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Methylphenidate Decreases Local Glucose Metabolism in the Motor Cortex

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BELL, R. D., G. M. ALEXANDER AND R. J. SCHWARTZMAN. *Methylphenidate decreases local glucose metabolism in the motor cortex.* PHARMACOL BIOCHEM BEHAV **18**(1) 1–5, 1983.—The local cerebral metabolism on glucose (*l*-CMRg) was evaluated in animals given methylphenidate (15 mg/kg) in order to investigate possible mechanisms of action of the drug. Significant increases in *l*-CMRg ($p>0.05$) were found in the globus pallidus, entopeduncular nucleus, substantia nigra, subthalamic nucleus, inferior olive, red nucleus, lateral cerebellar cortex, ventral lateral nucleus of the thalamus and the midbrain reticular formation. Significant decreases ($p>0.05$) in *l*-CMRg were found in the motor cortex. These results suggest possible mechanisms for methylphenidate's action in attention deficit disorders.

Methylphenidate Attention deficit disorder Cerebral metabolism

ATTENTION deficit disorder (ADD) is a term used to describe a variety of neuropsychiatric disorders in children [14, 16, 26, 37, 38]. It is clear that no one specific constellation of symptoms clearly defines ADD. However, there is a major subgroup that exhibits a symptom complex of decreased attention span, increased impulsivity, increased motor activity, and learning disability, which is frequently referred to as the hyperkinetic child syndrome (HCS) [18].

There is extensive evidence that stimulatory drugs, such as methylphenidate, are effective in the treatment of HCS [5, 19, 20, 35]. Despite their efficacy, the mechanism whereby these drugs enable children and adults to attend to individual tasks for a longer period of time has not been fully elucidated, although central nervous system dopaminergic pathways have been implicated [30,32]. The quantitative 2-deoxyglucose technique offers a unique opportunity to evaluate the effect of methylphenidate on local cerebral glucose metabolic rate (*l*-CMRg) of all neuronal structures of the central nervous system (CNS) simultaneously. Because of the close relationship between metabolic rate and functional activity, this method may be used to identify specific structures in the brain in which functional activity is altered.

The purpose of this study is to determine the effect of methylphenidate on *l*-CMRg in the CNS which may suggest its mechanism of action in the HCS.

METHOD

Animals

Male Wistar rats weighing between 250 and 350 grams were used in this study. The animals were allowed nothing except water for 12 hours prior to the start of the experiment in order to stabilize blood glucose.

Chemicals

2-(1-^{14}C)-Deoxy-D-Glucose (2DG) (New England Nuclear NEC-495, 45–50 mCi/mmole) was used in the study. Glucose concentrations were determined enzymatically with the Calbiochem Glucostat Pak Kit. Amersham, ACS scintillant was used to determine ^{14}C concentration. Ritalin HCl (Methylphenidate) was obtained from CIBA Pharmaceutical Company.

Experimental Protocol

Nine rats were used in this study. Five animals were used as controls and four were injected intraperitoneally with methylphenidate (15 mg/kg).

The femoral artery and vein were cannulated under nembutal anesthesia (35 mg/kgm). They were then placed in a rodent restrainer and allowed 12–16 hours to recover from the anesthesia.

A bolus of ^{14}C 2DG (7.5 μCi/100 g) was injected via the femoral vein catheter 40 minutes after the methylphenidate injection. Arterial blood samples (0.15 cc) were obtained at 15 second intervals for the first minute, then at 2, 4, 6, and 10 minutes. After 10 minutes, samples were taken every 5 minutes for the remainder of the study (45 minutes after the initial bolus injection). The samples were centrifuged and 20 μl of plasma was pipetted into scintillation vials. Ten ml of ACS scintillation solution was added to each vial and the radioactivity counted in a Beckman LS 7000 Scintillation Counter with automatic quench compensation by external standardization. An additional 20 μl was pipetted from the 2, 10, 20, 30, and 40 minute samples and the glucose concentration determined enzymatically.

