Serum Cholesterol Responses to Hypothalamic Stimulation and Fatty Acid Administration in the Rat[1] (36732)

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Serum cholesterol concentration is influenced by a wide variety of factors. In recent years there has been increasing evidence that hypercholesterolemia occurs in man after exposure to highly charged emotional situations (1–4). These observations may raise questions about the relationship between emotional "stress" and coronary heart disease (5). The role of dietary fat in serum cholesterol concentration is also of current interest (6). While it appears that ingested fat and psychic influences are independent factors, certain laboratory evidence suggests that in some instances they may be related. Experiments in dogs (7), cats (7), rabbits (8–10) and rats (11, 12) using electric stimulation of the hypothalamus combined with lipid feeding have resulted in hypercholesterolemia. Similar experiments in rabbits have also disclosed an increased atherogenicity (9, 10). In our own laboratory while the administration of a heavy cream meal (40% butter fat) through a gastric tube to lightly anesthetized rats followed by hypothalamic stimulation produced hypercholesterolemia, hyperglyceridemia and hyperphospholipidemia (11), neither lipid feeding alone, nor electric stimulation alone, elicted this response. In these experiments, therefore, both the presence of a fat load and hypothalamic stimulation proved to be essential and interacted in such a way as to elevate the serum lipids. Recently, we were also able to demonstrate that the mechanism of this hyperlipidemia in the rat was mediated through functional biliary obstruction. This was evidenced by an increase in the serum alkaline phosphatase activity, demonstration of dilated intrahepatic biliary canaliculi by electron microscopy and complete absence of hyperlipidemia when a free flow of bile was maintained by positioning a rigid cannula in the common bile duct during and after stimulation (12).

In addition to stimulation of the hypothalamus, another type of influence on serum lipid concentration by this area of the brain has been noted. Thus Friedman and Byers (14) were able to produce hypercholesterolemia, but not hyperglyceridemia in the rat by placing lesions in the ventral medial nucleus, the fornices and the medial portion of the lateral nucleus. In this type of preparation these authors were unable to find any evidence of biliary obstruction. More recently, Bernardis and Schnatz (15), produced elevation of serum cholesterol concentration with lesions only in the ventromedial nucleus of the rat but not in other hypothalamic areas, and in contrast to Friedman and Byers, also noted elevation of plasma glycerides. Several mechanisms for their observed responses were considered including the possibility of biliary obstruction, but no definitive conclusions were reached. Of interest in their report, however, is the attention drawn to the possibility that destruction of the ventromedial nucleus and stimulation of the lateral nucleus may be reciprocally related.

Since the test meal we used in our previous experiments was a mixture of saturated and unsaturated fats in various proportions, it was not possible to conclude to which of these the hypercholesterolemic response was related, or indeed whether or not more than one was involved. In the present investigation, we have selected three fatty acids ac-

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TABLE I. Influence of Duodenal Contents on Concentrations and Mean % Changes of Serum Cholesterol in Hypothalamically Stimulated Rats.

Treatment	No. of animals	Range in serum cholesterol conc (mg %)		Mean % change in cholesterol conc ± SE[a]
		Prestimulation	Poststimulation	
Stearic acid (18:0)	12	44–95	54–111	16.8 ± 3.7[b]
Oleic acid (18:1)	10	49–81	43–98	7.2 ± 6.4
Linoleic acid (18:2)	15	53–107	51–111	−0.2 ± 4.0
Mineral oil	8	60–95	57–100	−2.8 ± 6.4

[a] Mean % change in concentration $= (100/N)\ (\Sigma^{N}_{J=1}\ (C_f/C_i - 1)_j$ where C_f = final concentration poststimulation; C_i = initial concentration prestimulation; N = number of animals in each group.

[b] Statistically significant with respect to linoleic acid ($p < .005$) and with respect to mineral oil ($p < .01$).

cording to their double bond number and administered them individually. All animals then received hypothalamic stimulation and their serum cholesterol responses were compared.

Methods. Forty-five overnight-fasted male and female albino Sprague-Dawley rats weighing between 200 and 250 g were lightly ether-anesthetized and maintained in this condition throughout the experimental period. One milliliter of blood was obtained from the jugular vein by venipuncture and allowed to clot. Using a small midline abdominal incision the following substances were injected into the proximal duodenum with a 2 ml syringe and 25 gauge needle: (a) 300 mg of stearic acid in 0.75 ml mineral oil (12 rats), (b) 300 mg of oleic acid as the oil (10 rats), (c) 300 mg of linoleic acid as the oil (15 rats), (d) 0.75 ml mineral oil (8 rats). Substances a, b, c, were all fatty acids of the same carbon chain length and of at least 95% purity.[2] Crystalline stearic acid was melted in heated mineral oil and cooled to 75° before injection. All other injectables were also brought to the same temperature, in order to control the thermal effect on the variable under observation, *i.e.*, the serum cholesterol concentration. No untoward systemic influences were noted in any of the animals during administration. Thirty minutes later, stimulation was performed stereotaxically in the right lateral hypothalamic region (13). A second blood sample was obtained 30 min after this procedure. The rats were then killed and the brains were removed for determination of electrode position.[3] All blood specimens were centrifuged immediately after clotting and the sera were removed for total cholesterol determination (16). For analysis of the data, the pre- and poststimulation serum cholesterol concentrations, and the percentage differences in these concentrations for each animal were determined. The means of these percentage changes were tabulated for each group and compared by analysis of variance (17).

