *mg%* respectively. Beside, the peak was observed in about 10 min and the duration of the hypoglycaemic effect was 20 min only.

The time for peak effect in central administration and local application varied. The immediate hyperglycaemic effect in all the cases was observed within 2 min of insulin administration and the subsequent hypoglycaemic effect in almost all the cases persisted for about 70 to 90 min.

ICV administration of the inactive insulin and the preservative substances of insulin in different set of control animals showed no special change in BGL and BGL was found to be quite stable for 4 hrs.

DISCUSSION

In the present investigation the effects of centrally administered insulin on the peripheral BGL have been studied with the intention of exploring any nervous tract that might be influencing the BGL.

Unlike the well accepted hypoglycaemic action of insulin, central administration by the different routes studied has the peculiarity of biphasic action i.e. an initial transient hyperglycaemia followed by a sustained hypolycaemia. This biphasic response to centrally administered insulin is mediated through some central structure is evident by two observations: firstly, the I/V administration of insulin (0.1 U) had no initial hyperglycaemic effect and the nonsignificant hypoglycaemic peak was observed only after 10 min. Secondly, no change in BGL was observed when insulin was administered by ICV route in animals which had undergone spinal-cord transectomy along with vagosympathectomy. Moreover, Chowers *et al.* (4) have reported that insulin dose not cross the CSF—blood barrier.

Since no variation in the effects (initial hyper and subsequent hypo-) is observed either after vagosympathectomy or after spinal-cord transectomy alone, it can be safely stated that the efferents pass via both the spinal-cord as well as the vagosympathetic trunks in as much as the sympathetic fibers are present both in the spinal-cord and the vagi nerves (23.). This conclusion seems to be supported by the fact that in spinal-cord transected-vagosympathectomised animals, the effects did not appear after ICV administration of insulin dose. The study of local application of insulin on cerebellomedullary angles and at various other sites on the dorsal surface of the medullaoblongata (unpublished observations), the most probable site appears to be the cerebellomedullary angles as being the only sensitive central structure (7).

Out of oft reported four possibilities for the initial hyperglycaemia on central stimulation, our observations obviously do not support the views suggesting that it is partly mediated by glucagon and partly through liver (10) or due to direct neural (14,24) stimulation of the pancreatic alpha cells (6,18) or by epinephrine mediation (13), because hepatectomy as well the pancreatectomy alone did not lead to disappearance of this hyperglycaemic phase. The observations along with other reports (21) make it amply clear that insulin, somehow, stimulates some structures in the CNS which results in the liberation of catecholamines from the adrenal medulla as adrenalectomy results in the complete disappearance of the hyperglycaemic phase. Significant in this connection is the observation of Shore (22) who has reported that insulin depletes adrenal catecholamines, presumably through the central pathway.

There is the possibility of two types of central structures being present, one sensitive to glucagon secretion (2,10) which causes hyperglycaemia by stimulating the alpha cells of the pancreas, while the other central structure is sensitive to the action, through the adrenals, of insulin and is responsible for transient hyperglycaemia (3,22).

Many workers have suggested that the subsequent hypoglycaemia could be due to the release of insulin by the stimulation of beta cells of pancreas (12,25,27). On the other hand, many others do not consider that this insulin plays any part in the hypoglycaemia (5,15). Our observations confirm the latter view because pancreatectomy did not result in the disappearance of the hypoglycaemic effect.

The evidence that, hypoglycaemic response is indeed due to direct neural innervation of liver, therefore, rests entirely on the studies of previous workers (19,20.21) who have reported that neural stimulation causes decrease in BGL by suppressing the liver enzyme glycogen phosphorylase and glucose-6-phosphatase and activating the glycogen synthetase. Additional confirmation about the direct neural innervation of the liver has been obtained by our observation that hepatectomy seems to abolish the hypoglycaemic effect (Fig. 4).