Forty-five minutes after the bolus of 2DG, the animals were sacrificed by intravenous administration of 1 ml of

nembutal (60 mg/ml) and 2 ml of saturated KCl solution. The brain was removed, frozen in liquid nitrogen and cut into 20 μ sections. The sections were placed on microscope slides, dried for 4 hours at 60°C and exposed along with plastic ^{14}C standards on Kodak SB-5 X-ray film for 14 days. The sections were then stained with Thionin for histological verification of structures. The anatomical structures of interest were identified by reference to the Pellegrino rat atlas [23]. Each structure was read in at least three sections. A minimum of five readings were taken per structure per section per rat. The optical density of each structure was determined with a gamma microdensitometer with an aperature diameter of 0.1 mm. The ^{14}C concentration of each structure was computed from its optical density as compared to that of the standards. The local cerebral metabolic rate for glucose (*l*-CMRg) was calculated quantitatively from the 2DG tissue concentration, the arterial 2-deoxyglucose and glucose curves, and the rate and lumped constants evaluated by Sokoloff, *et al.* [33].

Statistics

The mean *l*-CMRg of neuroanatomical structures from the experimental animals was compared to controls by 1-way ANOVA.

RESULTS

The effect of methylphenidate on *l*-CMRg is shown in Table 1. Significant increases in *l*-CMRg were found in the substantia nigra, subthalamic nucleus, entopeduncular nucleus, globus pallidus, lateral cerebellar cortex, inferior olive, red nucleus and ventral lateral nucleus of the thalamus. A significant increase in *l*-CMRg was also found in the midbrain reticular formation but not the pontine reticular formation ($p<0.01$). Significant decreases in *l*-CMRg were found in the gigantopyramidal cells in area 4 of the motor cortex. These findings are illustrated in Fig. 1a where the decrease in *l*-CMRg in area 4 is shown. Figure 1b illustrates the increased *l*-CMRg in the entopeduncular nucleus and VL of thalamus. Figure 1c shows the increase in *l*-CMRg in the subthalamic nucleus. Figure 1d demonstrates the increase in *l*-CMRg in the substantia nigra and the red nucleus. No changes from control were found in the medial geniculate and inferior colliculus which demonstrates the specific action of methylphenidate on the motor systems.

TABLE 1

EFFECT OF METHYLPHENIDATE ON *l*-CMRg IN THE AWAKE RAT

Structure		Controls (N=5)	Methylphenidate (N=4)
Motor Cortex			
Tier	Area		
1	6	98.6 ± 4.5	89.0 ± 3.5
2	6	108.8 ± 6.2	98.5 ± 3.6
3	6	89.4 ± 4.9	87.3 ± 3.6
1	4	97.8 ± 6.4	78.0 ± 2.0*
2	4	104.2 ± 5.7	85.0 ± 4.0*
3	4	83.0 ± 5.5	71.3 ± 4.5
Thalamus			
VL		88.7 ± 3.0	110.0 ± 5.0†
VM		99.4 ± 4.8	120.0 ± 8.8
Caudoputamen			
Anterior		105.4 ± 4.1	109.0 ± 5.4
Mid		98.0 ± 4.9	95.8 ± 6.6
Globus Pallidus			
Medial		60.8 ± 5.4	85.0 ± 7.3*
Lateral		57.4 ± 2.8	74.0 ± 6.7†
Entopeduncular Nucleus		56.0 ± 2.6	72.0 ± 3.2†
Substantia Nigra		70.8 ± 3.3	105.0 ± 2.3†
Subthalamic Nucleus		81.4 ± 5.8	126.0 ± 9.5†
Nucleus Accumbens		59.2 ± 2.8	63.0 ± 5.0
Cerebellar Cortex			
Medial		59.2 ± 7.2	74.0 ± 7.3
Lateral		54.0 ± 2.7	68.0 ± 5.0*
Cerebellar Nuclei			
Dentate		94.6 ± 3.5	103.0 ± 4.2
Interpositus		94.4 ± 4.7	101.0 ± 4.6
Fastigial		78.8 ± 2.4	88.0 ± 3.8
Red Nucleus		72.2 ± 3.7	95.0 ± 3.4†
Inferior Olive		75.0 ± 1.8	88.0 ± 3.0†
Medial Geniculate		116.2 ± 5.8	119.0 ± 1.0
Inferior Colliculus		154.2 ± 9.0	149.0 ± 7.6
Reticular Formation			
Midbrain		67.7 ± 3.0	94.0 ± 3.5†
Pons		65.2 ± 3.5	73.6 ± 2.7

CMRg (mean±standard error) are given in μmoles/100 g/min.
*$p\leq0.05$; †$p\leq0.01$.

DISCUSSION

Several major hypotheses have evolved to explain the ability of stimulant drugs to improve the attention span in the HCS [20, 21, 32, 38].