Results. Table I shows the ranges of the pre- and poststimulation cholesterol concentrations and the mean percentage change of the individual differences in each group. Since mineral oil was used as a vehicle for stearic acid, its possible effects in altering serum cholesterol concentration were also evaluated, although this appeared unlikely in view of its failure to do so in previous experiments involving plasma triglyceride responses to hypothalamic stimulation (13). On comparison between the mean percentage changes, statistically significant differences were present between the stearic and linoleic

[2] These doses of substances a, b, c, were chosen because they represent the amounts of saturated and unsaturated fatty acids approximately present in 1 ml of heavy cream, which was used as the source of lipid feeding in previous experiments.

[3] Actually more than 45 animals were used. Only those with histologic evidence of electrode tips in the lateral hypothalamic regions were retained for the experiment.

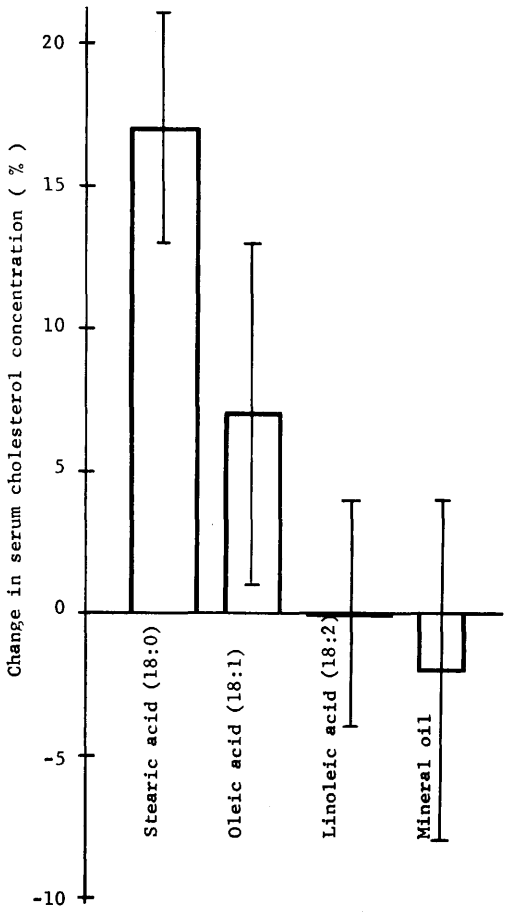

FIG. 1. Bar graph showing mean percentage change in serum cholesterol concentration ± standard error for each group shown in Table I.

acid groups ($p < .005$), and the stearic acid and mineral oil groups ($p < .01$). These results are exhibited graphically in Fig. 1.

Discussion. The data suggest that the magnitude of serum cholesterol elevation in response to hypothalamic stimulation is *inversely* related to the number of double bonds in the C_{18} fatty acid molecule present in the intestinal lumen. The mechanism by which these changes are brought about is presently unknown and is under study. Current investigations on the dietary control of serum cholesterol concentration assign an important lowering effect to polyunsaturated fats, which, following intestinal absorption, result in a net outflow of cholesterol from serum to tissue compartments (18). Although no studies of this type were performed in these experiments, such an event appears unlikely in view of the short time interval during which the hypercholesterolemic response was observed. In our previous reports with a fat load of heavy cream, the increase of serum cholesterol concentration was shown to be mediated through functional biliary tract obstruction (12). A similar mediation pathway is postulated for these experiments although it is not known why an inverse relationship between C_{18} double bond number and cholesterol elevation exists. A plausible explanation based on previous work suggests that variable degrees of biliary obstruction are involved.

The results of these experiments conform to our earlier findings relating a hypercholesterolemic response to the combined effects of lipid feeding and hypothalamic stimulation. They differ from the former and also from those of other investigators by indicating that the hypercholesterolemic response may be influenced by the type of lipid ingested. This information may be useful in studying atherogenesis induced with animal models employing brain stimulation and lipid diets. Although our studies are concerned with acute experiments the work of Gunn and associates suggest similar results in chronically stimulated rabbits on atherogenic regimes (9).

Summary. Responses of serum cholesterol concentration to electric stimulation of the lateral hypothalamus were determined in fasted rats given stearic, oleic or linoleic acid by direct duodenal instillation. Greatest percentage increase occurred in the saturated, with intermediate and zero responses in the mono- and diunsaturated fatty acid groups respectively, suggesting that the double bond number of the carbon chain influenced the response.

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