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Methylphenidate Effects on Activity-Stress Gastric Lesions and Regional Brain Noradrenaline Metabolism in Rats[1]

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GLAVIN, G. B. *Methylphenidate effects on activity-stress gastric lesions and regional brain noradrenaline metabolism in rats.* PHARMACOL BIOCHEM BEHAV **23**(3) 379–383, 1985.—Methylphenidate (5, 10, or 20 mg/kg/day) or saline were administered to rats in the activity-stress ulcer paradigm. Running-wheel activity and food consumption did not differ among groups. Methylphenidate produced dose-related increases in gastric ulcer severity, decreases in hypothalamic noradrenaline (NA) and increases in 3-methoxy-4-hydroxy-phenylethyleneglycol sulfate (MHPG-SO_4) in the hypothalamus, amygdala, hippocampus and thalamus. These results differ markedly from the effects seen with a related substance, d-amphetamine, and suggest different mechanisms of action for these drugs.

Methylphenidate Stress Ulcer Noradrenaline 3-Methoxy-4-hydroxy- phenylethyleneglycol sulfate

METHYLPHENIDATE is a mild central nervous system stimulant with structural, pharmacological, and behavioral properties somewhat similar to those of d-amphetamine [2]. While the mechanism of action of amphetamine appears to involve stimulation of dopamine (DA) synthesis [4,10], that of methylphenidate remains unclear. Fung and Uretsky [3] reported that methylphenidate does not stimulate DA synthesis *in vivo*, while Kuczenski and Segal [13] suggested that methylphenidate increased tyrosine hydroxylase activity (the rate-limiting step in neural catecholamine biosynthesis) in a synaptosome preparation.

Behavioral data also suggest differences in the effects of methylphenidate and d-amphetamine. Glavin *et al.* [6] reported that d-amphetamine given to rats in the activity-stress ulcer paradigm significantly elevated running-wheel activity, enhanced mortality, and markedly exacerbated gastric ulcer formation. The activity-stress model is one in which excess activity is a critical factor in the development of gastric disease and, ultimately, in the death of the aniaml [17]. Methylphenidate has seen extensive use in the management of attention deficit disorder ("hyperkinesis") in children, also characterized by excessive activity. Accordingly, the present study examined the effects of methylphenidate in this experimental model of excess activity in rats. In addition, we examined the effect of methylphenidate on regional brain noradrenaline metabolism. The activity-stress paradigm is associated with markedly enhanced noradrenaline turnover [20]. If methylphenidate exerts its effects primarily by enhancing noradrenaline synthesis and/or release, then this substance should exacerbate activity-stress-induced disease which should be accompanied by corresponding increases in brain noradrenaline turnover in one or more brain regions.

METHOD

Animals

One hundred and twenty male Wistar rats (Holtzman Co., Madison, WI) weighing 200 g (±10 g) at the start of the study were first randomly divided into 4 groups: saline control, and methylphenidate hydrochloride 5 mg/kg, 10 mg/kg or 20 mg/kg. Each of these drug groups was then further randomly divided in half with 15 animals remaining individually housed in standard laboratory cages (25×17.5×17.5 cm) and the other 15 animals being housed in standard activity-wheel cages (Wahmann Mfg. Co. Model LC-34) which include an adjoining cage (25×15×13 cm).

