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Brain Cholesterol XVIII: Effect of Methylphenidate (Ritalin) on [U-^{14}C] Glucose and [2-^{3}H] Acetate Incorporation (39070)

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In an attempt to elucidate sterol metabolism in adult brain, methylphenidate [phenyl-piperidyl-(2)-acetic acid methyl-ester], a central nervous system stimulant (1), was used as a perturbating agent. Structurally this drug is related to compounds that have a hypocholesterolemic effect (2–4) and also acts as a central nervous system stimulant. Previous reports (5–8) from this laboratory have dealt primarily with the effect of methylphenidate (MP) on brain cholesterol metabolism after chronic drug administration. Results after a single dose indicated sterol changes in the brain and maximal effects were reached in 24 h (8). The present report is a continuation of these studies using [2-^{3}H]acetate and [U-^{14}C]glucose as sterol precursors. The effect of a single drug injection on sterol brain levels and precursor incorporation during a 24-hr period is presented.

Materials and methods. [2-^{3}H]acetate (10 mCi/mmole) and [U-^{14}C]glucose (6.66 mCi/mmole) were purchased from New England Nuclear Corporation, mixed, and used simultaneously for this study. Radioactive precursors were dissolved in physiological saline and 0.5 ml injected intraperitoneally (IP).

Methylphenidate (Ritalin), donated by Ciba Pharmaceutical Company (H. Sheppard), was prepared as a 1% solution in saline and was diluted shortly before injection. Mice received 4 mg/kg IP of MP.

Eighty male Swiss mice (4–6 weeks old), weighing 19–22 g, were randomly distributed into four groups. Each group of 20 mice were subdivided into a saline or drug injected group. At zero-hour (9:00 AM) all animals were injected intraperitoneally with the drug or saline and placed in cages with five mice per cage. Food and water were given *ad libitum*. Animals were then killed at 2, 4, 12, and 24 h postdrug injection. Fifteen minutes prior to killing the animals, [2-^{3}H]-acetate (500 μCi/kg) and [U-^{14}C]glucose (40 μCi/kg) were injected simultaneously intraperitoneally into animals of both groups. Animals were killed by decapitation and exsanguination. Brains were surgically removed, washed with distilled water, blotted dry, quick frozen at $-70°$, and stored in an ice chest at $-30°$. Blood samples were taken, but due to the small amount of free plasma cholesterol and low radioactivity, the results were variable and are not reported. Their absence does not influence the interpretation of our results.

Tissues taken for analysis were weighed frozen (wet weight). Although both dry and nitrogen tissue values were taken, the different baseline values did not change the interpretation of the data based on wet weight alone. The tissues taken for analysis were homogenized in glass grinders and lipids were extracted with acetone:alcohol:ether (4:4:1). The lipid extracts were filtered free of protein and the nonesterified sterol precipitated by adding tomatine as previously described (9). Radioactive purity of the precipitated sterol was previously established (9, 10). The precipitated cholesterol was dissolved in glacial acetic acid and divided into two aliquots. One part was taken for colorimetric analysis, while the remainder was simultaneously assayed for carbon-14 and hydrogen-3 in a liquid scintillation counter (10).

Results. Animals were injected intraperitoneally with one level of drug (4 mg/kg) and killed after 15 min at four time intervals (2, 4, 12, and 24 hr). Fifteen minutes was at (in the case of acetate) or shortly before (in the case of glucose) peak time of precursor labeling. Each time interval had its own individual control. Since there was no significant difference in control values during the 24-hr period, the values were accumulated and used as a single mean and standard deviation. Brain values 2 hr after drug

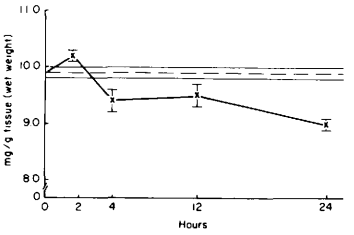

FIG. 1. Male mice were injected (ip) with 4 mg/kg of methylphenidate and killed at hourly intervals. The dashed and unbroken lines represent the mean and standard deviation, respectively, of normal brain sterol levels.

