Tissue Catecholamines in Hypercholesteremic Rabbits

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ABSTRACT

Hypercholesteremia was induced by cholesterol feeding of rabbits for 10 weeks. Gross examination of aorta of these animals showed an evidence of atheromatous lesions. The endogenous catecholamines in heart, adrenal gland, spleen, brain, liver and kidney of these hypercholesteremic rabbits were markedly reduced as compared to those of normal animals, respectively. There may exist some correlation between the serum cholesterol and tissue catecholamines.

INTRODUCTION

It has long been known that the infusion of epinephrine or norepinephrine causes rapid mobilization of unesterified fatty acids from the body tissues into the circulation (Havel and Goldfien, 1959; Schotz and Page, 1959; Goodman and Knobil, 1959a; Bogdonoff et al., 1961). On the other hand, Russek (1959) reported that deposition of cholesterol in the intima of blood vessels was accelerated and intensified by the administration of epinephrine, and also noticed that the catecholamine-phospholipid compounds appeared to possess a particular affinity for arterial tissues. Several compounds having adrenergic blocking activity such as reserpine (Somoza, 1958), dihydrogenated ergot alkaloids (Goodman and Knobil, 1959a), chlorpromazine, phenotamine and phentolamine (Hollister et al., 1957) have been found to depress the elevated serum cholesterol levels and the severity of atherosclerotic lesions in cholesterol-fed animals or in patients with various disorders of fat metabolism.

In the course of studies on catecholamines in various tissues, it was found that the cholesterol feeding of rabbits produced atheromatous lesions in the aorta and reduced markedly the catecholamine concentration in the heart and other tissues. This interesting finding suggest that there may be an intimate correlation between the catecholamines and hypercholesteremia. The present experiment was undertaken to examine the possible relationship between tissue catecholamines and serum cholesterol levels in rabbits in an attempt to gain access to the nature of endogenous catecholamines in the genesis of atherosclerosis.

MATERIALS AND METHODS

Female albino rabbits weighing approximately 2.0 kg were placed individually in wire bottom cages. All the animals were maintained in a controlled environment and also maintained on the standard diet for 2 weeks in order to establish base-line values for the parameters of this study. Hypercholesteremia was induced by feeding a diet containing cholesterol 1%.

Blood was taken from the marginal ear vein of rabbits in order to determine the serum cholesterol. The cholesterol was determined by
the method of Zak (1957). All animals were sacrificed at the end of 9 weeks of cholesterol feeding. Tissue extracts were prepared and their catecholamine contents assayed according to spectrophotofluorometric procedure described by Shore and Olin (1958). The aortae were taken out and examined grossly for evidence of atheroma.

RESULTS

A. Changes in Serum Cholesterol:

Table 1 represents a composite response of the serum cholesterol to the cholesterol feeding. The mean serum cholesterol value in rabbits, before initiation of the feeding with cholesterol diet, was 91±8.76 mg/100 ml. During the cholesterol feeding, the concentration of serum cholesterol markedly and rapidly increased during the 4 weeks reaching high level more than 10 folds as compared to that of control values. Thereafter, it gradually increased during the rest 6 weeks of cholesterol feeding.

All the animals killed at the end of the experimental period showed gross pathological evidence of atheromatous lesions in the aortae (Fig. 1).

Table 1. The concentrations of serum cholesterol in rabbits fed with cholesterol diet

<table>
<thead>
<tr>
<th>Control</th>
<th>Serum cholesterol (mg/100 ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2 wk</td>
</tr>
<tr>
<td>85</td>
<td>1580</td>
</tr>
<tr>
<td>145</td>
<td>750</td>
</tr>
<tr>
<td>105</td>
<td>1950</td>
</tr>
<tr>
<td>70</td>
<td>2110</td>
</tr>
<tr>
<td>65</td>
<td>912</td>
</tr>
<tr>
<td>80</td>
<td>800</td>
</tr>
<tr>
<td>115</td>
<td>975</td>
</tr>
<tr>
<td>120</td>
<td>1500</td>
</tr>
<tr>
<td>105</td>
<td>2700</td>
</tr>
<tr>
<td>60</td>
<td>650</td>
</tr>
<tr>
<td>91</td>
<td>1393</td>
</tr>
<tr>
<td>±8.76</td>
<td>±220</td>
</tr>
</tbody>
</table>

B. Tissue Catecholamine Contents:

At the end of 9 weeks following cholesterol feeding, rabbits were killed and the catecholamine contents of various organs were determined. As shown in Table 2, it may be noticed that cholesterol feeding markedly reduced the endogenous catecholamines in the heart, adrenal gland, spleen, brain, liver and kidney of rabbits. Especially, the catecholamine content in adrenal gland was reduced as much as 80% of the control value.