Procedure

A habituation period of 5 days followed, during which

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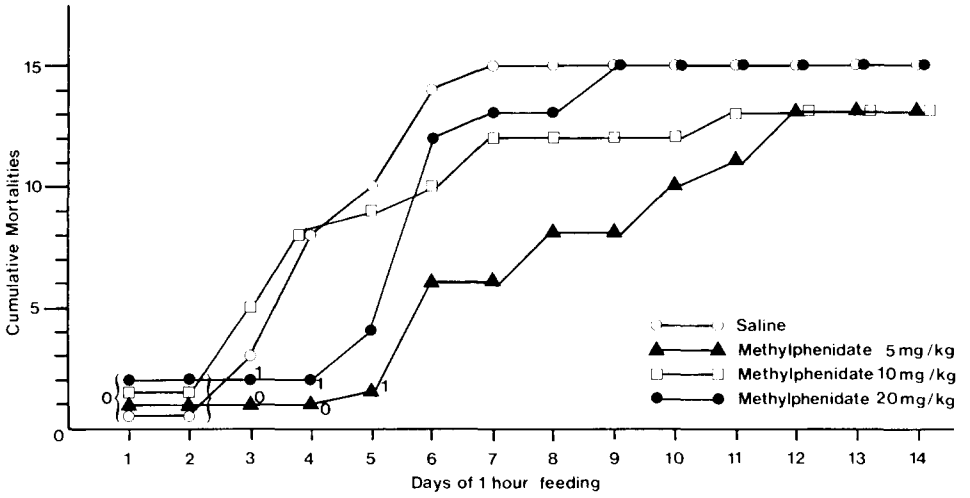

FIG. 1. Cumulative mortalities for wheel-housed rats in all drug conditions for the 14-day activity-stress period.

time food and water were available ad lib and no injections were given. Beginning on the fifth day, and continuing for the duration of the experiment, daily intraperitoneal injections began for all rats, and wheel-housed animals were fed for only 1 hr per day (0900–1000 hr). These animals were weighed each day, and their 1 hr food intake and activity wheel activity were also recorded. Injections were given after the 1 hr feeding period. Home cage-housed control rats were food-yoked to the wheel-housed rats, and were fed only that amount of food consumed by their wheel-housed partners. Previous experiments have shown that if a wheel-housed rat eats less than 1.0 g of food on a given day, it will die within 12 hr. Thus, in order to preserve brain tissues, if a rat ate less than 1.0 g of food on any experimental day, it was sacrificed by decapitation and examined as described below. Rats remaining after 14 days of 1 hr feeding were sacrificed and examined. Animals were decapitated at the same time each day (1000–1200 hr) and the brain rapidly removed and dissected over frozen carbon dioxide (CO_2) according to the method of Gispen *et al.* [5] into the following 8 regions: hypothalamus, amygdala, hippocampus, thalamus, midbrain (colliculi), pons plus medulla oblongata, cerebral cortex and basal ganglia (including caudate, putamen and globus pallidus). Brain tissue was stored at −45°C until assayed for noradrenaline (NA) and its major metabolite, 3-methoxy-4-hydroxy-phenylethyleneglycol sulfate (MHPG-SO_4) fluorometrically according to the method of Kohno *et al.* [12]. The stomach was excised, opened along the greater curvature and examined for ulcers by an observer who was naive with respect to experimental treatments. The number, location and cumulative length (in millimeters) of the ulcers were recorded by examination under a dissecting microscope fitted with a micrometer eyepiece.

All data were analyzed by analysis of variance unless otherwise indicated. When the analysis of variance was significant, individual group means were compared for significance using the Tukey HSD procedure [11].

RESULTS

Daily activity, food consumption and body weight remained similar in all groups of wheel-housed rats, and no significant differences were detected. An examination of mortality data (Fig. 1) suggested that methylphenidate at 5 mg/kg tended to prolong survival in the activity-stress paradigm, however, this trend was not significant.

Table 1 summarizes the gastric ulcer data. Only data from wheel-housed animals are shown since no instance of gastric ulcer was observed in home cage-housed rats in any drug group. All wheel-housed animals developed gastric glandular ulcers. No instance of either rumenal ulcer (indicative of starvation) or duodenal ulcer was observed. The number of ulcers per animal increased with increasing dose of methylphenidate, but not significantly ($p<0.11$). The cumulative length of ulcers (ulcer severity) increased significantly with increasing dose of methylphenidate, $F(3,56)=4.12$, $p<0.05$. Post-hoc (Tukey) tests revealed that rats given methylphenidate at 20 mg/kg and 10 mg/kg had significantly more severe ulcers than control rats or rats given the low dose of methylphenidate.

Activity-stress *per se* (saline-injected-wheel-housed rats compared to home cage controls) displayed lower NA levels in the cerebral cortex, pons and medulla, thalamus, hippocampus, amygdala and thalamus and higher MHPG-SO_4 levels in the cerebral cortex, pons and medulla, midbrain, thalamus, hypothalamus, hippocampus and amygdala (Figs. 2 and 3).