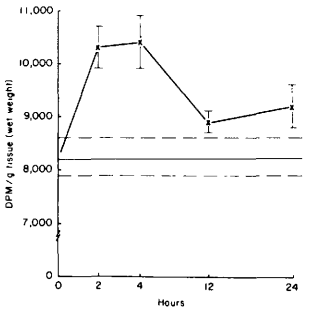

FIG. 2. Mice were injected with 4 mg/kg of methylphenidate. Animals were killed at 0, 2, 4, 12, and 24 hr after drug injection. Fifteen minutes prior to killing the animals, mice received [U-^{14}C] glucose (40 μCi/kg). Normal mean values are represented by unbroken lines.

injection tends to be slightly higher than normal, but after four hours show significant decreases at other time intervals (Fig. 1).

Metabolic studies were carried out in these same animals by the simultaneous injection of [U-^{14}C]glucose and [2-^{3}H]acetate. [U-^{14}C)glucose incorporation studies showed that there was an increase in glucose carbon-14 into brain sterol 2 and 4 hr after drug injection (Fig. 2). The values for the latter time intervals (12 and 24 hr post drug injection) were lower than peak values, but still slightly higher than normal. Consequently, the data suggest that in MP treated mice, more glucose was incorporated over normal amounts and that this effect is maximum 2–4 hr after drug injection.

The effect of MP on acetate incorporation is similarly significant (Fig. 3). However, the affect was in the opposite direction, a decrease, and the maximum drug effect was reached 12 hr after MP injection. Incorporation values at 2 and 4 hr are lower than normal, but not significant; while the 24-hr interval value is slightly higher than normal. The results suggest a decrease in [2-^{3}H]acetate incorporation into brain sterol and that the maximum effect (decrease) is reached 12 hr after drug injection.

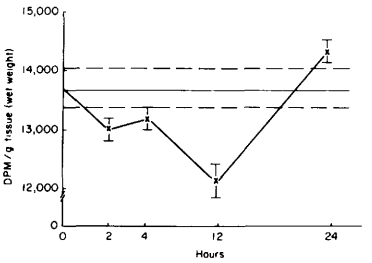

FIG. 3. Mice described in Fig. 1 also received [2-^{3}H]glucose (500 μCi/kg) under the same conditions.

Discussion. In earlier studies we have shown that brain metabolism of adult mice can be influenced by various conditions, i.e., starvation (11), aging (12), muscular dystrophy (13–15) and a central nervous system-stimulating drug, methylphenidate. Although the preceding "stresses" all indicate that cholesterol undergoes active metabolism, the relationship of this metabolism to physiological functions is not obvious.

The importance of these other studies to brain cholesterol metabolism has been reviewed (16). It has been our intention to study the effect of a drug on lipid metabolism in order to better understand factors effecting brain sterol metabolism. As previously stated, MP is chemically related to compounds having hypocholesteremic effect (2–4) and also affects brain metabolism (1). Although MP has been studied and used for over 20 yr (17), the primary basis for its mode of action remains uncertain. Only recently has the metabolism and biotransformation of the drug been studied in detail in man and other animals (18). Consequently, these studies may shed light not only on brain sterol metabolism, but also indicate possible biochemical alterations which give information on the mechanism of action of this clinically useful drug. The use of MP in different clinical situations (19) indicates a need for more information on the mode of action of this compound.

Early reports from this laboratory (5–8) dealt primarily with the effect of MP on brain cholesterol metabolism after chronic administration. Our previous result after a single injection of drug indicated that maximum effect in lowering the cholesterol levels brain book place after 24 hr (8). In these experiments, the effective dose level of drug was found to be 4 mg/kg. While a larger dose (20 mg/kg) also caused pronounced biochemical alteration, it was felt that the lowest dose which caused a change more readily indicated the primary effect rather than those changes taking place at higher doses or after chronic administration.

