

## Behavioral and Brain Catecholamine Depleting Actions of U-14,624, an Inhibitor of Dopamine $\beta$ -Hydroxylase<sup>1</sup> (34571)

P. F. VON VOIGTLANDER<sup>2</sup> AND K. E. MOORE

*Department of Pharmacology, Michigan State University, East Lansing, Michigan 48823*

There is increasing evidence to indicate that norepinephrine (NE) and dopamine (D) function as neurotransmitters in the central nervous system (1). Unfortunately, delineation of the functions of these two catecholamines has been hampered by difficulties in altering the brain concentration of one amine independently of the other. For example, reserpine and  $\alpha$ -methyltyrosine decrease and L-dopa and monoamine oxidase inhibitors increase the brain contents of both amines.

Several recent reports describe attempts to alter the brain content of NE without affecting the concentration of D. For example, the concentration of NE in the brain has been selectively increased, after depletion of catecholamines with  $\alpha$ -methyltyrosine, by the administration of *threo*-dihydroxyphenylserine, which is decarboxylated to NE (2). However, the NE formed may not be limited exclusively to noradrenergic neurons but is probably distributed to wherever L-aromatic amino acid decarboxylase is located within the brain. Selective lowering of the brain content of NE has been accomplished by administering drugs which inhibit dopamine  $\beta$ -hydroxylase. Disulfiram and its reduced metabolite, diethyldithiocarbamate, inhibit this enzyme and thereby effectively lower the brain content of NE. However, toxicity and peritoneal irritation limit the usefulness of these compounds for many behavioral studies (3, 4). Accordingly, there have been attempts to find more effective and less toxic inhibitors of dopamine  $\beta$ -hydroxylase.

Johnson *et al.* (5) have recently reported on the ability of a number of aromatic and

alkyl thiourea derivatives to inhibit dopamine  $\beta$ -hydroxylase and thereby lower brain levels of NE. The present report describes the effects of one of these compounds, U-14,624 (1-phenyl-3-(2-thiazolyl)-2-thiourea), on spontaneous locomotor activity and on the brain concentrations of catecholamines in mice.

*Methods.* Albino male mice (Spartan Farms), weighing 20–30 g, were used throughout this study. U-14,624 was prepared as a suspension in 1 % methylcellulose so that the desired dose was administered intraperitoneally, or orally, in a volume of 0.01 ml/g. Mice serving as appropriate controls received equivalent volumes of 1 % methylcellulose. Prior to oral administration (stomach tube) the mice were fasted for 12 hr. In the diet study, 0, 0.01, 0.3, or 1 % U-14,624 was added to a ground diet (Wayne Lab-blox) for 24 hr.

Spontaneous locomotor activity was determined in actophotometer cages (Woodward Research Corp.). Two mice were placed in each cage and, after a 10-min period acclimation, activity was recorded for 10 min (during the 10–20-min period after the mice were placed in the cages). The mice were then decapitated and three or four whole brains were pooled for the analysis of NE and D (6).

The LD<sub>50</sub> for U-14,624 was determined as described by Litchfield and Wilcoxon (7) following the intraperitoneal injection to groups of 12 mice of 400, 500, 600, 700, 800, and 1000 mg/kg; deaths occurring within 48 hr were recorded. Values of motor activity and brain catecholamines were analyzed statistically using Student's *t* test, group comparison (8).

*Results.* Following a single intraperitoneal injection of 100 mg/kg of U-14,624, there was

<sup>1</sup> Supported by U.S. Public Health Service Grant AM 11083.

<sup>2</sup> Supported by U.S. Public Health Service Training Grant GM 01761.

