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Brain cholesterol—IX.
Effect of methylphenidate on the incorporation of specifically labeled glucose

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THE lowering of brain cholesterol levels in adult mice through administration of methylphenidate (Ritalin) has been demonstrated in previous studies.[1-3] The current series of experiments are attempts to elucidate the biochemical mechanism involved in brain cholesterol formation from carbohydrate precursors and the effect of methylphenidate on this process. The following preliminary report is an account of the consequences of two drug doses on the biosynthesis of cholesterol in adult mice from glucose-1, glucose-2, glucose-6-^{14}C, and acetate-2-^{3}H.

A carbon-14 labeled sugar and tritiated acetate were injected simultaneously into mice. The specifically labeled sugars (glucose-1-^{14}C, 0·648 mc/mmole; glucose-2-^{14}C, 1·0 mc/mmole; and glucose-6-^{14}C, 0·83 mc/mmole) were injected at a level of 20 μc/kg. Acetate-2-^{3}H (10 mc/mmole) was injected at a dose of 400 μc/kg.

Ninety male Swiss mice (7–9 weeks old, weighing 21–23 g) were distributed randomly into three groups. Animals were injected i.p. either with saline or drug (4 mg/kg and 20 mg/kg) for 5 consecutive days. A final dose of the drug was given on the 6th day, 1 hr prior to sacrifice; 15 min before killing the mice, the animals were injected simultaneously with ^{14}C-sugar, specifically labeled (20 μc/kg), and acetate-2-^{3}H (400 μc/kg). The lipids were extracted from the tissues with acetone, alcohol, and ether (4:4:1) and the resulting extract used for cholesterol analysis as previously described.[4] The doubly-labeled sterol was assayed in a liquid scintillation counter.[5]

The effect of methylphenidate on the level of free cholesterol

In these preliminary studies, only free cholesterol was analysed. Data on animals in three groups, each containing 30 animals, are presented in Table 1. The effect of methylphenidate on cholesterol

TABLE 1. EFFECT OF METHYLPHENIDATE ON TISSUE FREE CHOLESTEROL*

Organ	Experiment		
	I	II	III
Liver			
Control	1·96 ± 0·09	2·01 ± 0·03	2·49 ± 0·26
4 mg/kg	1·85 ± 0·06	2·04 ± 0·06	2·00 ± 0·07
20 mg/kg	1·78 ± 0·05	1·69 ± 0·07	1·96 ± 0·08
Spleen			
Control	2·25 ± 0·16	2·09 ± 0·13	2·54 ± 0·13
4 mg/kg	2·20 ± 0·08	2·00 ± 0·10	2·16 ± 0·14
20 mg/kg	1·91 ± 0·07	2·14 ± 0·11	2·08 ± 0·30
Brain			
Control	9·13 ± 0·28	9·80 ± 0·63	9·91 ± 0·45
4 mg/kg	7·21 ± 0·35	7·65 ± 0·47	8·38 ± 0·38
20 mg/kg	7·65 ± 0·33	9·61 ± 0·43	9·82 ± 0·64

* Milligrams per gram wet weight tissue; means ± standard error; n = 10.

tissue levels is variable and dose dependent. Liver and spleen values, even in groups receiving 20 mg drug/kg, are generally, but not significantly, lower than control values. The only pronounced and consistent effect on sterol tissue levels was found for brain cholesterol values after administration of 4 mg drug/kg.

The effect of methylphenidate on glucose incorporation

Glucose, labeled specifically in the 1-, 2-, or 6-position with ^{14}C, was injected separately into three groups, each containing 30 animals (Table 2).

TABLE 2. THE INFLUENCE OF METHYLPHENIDATE ON PRECURSOR INCORPORATION INTO TISSUE FREE CHOLESTEROL (D/M/g tissue (wet)*)

Organ	Radioactive Nutrient			
	Glucose-1-^{14}C	Glucose-2-^{14}C	Glucose-6-^{14}C	Acetate-2-^{3}H†
Liver				
Control	3836 ± 1083	899 ± 117	1813 ± 421	3026 ± 538
4 mg/kg	824 ± 167	1844 ± 383	973 ± 217	2710 ± 496
20 mg/kg	947 ± 137	223 ± 25	815 ± 152	2533 ± 481
Spleen				
Control	182 ± 78	549 ± 113	440 ± 71	3158 ± 425
4 mg/kg	638 ± 132	418 ± 57	406 ± 71	3840 ± 747
20 mg/kg	375 ± 105	282 ± 34	654 ± 137	3100 ± 456
Brain				
Control	3305 ± 491	1706 ± 94	2225 ± 409	2214 ± 312
4 mg/kg	4167 ± 848	1869 ± 269	1663 ± 371	1664 ± 391
20 mg/kg	1275±384	1224±1224	2136±258	1916±182.