To investigate further the problem of drug effects on brain cholesterol metabolism, and not merely sterol levels, a more systematic approach was initiated. In the present report, the effect of acute drug administration on [2-^{3}H]acetate and [U-^{14}C]glucose incorporation into brain free sterol was considered. A limitation of our experimental protocol is the fact that experiments were carried out over a 24-hr period. Diurnal changes normally taking place during this period complicate our interpretation of drug effects and normal variation (20). Because of these limitations, all drug groups are compared to their own hourly control. Our data for control brain values, however, did not seem to be influenced by this type of variation, and control values from different time intervals are grouped together. With these experimental limitations in mind, the following observation on the data can be made.

A single injection of methylphenidate (4 mg/kg) caused a continuous change in the concentration of brain free cholesterol until a maximum decrease was reached after 24 hr. This agrees with our previous findings (8).

It is of interest that the slight rise in sterol brain levels after two hours found previously (8) was also duplicated in the present experiment. Our finding continues to support the concept that membrane cholesterol levels may regulate neurol nerve impulse activity and may help explain certain drug effects. Metrazol has been shown to also inhibit cholesterol synthesis (21). Additional support to this concept comes from the fact that chlorpromazine (22) and many barbiturates (23) increase the amount of lipids in tissues. More recently, Kopeloff and Alexander have associated low sterol levels with spontaneous activity and/or convulsive response after low levels of pentamethylenetetrazol (24).

The specific mode of sterol lowering by MP action cannot be surmised at this time. The incorporation studies with radioactive acetate and glucose shed some light on this problem, but the interpretation of the incorporation experiments are not simple. While [2-^{3}H]acetate indicates a decreased incorporation rate, [U-^{14}C]glucose shows an increased rate of incorporation in brain sterols. Whether these decreases and increases reflect changes in biosynthesis or changes in pool sizes cannot be determined. Even if levels of glucose and acetate were measured, the pool size, directly related to biosynthesis, generally cannot be determined. This is a limitation of most pulse-tracer experiments.

Incorporation experiments with uniformly labeled glucose are even more difficult to interpret since glucose has at least two major pathways for metabolism. An added complicating factor is that MP has been shown to inhibit glycolysis by blocking sugar passage into yeast (25, 26). The conclusion was reached from these experiments that the cell membrane was the site of MP action. Even with these limitations in mind, the use of methylphenidate as a probe for brain sterol metabolism clearly supports the following concept. The MP lowers brain sterol levels in all of our previous studies (5–8), as well as in the present study. The mechanism by which this is accomplished, while not known, may involve sterol metabolism only as an ultimate rather than a primary affect. This is indicated by failure of two cholesterol precursors to show the same changes in incorporation after drug administration. Regardless of the mechanism involved, the lowering of sterol in the brain membrane could well account for methylphenidate's pharmacological effects.

Finally, contrary to earlier concepts regarding brain lipid metabolism (see 18 for critical review), the lipids of the central nervous system are in a dynamic rather than static state (27).

This interpretation of the data, while con-

sistent with the facts of this and other experiments, may be limited since drug effects were measured in whole brain. Consequently, affects on specific sterol compartment(s) were not measured. Since whole and not discrete brain areas were studied, a more regional approach to changes in specific brain areas, cell populations, or even organelles needs to be undertaken if we hope to elucidate further drug action and/or brain cholesterol metabolism. The present protocol can provide a model for useful future studies with this compound.

Summary. The effect of a single injection of methylphenidate (Ritalin, 4 mg/kg) on precursor ([2-^{3}H]acetate and [U-^{14}C]glucose) incorporation into brain cholesterol was studied. The drug caused a steady decrease in the concentration of brain cholesterol during the 24-hr period examined. Incorporation studies during this time with [U-^{14}C]glucose indicated higher than normal incorporation for all time periods studied. The most significant incorporation increases took place 2 and 4 hr after drug injection. Experiments using [2-^{3}H]acetate as the sterol precursor gave incorporation values which tended (not significantly) to be lower than control values at 2 and 4 h. The values after 12 hr were less than normal, while the 24-hr group indicated an increase to or slightly higher than normal values.