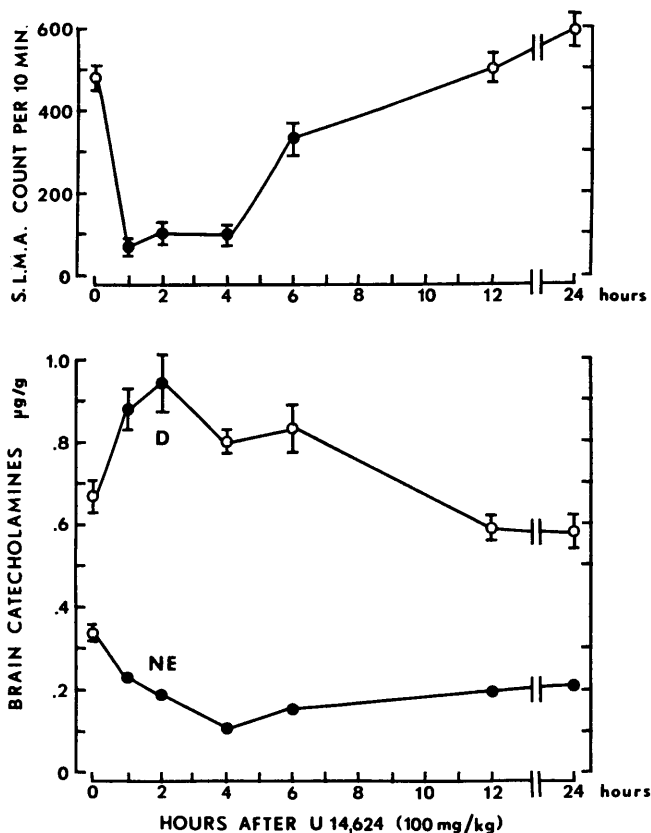


FIG. 1. Time course of the effects of a single intraperitoneal injection of U-14,624 on spontaneous locomotor activity (SLMA) and on the brain content of norepinephrine (NE) and dopamine (D). Each point and vertical line represents the mean  $\pm$  1 SE of 12 determinations of motor activity and 6 determinations of brain catecholamines. Solid points represent those values differing significantly ( $p < .01$ ) from the untreated control (zero time).

a prompt and sustained depression of motor activity; this effect was accompanied by a significant reduction in the concentration of NE and an increase in the concentration of D in the brain (Fig. 1). The effects of various doses of U-14,624 on spontaneous locomotor activity and brain catecholamine levels are summarized in Table I. Four hr after intraperitoneal injections, U-14,624 caused a dose-dependent reduction in motor activity and in the brain content of NE; at this time only 200 mg/kg of U-14,624 significantly increased the dopamine content. There was also a dose-dependent decrease in motor activity and brain NE concentrations 4 hr after the oral administration of U-14,624. However, for an equivalent reduction of brain NE there was a decidedly greater defi-

cit in motor activity after intraperitoneal than after oral administration. For example, 100 mg/kg ip and 200 mg/kg po of U-14,624 produced equivalent reductions of brain NE concentrations but they caused an 80 and 33 % reduction in motor activity, respectively. The greater depression of motor activity following intraperitoneal administration may result from peritoneal irritation caused by this insoluble drug.

The addition of U-14,624 to the diet of mice for 24 hr caused a dose-dependent decrease in brain NE concentrations (Table II); there was no change in brain concentrations of dopamine and motor activity was not altered. Food intake decreased as the percentage of U-14,624 in the diet increased, but only with the highest concentration of the

TABLE I. Spontaneous Locomotor Activity (SLMA) and Brain Levels of Norepinephrine (NE) and Dopamine (D) 4 hr After the Intraperitoneal or Oral Administration of Various Doses of U-14,624.<sup>a</sup>

Route of administration	Dose (mg/kg)	SLMA (counts/10 min)	NE ( $\mu\text{g/g}$ )	D ( $\mu\text{g/g}$ )
ip	0	475 $\pm$ 32	0.34 $\pm$ 0.02	0.67 $\pm$ 0.04
	12.5	441 $\pm$ 54	0.28 $\pm$ 0.01 <sup>b</sup>	0.61 $\pm$ 0.04
	25	330 $\pm$ 48	0.27 $\pm$ 0.01 <sup>b</sup>	0.63 $\pm$ 0.03
	50	286 $\pm$ 37 <sup>b</sup>	0.16 $\pm$ 0.01 <sup>b</sup>	0.61 $\pm$ 0.03
	100	94 $\pm$ 35 <sup>b</sup>	0.11 $\pm$ 0.01 <sup>b</sup>	0.80 $\pm$ 0.03
	200	13 $\pm$ 5 <sup>b</sup>	0.12 $\pm$ 0.01 <sup>b</sup>	0.96 $\pm$ 0.06 <sup>b</sup>
oral	0	494 $\pm$ 40	0.30 $\pm$ 0.02	0.57 $\pm$ 0.04
	50	468 $\pm$ 22	0.22 $\pm$ 0.01 <sup>b</sup>	0.67 $\pm$ 0.03
	100	402 $\pm$ 34	0.17 $\pm$ 0.01 <sup>b</sup>	0.64 $\pm$ 0.07
	200	340 $\pm$ 52	0.12 $\pm$ 0.01 <sup>b</sup>	0.77 $\pm$ 0.07
	400	220 $\pm$ 35 <sup>b</sup>	0.10 $\pm$ 0.02 <sup>b</sup>	0.60 $\pm$ 0.04

<sup>a</sup> Each value represents the mean  $\pm$  1 SE of 12 determinations of motor activity and 6 determinations of brain catecholamine levels.