REFERENCES

1. Bernard, C. Chiens rendus diabetiques. *Compt. Rend. Soc. Biol.*, **1** : 60, 1849.
2. Bloom, S.R., A.V. Edwards and N.J.A. Vaughan. The role of the autonomic innervation in the control o glucagon release during hypoglycaemia in the calf. *J. Physiol.* (London), **236** : 611-623, 1974.
3. Britton, S.W. Studies on the conditions of activity in endocrine glands (XVIII). The nervous control o insulin secretion. *Am. J. Physiol.*, **74** : 291-307, 1925.
4. Chowers, I., S. Lavy and L. Halpern. Effect of insulin administered intracisternally on the glucose lev of the blood and the cerebrospinal fluid in vagotomised dogs. *Expt. Neuro.*, **14** : 383-389, 1966.
5. Daniel, P.M. and J.R. Henderson. The effect of vagal stimulation on plasma insulin and glucose levels i the baboon. *J. Physiol.*, **192** : 317-327, 1967.
6. Ezdinli, E.J., R. Javid, G. Owens and J.E. Sokal. Effect of high spinal-cord section on epinephrine hyper glycaemia. *Am. J. Physiol.*, **214** : 1019-1024, 1968.
7. Feldberg, W. A Pharmocological approach to the brain from its inner and outer surface, London, Edwar Arnold (Publisher) Ltd. p. 18, 1963.
8. Feldberg, W. and S.L. Sherwood. A permanent cannula for intraven:ricular injection in cat. *J. Physiol.*, **120** : 3P-5P, 1953.
9. Oser, B.L. Hawk's Physiological Chemistry. ed. XIV, Bombay-New Delhi, Tata McGraw-Hill Publishing Company Ltd. p. 1052, 1965.
10. Frohman, L.A. and L.L. Bernardis. Effect of hypothalmic stimulation on plasma glucose, insulin and glucago levels. *Am. J. Physiol.*, **221** : 1596-1603, 1971.
11. Greco, A.V., G. Ghirlanda, G. Fedeli, G. Gambassi. Insulin in the cerebrospinal fluid of man. *European Neurol.*, **3** : 303-307, 1970.
12. Kaneto, A., K. Kosaka and K. Nakao. Effects of stimulation of the vagus nerve on insulin secretion. *Endocrinology*, **80** : 530-536, 1967.
13. Leclercq-Meyer, V., G.R. Brisson and W.J. Malaisse. Effect of adrenaline and glucose on release of glucagon and insulin *in vitro*. *Nature—New Biol.*, **231** : 248-249, 1971.
14. Lefebvre, P.J. and R.H. Unger. Glucagon Molecular Physiology, Clinical and Therapeutic Implications. Oxford, Pergamon, p. 80, 1972.
15. Morgolis, R.U. and N. Altszuler. Effect of intracisternally administered insulin 131I in normal and vagotomised dogs. *Proc. Soc. Exptl. Biol. Med.*, **127** : 1122-1125, 1968.
16. Morgolis, R.U. and N. Altszuler. Insulin cerebrospinal fluid. *Nature*, **215** : 1375-1376, 1967.
17. Rafaelson, O.J. Insulin action on the certal nervous system. *Acta Med. Scand. Suppl.*, **476** : 75-84, 1967.
18. Rosenberg, F.J. and V. Distefano. A central nervous system component of epineprine hyperglycaemia. *Am. J. Physiol.*, **203** : 782-788, 1962.
19. Shimazu, T. Glycogen synthetase activity in liver : Regulation by autonomic nerves. *Science*, **156** : 1256-1257, 1967.
20. Shimazu, T. and A. Fukuda. Increased activities of glycogenolytic enzymes in liver after splanchnic nerve stimulation. *Science*, **150** : 1607-1608, 1965.
21. Shimazu, T., A. Fukuda and A. Ban. Reciprocal influences of the ventromedial and lateral hypothalamic nuclei on blood glucose level and liver glycogen content. *Nature* (London), **210** : 1178-1179, 1966.
22. Shore, P.A. Release of serotonin and catecholamines by drugs. *Pharm. Rev.*, **14** : 531-550, 1962.
23. Sisson, S. The Anatomy of The Domestic Animals. Revised by Grossman, J.D., ed. IV, Bombay, Asia Publishing House, p. 826, 1961.
24. Steiner, D.F. and N. Freinkel. Hand Book of Physiology. Endocrine Pancreas. Washington, D.C., Sec. 7, Vol. I, *Am. Physiol. Soc.*, p. 721, 1972.
25. Szabo, O. and A.J. Szabo. Evidence for an insulin-sensitive receptor in the central nervous system. *Am. J. Physiol.*, **223** : 1349-1353, 1972.
26. Vallance-Own, J. Insulin inhibitors and antagonists. *Advances in metabolic disorders*, **1** : 191-217, 1964.
27. Woods, S.C. and D. Porte, J.Y. Neural control of the endocrine pancreas. *Physiol. Rev.*, **53**, No. 3 : 596-619, 1974.