These data suggest that the pharmacological effect of methylphenidate may be due to lowering of brain cholesterol levels directly or on some more basic metabolic process leading to a decreased level of membrane sterols.

1. Meier, R., Gross, F., and Tripod, J., Klin. Wochenschr **32,** 445 (1954).
2. Cottet, J., Redel, J., Drumm-Heller, C., Tricaud, M. E. Bull. Acad. Nat. Med. **441,** 25 (1953).
3. Bizzi, L., Garattini, S., and Pacilli, N., Boll. Soc. Ital. Biol. Sper. **32,** 95 (1956).
4. Garattini, S., Paoletti, P., and Paolette, R. G., Int. Biochem. **6,** 429 (1956).
5. Kabara, J. J., McLaughlin, J. T., and Riegel, C. A., *in* "Drugs Affecting Lipid Metabolism" (S. Garattini and R. Paoletti, eds.), pp. 221 Elsevier Publishing Company (1961)
6. Kabara, J. J., and Riegel, C. A., Biochem. Pharm. **14,** 1928 (1965).
7. Kabara, J. J., Proc. Soc. Exp. Biol. Med. **118,** 905 (1965).
8. Kabara, J. J., Alvares, A. P., Riegel, C. A., and McLaughlin, J. T., Proc. Soc. Exp. Biol. Med. **120,** 37 (1965).
9. Kabara, J. J., McLaughlin, J. T., and Riegel, C. A., Anal. Chem. **33,** 305 (1961).
10. Kabara, J. J., and McLaughlin, J. T., J. Lipid Res. **2,** 283 (1961).
11. Kabara, J. J. In proceedings VII Congress on World Federation of Neurology, Rome, Italy (1961).
12. Kabara, J. J., Prog. Brain Res. **9,** 155 (1964).
13. Kabara, J. J., Tex. Rep. Biol. Med. **22,** 126 (1964).
14. Kabara, J. J., Tex. Rep. Biol. Med. **22,** 134 (1964).
15. Kabara, J. J., Tex. Rep. Biol. Med. **22,** 143 (1964).
16. Kabara, J. J., *in* "Neurological Aspects of Maturation and Aging" (D. H. Ford, ed.), Elsevier Publishing Company (1973).
17. Meier, R., Gross, F., and Tripod, J., Ritaline. Klin. Wochenschr. **32,** 445 (1954).
18. Faraj, B. A., Israili, A. H., Perel, J. M., Jenkins, M. L., Holtzman, S. G., Cucinell, S. A., Dayton, P. G., J. Pharmacol. Exp. Ther. **191,** 535 (1974).
19. Esplin, D. W., Zablocka-Esplin, B., *in* "The Pharmacological Basis of Therapeutics" (L. S. Goodman and A. Gilman, ed., The Mac Milan Company, New York, Fourth Edition (1970).
20. Shapiro, D. J., and Rodwell, V. W., J. Biol. Chem. **246,** 3210 (1971).
21. Alexander, G. J., and Alexander, R. B., Proc. Soc. Exp. Biol. Med. **115,** 229 (1964).
22. Grassi, E., Paoletti, P., Paoletti, R., J. Neurochem. **6,** 73 (1960).
23. Conference on Evaluation and Mechanism of Drug Toxicity, New York Academy of Science, New York, New York, March (1964).
24. Kopeloff, L. M., and Alexander, G. J., Life Sci. **10,** 869 (1971).
25. Spoerl, E., and Doyle, R. J., J. Bact. **96,** 744 (1968).
26. Spoerl, E., J. Bact. **105,** 1168 (1971).
27. Dhopeshwarkar, G. A., Mead, J. F., Adv. Lipid Res. **11,** 109 (1973).

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