* Mean ± SE (where n = 30)
† Mean ± SE (where n = 90)

The effect of drug administration on the incorporation of glucose-1-^{14}C into cholesterol of various tissues was different for each tissue. In the liver, both drug levels caused a decreased incorporation of radioactivity, as compared to control values. Spleen values indicated increased radioactivity. In the brain, the incorporation of glucose-1-^{14}C was decreased only with the lower drug dose.

After drug injection with glucose-2-^{14}C, a marked variation of incorporation into liver sterol was measured. At low levels of drug concentration there was a significant increase in amount of carbons incorporated into liver cholesterol. A decrease was recorded with higher dosage. The biosynthesis of cholesterol in the spleen was markedly lowered under drug influence, with this precursor. In the brain, variation from normal values was not clearly demarcated in the group receiving the smallest amount of drug. Animals receiving the higher level of drugs showed some decrease in incorporation.

Incorporation of glucose-6-^{14}C into cholesterol presumably takes place in the breakdown of glucose-6 to acetyl-CoA, an acetyl derivative labeled in the methyl position. In the liver, the incoporation of this precursor in drug-injected groups was substantially less than control values; spleen data indicated only a slight increase at the higher drug level. Brain cholesterol showed a decrease of incorporation with the lower drug dose and little difference at the higher dose level.

The effect of methylphenidate on acetate-2-^{3}H incorporation

The same animals given specifically labeled glucose-^{14}C precursors also were injected with acetate, labeled in the methyl position with tritium. Results of these experiments are summarized in Table 2.

The incorporation of methyl-labeled acetate into liver sterol was decreased under the influence of the drug. The difference was not significant and was slightly more noticeable at the higher dose level.

At drug levels used (4 mg/kg and 20 mg/kg), no appreciable difference was noted for the incorporation of labeled acetate into spleen free cholesterol.

Incorporation of the tritiated fatty acids into brain free cholesterol imitated the changes in quantitative levels of this sterol. At the low drug level, a decrease in incorporation of methyl-leveled acetate was measured. The differences between controls and animals receiving the high-level drug doses were not considered noteworthy.

In previous studies we have shown that brain metabolism of adult mice can be influenced by various conditions—e.g. cancer,[6] starvation,[7] aging,[8] muscular dystrophy,[9–11] and a central nervous system-stimulating drug, methylphenidate.[1–3] Although the preceding "stresses" all indicate that cholesterol undergoes active metabolism, the relationship of this metabolism to physiological functions is not obvious.

The differential incorporation of glucose-2-^{14}C and glucose-6-^{14}C into tissue cholesterol followed the pattern indicated by their conversion to carboxyl- and methyl-labeled acetate respectively.[8] Similarity between data from specifically labeled glucose and acetate suggests that glycolysis of glucose is not being affected.

At this time it is impossible to differentiate the mechanism of drug action with the limited data at hand. There are two apparent areas for further investigation. The first deals with the effect of methylphenidate on fixation reactions of CO_2 involved in cholesterol formation. The second possibility, the differential incorporation of methyl- and carboxyl-labeled acetate into the sterol nucleus, suggests some change in the tricarboxylic acid cycle. Further work is necessary before the mechanism of methylphenidate on brain cholesterol can be elucidated.

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Fate of intravenously injected iodate and periodate

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BECAUSE its relation with thyroid physiology iodide metabolism has been thoroughly studied but little[1–3] is known about the distribution and elimination of iodate and periodate after intravenous administration.

The ^{131}I-iodate has been prepared by oxidation of ^{131}I-iodine with an excess of sodium chlorate[4] and the ^{131}I-metaperiodate by oxidation of the ^{131}I-iodate with chlorine in alkaline solution.[5] The radiochemical purity of these labelled compounds was assayed chromatographically. 20 μc of ^{131}-I-labelled iodate (specific activity 5 μc/mg, or 10 μc of ^{131}I-labeled potassium metaperiodate (specific activity 10 μc/mg) dissolved in 0·1 ml of distilled water, were injected into adult Wistar rats through the tail vein. Groups of 5 animals (three males and two females) were sacrificed at different intervals and the activity in organs and tissues was determined with a scintillation counter. After this, the organs were homogenized 0·01 N in sodium hydroxide and the supernatant sample analyzed by ascending chromatography on paper Whatman 3 MM and using *n*-propanol: water: 15 N ammonium hydroxide (30 : 10 : 5). With this solvent the R_f values are: Iodate 0·14–0·20, metaperiodate 0·00–0·02 and iodide 0·56–0·62.