The first hypothesis, that hyperkinetic children are physiologically underaroused [28], is supported by observations that these children have higher and smaller fluctuations of their skin resistance which is characteristic of low arousal states [27,28]. This physiological state is reversed by treatment with stimulant drugs. Using other physiological indexes of arousal and sensory processing such as auditory evoked responses, galvanic skin conductance sensimotor EEG rhythm and EMG, Shouse and Lubar were able to discriminate a subgroup of hypoaroused hyperkinetic subjects that had the greatest pretreatment symptom severity and the most favorable response to methylphenidate [32].

The second hypothesis is based on the fact that children with HCS have increased motor activity. There is also a positive relationship between baseline levels of motor overactivity and subsequent susceptibility to stimulant drugs [34]. It has, therefore, been postulated that the difficulty lies in the motor system, in sensory motor integration, or in ability to maintain attention span.

Since methylphenidate affects predominantly dopamine uptake, involvement of dopamine pathways in HCS has been postulated [24,25]. Alteration in cerebrospinal fluid monamine metabolites in children with ADD has been demonstrated [29,31]. Neonatal animal studies which partially deplete brain dopamine with 6-hydroxydopamine produce hyperactive animals. Their motor activity can be decreased and their maze learning improved by treatment with methylphenidate [30].

Both hypotheses are supported by our data. The observed increase in *l*-CMRg in the midbrain reticular formation suggests that methylphenidate may produce its effects through the ascending reticular activating system [17]. This would certainly be compatible with its known arousal affects in narcolepsy and syndromes of excessive daytime sleepiness [39].

