# CENTRALLY MEDIATED HYPOCHOLESTEROLAEMIC RESPONSE OF INSULIN IN DOGS

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**Summary:** Insulin on intravenous administration in dogs caused a rise in blood cholesterol level. This may be due to its direct action on liver or other peripheral structures. On the other hand, insulin administered into the lateral cerebral ventricles in normal as well as in spinal and vagotomized dogs resulted in a lowering of blood cholesterol. In cross circulation studies insulin administered into lateral cerebral ventricles of donor dogs produced a hypocholesterolaemia in the recepient dogs without significant changes in blood cholesterol of donor dogs. It is suggested that some substance may be liberated from some parts of central nervous system due to an action of centrally administered insulin. This substance in turn causes hypocholesterolaemia by acting on liver or some other peripheral structures in dogs.

**Key words:** Insulin intracerebroventricular route spinal and vagotomy hypocholesterolaemia

## INTRODUCTION

Cholesterol plays a significant role in the pathogenesis of arterial atherosclerosis (4). In the body cholesterol is distributed in two forms (1) free and (ii) esterified with fatty acids. It is assumed that it is the esterified form which changes with dietary, hormonal and other variations (1).

The incidence of severity of arterial sclerosis is enhanced by the presence of diabetes mellitus and this may be due to increased serum cholesterol levels (4). This would suggest that insulin lowers blood chloesterol, but Penhos *et al.* (8) reported that there was no change in blood and liver cholesterol when insulin was perfused in rats. On the other hand, increasing serum insulin level to double the normal value by diverting the pancreatic venous blood into the vena cava and feeding sucrose resulted in a two fold increase in serum cholesterol, phosphlipids and triglycerides in dogs (3). Sloan *et al.* (9) demonstrated that hyperinsulinaemia and hypertriglyceridemia are often associated with atherosclerosis and they proposed that these may be the etiological factors of atherosclerosis. Stout (10) reported that insulin stimulates the incorporation of radioactive sodium acetate into lipids, cholesterol and phospholipids to a significant extent.

The effect of insulin on blood cholesterol thus appears to be disputed. Since insulin readily crosses the blood brain barrier (5,6), it is quite possible that at least, part of the insulin action might be due to its central action. Keeping this in mind, the effects of centrally administered insulin on blood cholseterol have been studied.

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## MATERIALS AND METHODS

Dogs of either sex weighing between 8 to 12 *kg* were used in the present study. They were anaesthetized with nembutal (30 *mg/kg* body weight) dissolved in normal saline at room temperature. The anaesthesia was maintained by subsequent intravenous nembutal, if necessary. In all the dogs constant ventilation of the lungs was maintained by intubating the trachea and connecting it to a pulmoflator. The femoral vein was exposed and a polythene cathetor was indwelled for taking the successive samples of blood and to infuse saline and drug whenever required. The standard dose of insulin I.P. (Boots Company India Ltd.) used was 0.05 *ml* (2.00 units) intravenously, diluted to a volume of 2 *ml* with normal saline. Intravenous injections were made through a polythene cathetor inserted unto the formal vein. The dose of insulin injected into the lateral cerebral ventricle was 0.25 units in a volume not more than 0.1 *ml*. A fresh solution was prepared before administration.

Cannulation of lateral cerebral ventricle was done according to the technique of Bhargava and Tangri (2). Bilateral vagotomy was done in ten dogs by exposing and sectioning both the vagi high in the neck. In some animals spinal transection was done at the level of $C_2$. During the surgical procedure about 300 *ml* of normal saline was infused by drip.