Table 2. Tissue catecholamine contents of rabbits fed with cholesterol diet

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Catecholamines (µg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before cholesterol</td>
</tr>
<tr>
<td>Heart</td>
<td>1.54±0.032</td>
</tr>
<tr>
<td>Adrenal gland</td>
<td>365.00±33.00</td>
</tr>
<tr>
<td>Spleen</td>
<td>0.79±0.089</td>
</tr>
<tr>
<td>Brain</td>
<td>0.47±0.031</td>
</tr>
<tr>
<td>Liver</td>
<td>0.45±0.026</td>
</tr>
<tr>
<td>Kidney</td>
<td>0.56±0.028</td>
</tr>
</tbody>
</table>

*P < 0.01
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![Graphs showing catecholamine contents in adrenal gland, brain, and spleen](image)

**Fig. 2.** The relationship between the catecholamine contents of adrenal gland, brain, spleen and serum cholesterol of rabbits.

Examination of the relationship between the serum cholesterol and the tissue catecholamines revealed that as the serum cholesterol increases, the tissue catecholamine content decreases. There was significant correlation between the serum cholesterol and catecholamine content of the adrenals, of the brain and of the spleen with a correlation coefficient ($r$) of $-0.81$, $-0.98$ and $-0.55$, respectively (Fig. 2).

**DISCUSSION**

Since the initial demonstration by Anitschkow (1913) that atherosclerosis could be induced in the rabbit by cholesterol feeding, this animal has been widely used for such studies. Generally the experimental atherosclerosis in rabbits has been produced by employing a diet containing between 1 and 3% of cholesterol for about 2 months (Filos et al., 1956; Rona et al., 1959; Cook et al., 1954). In this experiment, hypercholesteremia was uniformly produced in the rabbit fed 1% cholesterol diet for 10 weeks and concomitantly there was marked evidence of gross atheromatous plaques of the aorta in these animals. This result is well consistent with those by Scebat et al. (1961), Lemmon et al. (1954) and Cook et al. (1954) who have provided experimental evidence for the occurrence of atherosclerosis in the hypercholesteremic rabbits.

It is of interest that the catecholamine contents in the adrenal gland, heart, brain, spleen, kidney and liver of hypercholesteremic rabbits were profoundly reduced as compared to those of normal animals. Furthermore, examination of relationship between tissue catecholamines and serum cholesterol suggested that there was a significant correlation between these two.

It is generally well accepted that catecholamines are potent mobilizers of fatty acid from adipose tissues and this action apparently results from an activation of lipase within the adipose tissue cells (Rizack, 1961; Hollenberg, 1961). In addition, Havel (1964) proposed that the tonic activity of sympathetic nerves in this tissue promotes the release of fatty acids in the normal animal and modulation of this activity is a prime factor in the control of the rate of hydrolysis of lipid. Furthermore, Kaplan et al. (1957) reported that 24 hrs after the administration of epinephrine in oil given to dogs there were significant elevations in the plasma cholesterol concentrations. This observation was confirmed by Shafir et al. (1959) and Gans (1966) who demonstrated that the continued daily administration of epinephrine resulted in sustained hypercholesteremia. These experimental results attained physiologic significance because various types of emotional strain in man were shown to be accompanied by
simultaneous increases in the plasma cholesterol concentrations and an excretion of catecholamines into the urine (Friedman et al. 1958, 1960). Of special interest is the work of Rabb(1958) reporting that changes in the catecholamine content of the blood vessel wall may play a significant role in the development of hypertensive vascular disease. On the basis of these evidences, it is conceivable that the sympathetic nervous system, through liberation of norepinephrine in adipose tissue, has a central role in the control of the rate of turnover and of the concentration of lipid in blood plasma. Considering this experimental result that the catecholamine contents in tissue were inversely decreased in relation to the increase in the serum cholesterol, it is reasonable to assume that catecholamines normally stored within sympathetic nervous tissue or other depots are released to protect against the deposition of excess cholesterol to the body tissue.

Further studies concerning the possible role of tissue catecholamines in the development of atherosclerosis are now under progress.

REFERENCES