Among activity-stress (wheel-housed) rats, methylphenidate tended to decrease NA in the hypothalamus and amygdala relative to saline-injected wheel-housed rats and relative to home cage-housed controls (Fig. 2). For example, relative to saline-injected animals, methylphenidate at 5 and 10 μg/kg significantly reduced NA level in the hypothalamus, while all doses of methylphenidate resulted in significantly lower NA levels in the amygdala.

Relative to home cage controls, significant elevations in MHPG-SO_4 (Fig. 3) occurred in the hypothalamus, $F(4,70)=3.17$, $p<0.05$, amygdala, $F(4,70)=2.91$, $p<0.05$, hippocampus, $F(4,70)=3.80$, $p<0.05$, and thalamus, $F(4,70)=3.44$, $p<0.05$, suggesting accelerated NA metabolism in these brain regions of the stressed rats. These effects were not uniformly related to the dose of methylphenidate. For example, relative to saline-injected animals, methylphenidate at all doses significantly increased MHPG-SO_4

TABLE 1

SUMMARY OF STOMACH PATHOLOGY* FOR THE ACTIVITY-STRESS GROUPS

Group	No. of Rats	No. of Rats With Ulcers	Mean (±S.E.) No. of Ulcers	Mean (±S.E.) Cumulative Length of Ulcers (mm)
Saline	15	15	8.9 (8.1)	16.3 (6.7)
Methylphenidate 5.0 mg/kg	15	15	10.7 (9.6)	24.1 (7.1)†
Methylphenidate 10.0 mg/kg	15	15	11.0 (9.8)	29.0 (8.3)†
Methylphenidate 20.0 mg/kg	15	15	15.4 (10.9)	55.8 (8.8)†

*All data refer to glandular ulcers.
†Significantly different from saline group, $p<0.05$.

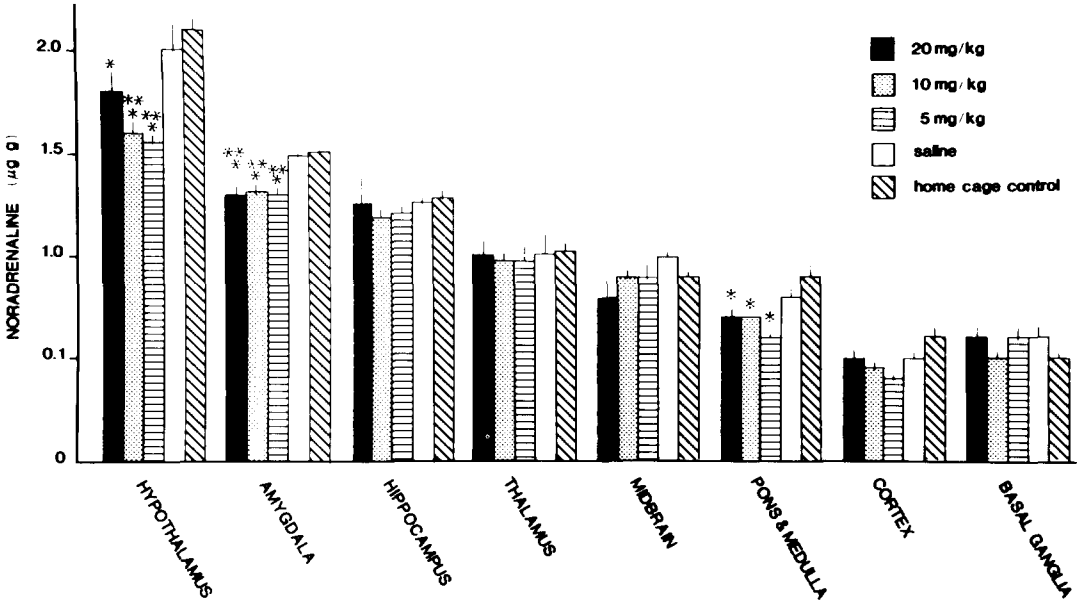

FIG. 2. Mean (±S.E.) noradrenaline levels in eight brain region of wheel-housed (n=15 per group) and home-cage-housed (n=15 randomly selected) rats. *Significantly different from home cage controls ($p<0.05$). **Significantly different from saline-injected-wheel-housed rats ($p<0.05$).

