EFFECT OF CARBACHOL AND ADRENALIN ON CHOLESTEROL BIOSYNTHESIS IN THE RAT LIVER

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Subcutaneous injection of carbachol (0.01 mg/100 g body weight) retards the incorporation of acetate-1-C^{14} into cholesterol in the rat liver. Injection of adrenalin (0.1 mg/100 g body weight) had no significant effect on the rate of cholesterol biosynthesis.

Besides their specific role in the transmission of nervous impulses, acetylcholine and adrenalin have a direct effect on metabolism, especially lipid metabolism.

Acetylcholine stimulates the phospholipid metabolism of the pancreas and brain [7-9]. In the presence of eserine, it sharply inhibits renewal of phosphate in lecithin and cephalin of liver slices [1]. After injection of atropine, which blocks M-cholinergic systems, the intensity of cholesterol renewal in the blood and liver of guinea pigs is reduced [2, 3]. Adrenalin slows the incorporation of acetate-1-C^{14} into tissue lipids [10] increases the liberation of free fatty acids into the medium [6] and inhibits incorporation of palmitate-1-C^{14} into triglycerides [11]. The author has shown that acetylcholine in the presence of eserine, and also carbachol, retards cholesterol biosynthesis from acetate-1-C^{14} in rat liver slices. This inhibitory effect was also given by noradrenalin, but adrenalin produced no significant changes [4].

The object of the present investigation was to study the effect of carbachol and adrenalin on the rate of cholesterol biosynthesis in vivo.

EXPERIMENTAL METHOD

Experiments were carried out on male rats weighing 180-220 g, lightly anesthetized with ether, which received injections of acetate-1-C^{14} (35 μCi/100 g body weight) followed by carbachol (0.1 mg/100 g) or adrenalin (0.1 mg/100 g) in 0.1 ml physiological saline. The rats were sacrified 30 and 60 min after injection of acetate-1-C^{14}. The liver (400 mg) was washed to remove blood, and then homogenized with 3 ml physiological saline in a Potter homogenizer. Lipids were extracted from the homogenate by a mixture of isopropyl alcohol and heptane (4:1) or chloroform and methanol (2:1). The specific radioactivity of the cholesterol was determined by a modification of the method of Sperry and Webb [4]. In addition, 0.2 ml of liver homogenate was analyzed for incorporation of acetate-1-C^{14} into the liver tissue, and the specific radioactivity of the tissue was determined. The relative specific radioactivity of cholesterol was found from the ratio between the specific radioactivities of cholesterol and liver.

EXPERIMENTAL RESULTS

In the experiments of series I the effect of carbachol on the rate of incorporation of acetate-1-C^{14} into cholesterol in the rat liver was investigated. Unlike acetylcholine, carbachol is not quickly broken down in the body, but at the same time it possesses a similar physiological action. The results in Table 1 show that 30 min after injection of acetate-1-C^{14} and carbachol the specific radioactivity of cholesterol was reduced by 33.3% compared with the control ($P < 0.02$). Incorporation of acetate-1-C^{14} into the liver tissue was the same as in the control rats. The relative specific radioactivity of cholesterol was reduced

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TABLE 1. Rate of Cholesterol Biosynthesis in Rat Liver after Injection of Carbachol (M±m)

Group of animals	SR of cholesterol	SR of liver	RSR	SR of cholesterol	SR of liver	RSR
	30 min after injection of acetate-1-C^{14}			60 min after injection of acetate-1-C^{14}		
Control (n=7)	1 704±102	28,8±2,51	65,4±6,54	789±63	16,2±1,54	47,4±2,99
Experimental (n=7)	1 137±156	30,7±2,77	37,4±5,18	482±97	16,0±2,23	29,7±4,72
P	<0,02	>0,05	<0,01	<0,05	>0,05	<0,01
% difference from control	−33,3	—	−42,7	−38,9	—	−37,6

Note. Here and in Table 2 specific radioactivity (SR) of cholesterol is expressed in pulses/min/mg cholesterol; SR of liver in pulses/min/mg fresh liver; relative specific radioactivity (RSR) = SR of cholesterol/SR of liver.

TABLE 2. Rate of Cholesterol Biosynthesis in Rat Liver after Injection of Adrenalin (M±m)

Group of animals	SR of cholesterol	SR of liver	RSR	SR of cholesterol	SR of liver	RSR
	30 min after injection of acetate-1-C^{14}			60 min after injection of acetate-1-C^{14}		
Control (n=7)	1 451±136	29,7±3,43	54,6±9,62	559±68	17,4±1,79	32,3±5,22
Experimental (n=7)	1 139±98	21,3±1,97	55,6±6,17	873±144	18,4±1,96	51,4±11,37
P	>0,05	>0,05	>0,05	>0,05	>0,05	>0,05

by 42.7% ($P < 0.01$). Incorporation of acetate-1-C^{14} into cholesterol 60 min after injection of carbachol also was reduced. The decrease in specific radioactivity of cholesterol was 38.9% ($P < 0.05$), and the decrease in its relative specific activity was 37.6% ($P < 0.01$). Just as after exposure for 30 min, incorporation of acetate-1-C^{14} into liver tissue showed no difference from the control as a result of carbachol administration.

The results of experiments to study the effect of adrenalin are given in Table 2. No statistically significant difference in the specific radioactivity of cholesterol compared with the control rats was found either 30 or 60 min after injection of acetate-1-C^{14}. A slight tendency was observed for the specific radioactivity of the liver tissue to fall 30 min after injection of acetate-1-C^{14} and adrenalin, and this led to a tendency for the relative specific radioactivity of cholesterol to decrease. These tendencies evidently were connected with slowing of the supply of labeled acetate from the blood to the liver cells because of vasoconstriction occurring immediately after the injection of adrenalin.

In the study of cholesterol metabolism it is important to examine not only the cholesterol concentration in the blood and tissues, but also the rate of its biosynthesis and liberation from the cells. It is considered that the cholesterol of the liver and blood constitutes a single closed metabolic system, i.e., all the cholesterol of the liver is metabolized into cholesterol of the blood, and vice versa [5]. A change in the rate of cholesterol biosynthesis in the liver must therefore exert a significant effect on its concentration in the liver tissue itself and in the blood. Mediators of the nervous system, and especially acetylcholine and noradrenalin, can evidently play an important role in this process. The results now obtained supplement those of experiments in vitro [4], and it can be concluded from them that acetylcholine and carbachol exert an inhibitory action on the rate of cholesterol biosynthesis, whereas adrenalin has no marked action on this process.

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