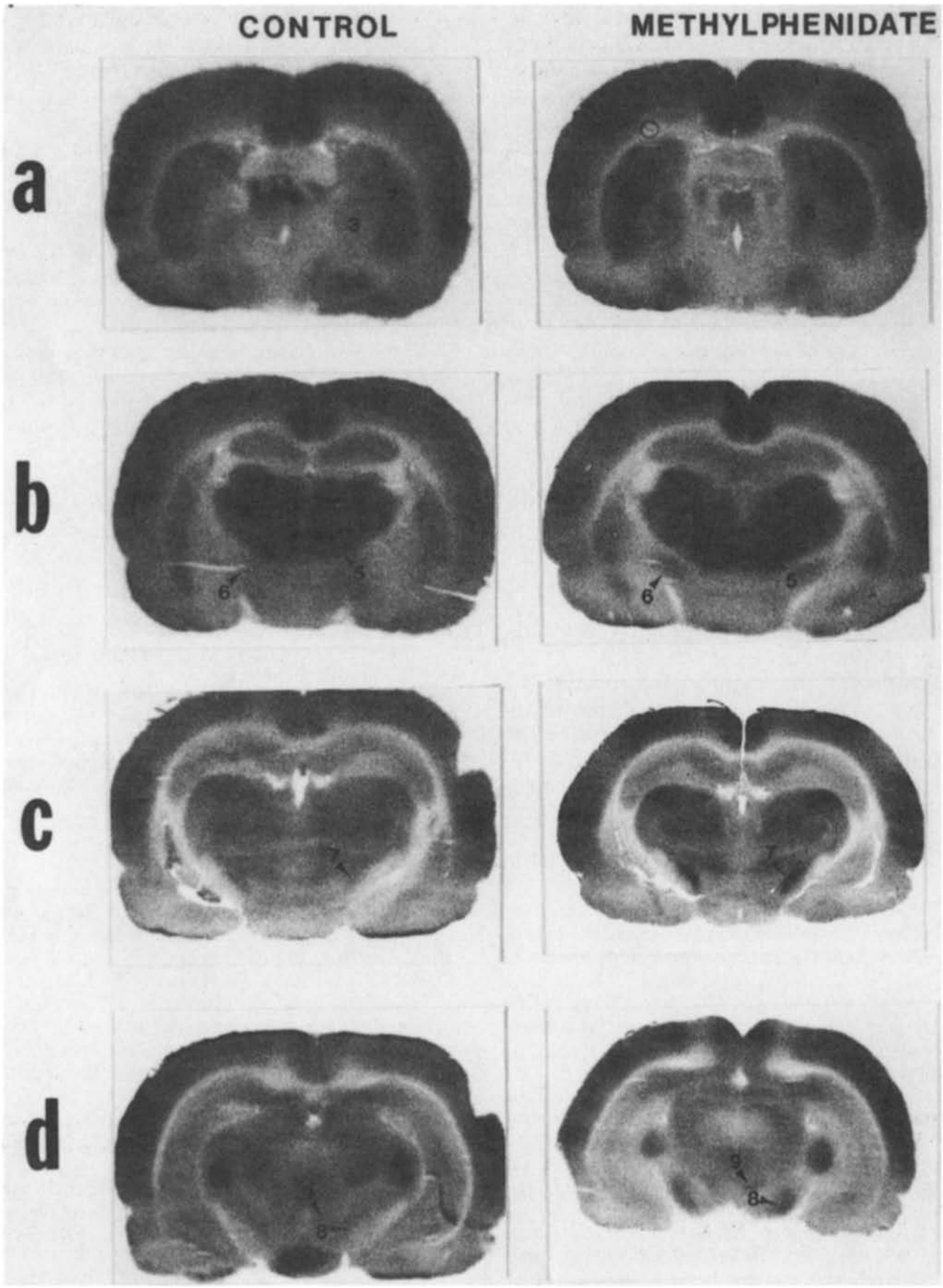

FIG. 1. This figure shows selected autoradiographic sections in controls and in animals with methylphenidate-induced stereotypy. The following structures are shown: (a) 1. motor cortex, 2. caudoputamen, 3. global pallidus, (b) 4. ventral lateral nucleus of the thalamus, 5. ventral medial nucleus of the thalamus, 6. entopeduncular nucleus, (c) 7. subthalamic nucleus, (d) 8. substantia nigra, 9. red nucleus.

Our data demonstrate an increase in *l*-CMRg of known dopaminergic systems, specifically the nigrostriatal, nigrocerebellar, and perhaps nigrocortical projections [7,12].

These results are similar to the results of Wechsler *et al.* who used different dopamine agonists d- and *l*-amphetamine [36]. They demonstrated increases in *l*-CMRg in the major components of the extrapyramidal motor system; i.e., zona reticulata and zona compacta of the substantia nigra, subthalamic nucleus, caudate nucleus, globus pallidus, red nucleus and the ventral nucleus of the thalamus. These effects were more pronounced with d- than *l*-amphetamine. There was, however, no noted decrease in *l*-CMRg in the motor cortex or increase in *l*-CMRg in the midbrain reticular formation, the cerebellum or the inferior olive. There are several reasons for this. First, from looking at our autoradiographs, it was evident that the optical density was decreased in the motor cortex. Because of this, we evaluated the cortex in three tiers according to the known organization of projections from the thalamus [3,13]. Wechsler *et al.* [36] evaluated the entire cortex. Second, *l*-CMRg in the inferior olive and midbrain reticular formation were not measured and therefore cannot be compared.

The major unexpected finding from our data was the marked decrease in *l*-CMRg in the gigantopyramidal cells of area 4 of the motor cortex. This suppression of *l*-CMRg may be explained by effects of the following projections: (1) pallidothalamocortical projections, (2) nigrothalamocortical projections, (3) cerebellothalamocortical projections, (4) direct nigrocortical projections.

Three of these pathways, the nigrothalamocortical, the pallidothalamocortical, and the cerebellothalamocortical pathways project to the motor cortex via the thalamus. The nigrothalamocortical pathway is thought to be non-dopaminergic and projects mainly to the ventral medial nucleus (VM) of the thalamus [6, 8–10, 22]. VM in turn projects diffusely to the entire cortex [3,11]. It, therefore, seems unlikely that this pathway is involved in the reduction of *l*-CMRg in area 4 of the motor cortex. The major outflow of the globus pallidus, the entopeduncular nucleus (globus pallidus interna), projects mainly to the VM but also to the ventral lateral nucleus of the thalamus (VL) [2,15]. The possibility that this pathway is involved through VL of thalamus cannot be eliminated from the existing data.

The VL nucleus receives the major projections from the cerebellum and projects primarily to the middle tier of neurons in area 4 of the motor cortex [4,9]. Since both of these pathways (i.e., inferior olive to cerebellum to red nucleus to thalamus to cortex) demonstrated an increase in *l*-CMRg, it seems likely that they are involved in the inhibition of *l*-CMRg in the motor cortex.

The last anatomical projection that may be inhibitory to the motor cortex is direct nigrocortical pathway. Identification of inhibitory dopamine receptors in the motor cortex would support the hypothesis that this pathway is involved [1,22].

Regardless of the anatomical projections responsible for the decrease in *l*-CMRg in the motor cortex after the administration of methylphenidate, the observation that metabolic activity is decreased suggests a possible mechanism of stimulatory drugs which produce an increase in the attention span in the HCS.

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