level in the hypothalamus; methylphenidate at 5 and 20 mg/kg significantly elevated MHPG-SO_4 in the amygdala; methylphenidate at 5 and 10 mg/kg elevated MHPG-SO_4 in the hippocampus while only the low (5 mg/kg) dose of methylphenidate elevated MHPG-SO_4 in the thalamus.

DISCUSSION

Methylphenidate did not significantly influence running-wheel activity, yet significantly exacerbated activity-stress-induced gastric ulceration. Noradrenaline metabolism (as reflected by *increased* MHPG-SO_4 levels and *decreased* NA levels) was altered by methylphenidate primarily in the hypothalamus, thalamus, hippocampus and amygdala. It is interesting that accelerated NA turnover in a much smaller number of brain regions is influenced by methylphenidate than by d-amphetamine [6]. D-amphetamine markedly increases running-wheel activity and mortality in rats placed into the activity-stress paradigm while methylphenidate tended to have the opposite behavioral effects. Both drugs, however, exacerbate stress ulcer formation. These results tend to support the hypothesis that these two stimulants exert their effects by different mechanisms [3].

The precise nature of the relationship between regional brain NA activity and peripheral gastric function/disease is difficult to specify, however, at least one brain region—the hypothalamus—is strongly implicated. Grijalva *et al.* [8] showed that lateral hypothalamic lesions produce several disorders including gastrointestinal pathology, possibly as a consequence of hypersecretion of gastric acid [9]. Nobrega *et al.* [16] noted that both lateral and ventromedial hypothalamic lesions produce gastric lesions as well as "hyperactivity." Interestingly, the excess motor activity following the