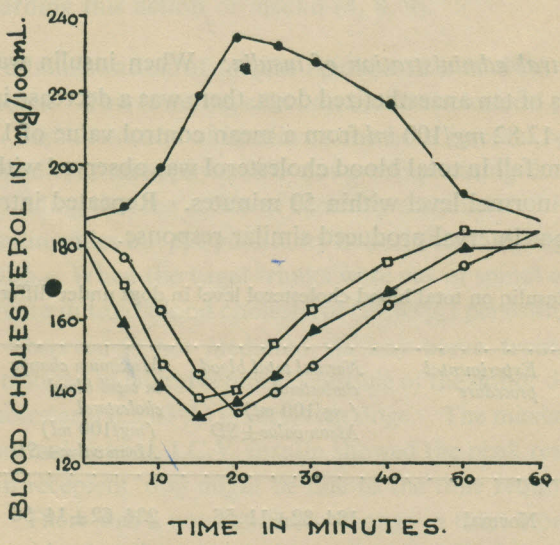


Fig. 1: Showing the effect of insulin on blood cholesterol level in dogs.

1. Intravenous(2.00 units).
2. Intracerebroventricular(0.25 units).
3. Intracerebroventricular in spinal vagotomized dogs (0.25 unit).
4. Intracerebroventricular in donor dogs in cross circulation experiments (0.25 unit). (cholesterol level of recepient dogs)

In five sets of cross circulation experiments, two dogs were used in each set. After anaesthetizing them, they were put on two different operation tables side by side. In both the dogs

internal carotid arteries and internal jugular veins were exposed and cannulated by polythene tubes in such a manner that both the internal jugular vein and internal carotid artery of one dog were connected to the internal jugular veins and internal carotid arteries of other dog, respectively. The flow of venous blood of dog 'A' (Donor) was towards veins of dog 'B' (recepient) and flow of arterial blood of dog 'B' was towards dog 'A'. The ultimate purpose was to utilise the head (brain) of dog 'A' and periphery of dog 'B'. Now a intraventricular cannula was inserted in dog 'A' by the procedure already mentioned. Heparin (3-4 *mg/kg* of body weight) was administered intravenously to prevent clotting.

The samples of blood were taken in plain tubes at five minutes interval for 30 minutes; then ten minutes interval for next 30 minutes. The total blood cholesterol was determined according to the technique of Sackett (1925) as described by Varley (11).

## RESULTS

1. *Effect of intravenous administration of insulin:* When insulin was administered intravenously in ten normal dogs, there was an increase in mean total blood cholesterol level to 234.62 $\pm$14.56 *mg*/100 *ml* from a control mean value of 184.82$\pm$11.56 *mg*/100 *ml*. The maximum increase in blood cholesterol level was observed within 20 minutes, reaching to its normal level within one hour.

2. *Effect of central administration of insulin:* When insulin was administered into the lateral cerebral ventricles of ten anaesthetized dogs, there was a decrease in mean total blood cholesterol level to 132.56$\pm$12.82 *mg*/100 *ml* from a mean control value of 178.72$\pm$13.87 *mg*/100 *ml* of blood. The maximum fall in total blood cholesterol was observed within 15 minutes and then it gradually returned to normal level within 50 minutes. Repeated intraventricular administration of insulin at one hour interval produced similar response.

TABLE I: Showing effect of insulin on total blood cholesterol level in dogs under different experimental procedures.

| *No. of experiment* | *Route of administration* | *Experimental procedure* | *Normal total blood cholesterol (mg/100 ml) Mean value$\pm$SD* | *Maximum change in total blood cholesterol (mg/100 ml) Mean value$\pm$SD* | *Change in percentage* | *Time required for maximum charge* |
|---|---|---|---|---|---|---|
| 10 | Intravenous | Normal | 184.82$\pm$11.56 | 234.62$\pm$14.56 | +26.90 | 20 minutes |
| 10 | Intracerebro-ventricular | Normal | 178.72$\pm$13.87 | 132.56$\pm$12.82 | —25.84 | 15 minutes |
| 10 | Intracerebro-ventricular | Spinal Section and bilateral vagotomy | 181.73$\pm$12.89 | 136.85$\pm$10.94 | —24.69 | 15 minutes |
| 5 sets | Intracerebro-ventricular | Cross circulation | | | | |
| | | Donor | 186.28$\pm$14.92 | 177.54$\pm$15.26 | —4.69 | 20 minutes |
| | | Recepient | 181.74$\pm$11.65 | 134.44$\pm$12.76 | —25.03 | 20 minutes |