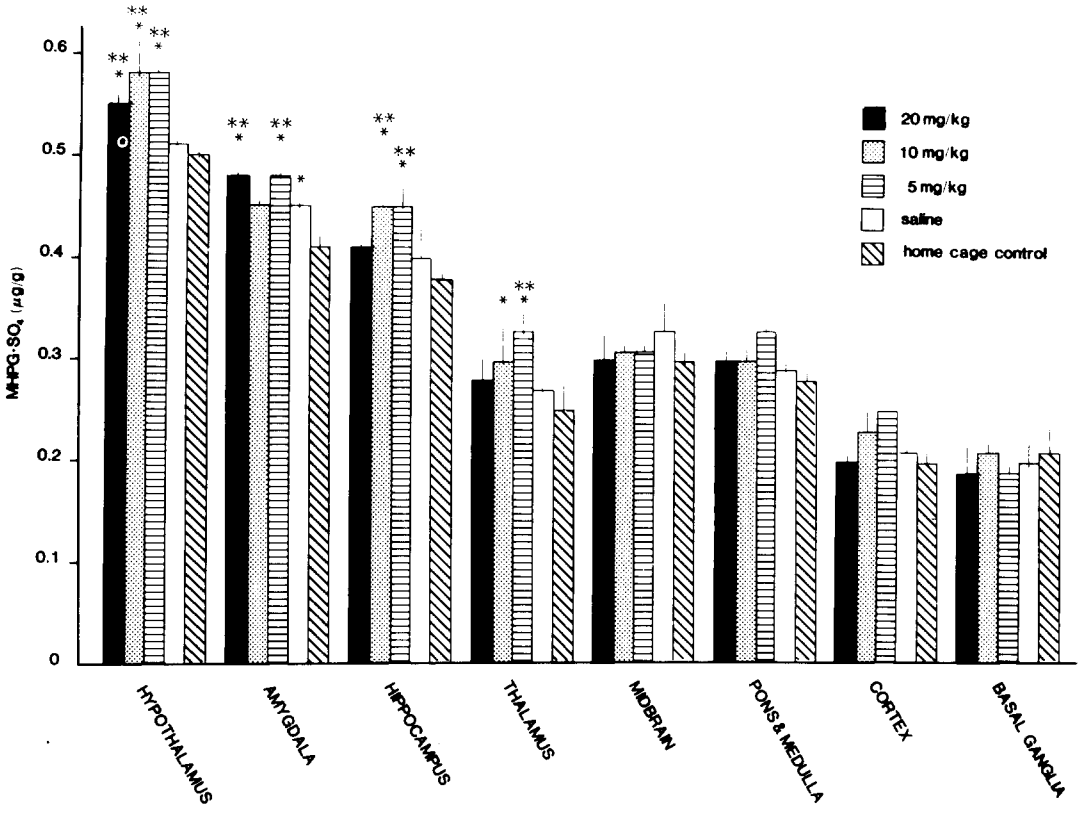

FIG. 3. Mean (±S.E.) 3-methoxy-4-hydroxy-phenylethyleneglycol sulfate (MHPG-SO_4) levels in eight brain regions of wheel-housed (n=15 per group) and home-cage-housed (n=15 randomly selected) rats. *Significantly different from home cage controls ($p<0.05$). **Significantly different from saline-injected-wheel-housed rats.

brain lesions correlated positively with ulcer severity. In a subsequent study, Nobrega and Wiener [15] found that catecholamine agonists (d-amphetamine, desipramine) reduced the extent of gastric ulceration following medial hypothalamic lesions, while catecholamine antagonists (phentolamine, propanolol) augmented ulcer severity in hypothalamically-lesioned rats. Neurochemical studies also implicate the hypothalamus, and particularly NA in the hypothalamus, as playing a role in the etiology of stress-induced gastric ulcers. Tanaka *et al.* [18] showed that acute restraint stress produces NA depletion and MHPG-SO_4 elevation (that is, enhanced NA turnover) most rapidly in the hypothalamus as compared to the cerebral cortex, hippocampus, basal ganglia and brainstem. Activity-stress also produces marked enhancement of NA turnover in many brain regions [20] with the hypothalamus showing progressive increasing NA depletion over the daily course of activity-stress [19]. In general, it appears that intact brain NA activity is required for effective "coping" with stress, expressed in terms of gastric ulcer severity. For example, selectively depleting brain NA level using a combination of vesicular depletion with RO4-1284 (2-hydroxy-2-ethyl-3-isobutyl-9,10-dimethoxy-1,2,3,6,7-hexahydro-11bH-benzo(a)quinolizine HCl) and synthesis inhibition using the dopamine beta-hydroxylase inhibitor FLA-63 (a procedure which does *not* affect DA and 5-HT levels) markedly exacerbates restraint ulcer (Glavin, 1985, in press). In addition, a modest relationship (r=+0.71) was obtained between restraint-induced gastric ulcer frequency and the acceleration of whole brain NA metabolism produced by the above procedure [7]. Methylphenidate may exert its effects by augmenting brain NA turnover and hence reducing NA levels in certain regions, particularly the hypothalamus. Studies are in progress to determine (a) in which brain region or regions NA depletion most strongly correlates with gastric ulcer formation and (b) in which brain region or regions tritiated methylphenidate and its metabolites preferentially accumulate.

In the present study, NA metabolism in the cortex as a whole was not substantially affected by methylphenidate. It may be, however, that discrete areas within the cortex are differentially affected by various compounds. For example, Bell *et al.* [1] noted that methylphenidate reduced glucose metabolism in the motor cortex, while increasing glucose utilization in several other brain areas, especially the midbrain reticular formation. The authors suggested that methylphenidate exerts its effects via the ascending reticular activating system and functions as an "arouser" in this fashion. A similar "arousal" function has been postulated to exist for brain NA [14], yet widespread influence of methylphenidate on brain NA metabolism was not detected in the present study. It may be that our assay method (fluorometric) is not sensitive enough to detect the effects of methylphenidate upon NA metabolism in extensive brain regions, and we are currently investigating this phenomenon with a new and highly sensitive HPLC procedure [21].

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