3. *Effect of central administration of insulin following spinal section and bilateral vagotomy:* In ten anaesthetized dogs with spinal section and vagotomy, insulin administered into lateral cerebral ventricle caused a decrease in mean total blood cholesterol to 136.85±10.94 *mg*/100 *ml* from a control mean value of 181.73±12.89 *mg*/100 *ml* of blood. The maximum decrease in total cholesterol was observed within 15 minutes reaching to normal level within 50 minutes.

4. *Effects of intracerebroventricular administration of insulin in cross circulation experiments:* When insulin was administered into the lateral cerebralventricle of donor dogs a fall in mean total blood cholesterol level to 134.44±12.76 *mg*/100 *ml* from a control mean value of 181.74±11.65 *mg*/100 *ml* in recepient dog was observed. The maximum fall was obtained within 20 minutes; then there was a gradual return to its normal level within one hour. There was a nonsignificant fall in mean total blood cholesterol to 177.54±15.26 *mg*/100 *ml* of donor dog, from a control mean blood cholesterol level of 186.28±14.92 *mg*/100 *ml*.

## DISCUSSION

When insulin was given by peripheral route it caused an elevation in total blood cholesterol level by 26.99%. The highest peak was obtained within 20 minutes and the effect lasted for one hour. This may be due to direct action of insulin on liver or other structures. Controversial reports are available regarding this action of insulin (4, 8, 9).

However, central administration of insulin resulted in a 25.84% decrease in total blood cholesterol within 15 minutes. This lowering effect on blood cholesterol persisted for 50 minutes. Repeated intraventricular administration of insulin at one hour interval resulted in a similar type of response. This indicated that tachyphylaxis is not a complicating factor under these experimental conditions. The finding that the action of centrally administered insulin was just opposite to that of intravenous insulin, rules out the possibility of peripheral leakage of intracerebroventricularly administered insulin. When the vagal trunks were cut in spinal animal, central administration still caused a decrease in total blood cholesterol (24.69%) persisting for 50 minutes. This indicates that the effect observed is not mediated via any nerve trunk. In cross circulation experiments, insulin administered in lateral cerebral ventricle of the donor dogs, caused a significant decrease in total blood cholesterol (25.03%) in recepient dogs. The maximum effect was observed within 20 minutes. In normal animal, I.C.V. insulin showed the peak response after 15 minutes. The delay of 5 minutes in recepient dog might be due to the time required by circulation from donor to recepient dogs. There was a nonsignificant change in total blood cholesterol of donor dogs (4.69%) again indicating that the hypocholesterolaemic effect is not mediated via any nerve trunk.

Since insulin is known to pass blood brain barrier and is shown to be present in cerebrospinal fluid (5,6), all the above findings suggest that centrally administered insulin causes a liberation of some chemical substance which in turn causes hypocholesterolaemia in dogs. The exact nature of this substance could not be ascertained.

These findings could, to some extent, provide an explanation of the paradoxical observation that in diabetics the blood cholesterol is usually raised whereas the peripheral administration of exogenous insulin itself raises the cholesterol level. It may be surmised that in diabetics, the deficiency of insulin is more 'sensed' by central nervous structures with the resultant rise in blood cholesterol. It may be noted in this context that the dose necessary for showing an effect was 0.25 unit by intracerebroventricular route which is very small as compared to 2.00 units by intravenous route required to produce a rise in cholesterol. In other words, insulin deficiency in the central nervous system is the decisive factor in diabetes which causes hypercholesterolaemia, the hypercholesterolaemic action of peripheral insulin being purely a pharmacological action

It is worthwhile studying the blood cholesterol levels in cases of hyperinsulinaemia.

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