<sup>b</sup> Values that are significantly different ( $p < .01$ ) from controls (no drug).

drug (1%) was there a significant weight loss.

The behavioral and catecholamine depleting actions of U-14,624 do not appear to be secondary to toxicity; no deaths occurred following the administration of the doses listed in Table I although the animals were quite depressed after the 200 mg/kg ip of this drug. After intraperitoneal injections, the LD<sub>50</sub> for U-14,624 was 680 mg/kg (95% confidence limits of 630–720 mg/kg). No fatalities occurred with oral doses up to 1000 mg/kg of U-14,624.

*Discussion.* Johnson *et al.* (5) have recently reported that U-14,624 significantly reduced NE and increased D contents in mouse and rat brain 4 hr after a single intraperi-

toneal injection of 200 mg/kg. These workers also noted that U-14,624 caused ptosis and overt depression. The results of the present study confirm and extend these observations.

In mice, U-14,624 reduced brain stores of NE but did not alter, and in some instances increased, the brain content of D. These effects would be expected if this drug blocked catecholamine synthesis at the step catalyzed by dopamine  $\beta$ -hydroxylase. Following oral and intraperitoneal administration of U-14,624 the depression of motor activity was dose dependent and temporally related to the depletion of NE. However, it would appear, from the results of the diet study, that the two events are not casually related.

It has been previously demonstrated that

TABLE II. Effects of a 24-hr Diet of U-14,624.<sup>a</sup>

U-14,624 in diet (%):	0	0.1	0.3	1
% Change in body wt	+13	+4	+3	-6
% Of control food intake	—	84	75	37
Drug intake (mg/kg)	—	263	773	1218
Motor activity (counts/10 min)	630 $\pm$ 28	613 $\pm$ 27	627 $\pm$ 22	659 $\pm$ 15
Brain norepinephrine ( $\mu\text{g/g}$ )	0.32 $\pm$ 0.01	0.26 $\pm$ 0.02 <sup>b</sup>	0.18 $\pm$ 0.01 <sup>b</sup>	0.12 $\pm$ 0.01 <sup>b</sup>
Brain dopamine ( $\mu\text{g/g}$ )	0.85 $\pm$ 0.05	0.80 $\pm$ 0.03	0.83 $\pm$ 0.06	0.77 $\pm$ 0.07

<sup>a</sup> The values on the first 3 lines represent mean values obtained from 3 cages of 6 mice for each diet. Values for motor activity represents the mean  $\pm$  1 SE as determined from 9 separate determinations and values for brain catecholamine concentration represent the mean  $\pm$  1 SE as determined from 4 to 6 separate analyses.

<sup>b</sup> Values that are significantly different ( $p < .01$ ) from those obtained with mice of the control diet.

complications arising from irritation associated with the intraperitoneal administration of disulfiram could be avoided by administering this drug in the diet (3). The same is true for U-14,624. The addition of this experimental drug to the 24-hr diet of mice caused a significant reduction in the brain concentration of NE but did not alter spontaneous locomotor activity (Table II). It would appear, therefore, that the marked behavioral depression that follows intraperitoneal injections, and the moderate depression that follows oral administration of U-14,624 (Table I), may result primarily from peritoneal or gastric irritation. This does not necessarily mean that U-14,624 is without central depressant properties since we have observed that low doses of this drug in rats disrupts conditioned avoidance responding in a shuttle box. This latter test is generally less affected by peritoneal irritation than is motor activity.

Numerous investigators have attempted to determine the relative importance of NE and D in the maintenance of central nervous system functions [for summary see Ref. (4)]. The addition of  $\alpha$ -methyltyrosine to the diet of mice depresses spontaneous locomotor activity and depletes the brain of both NE and D (9). Since, in similar test situations, disulfiram (3) and U-14,624 (Table II) can deplete brain stores of NE without altering motor activity or the brain content of D, it is tempting to postulate that depression of motor activity is associated with a depletion of D in the brain. However, until reasons for conflicting evidence [see Ref. (10)] are resolved, it is premature to invoke selectively dopaminergic or noradrenergic mechanisms in behavioral functions.

Further studies with inhibitors of dopamine  $\beta$ -hydroxylase may help to determine the functions of NE and of D in the central nervous system. For this purpose U-14,624 appears to have some advantages over disulfiram. With doses which are equieffective in lowering the brain NE content U-14,624 is less toxic and, when added in the diet, is less likely to reduce food intake than is disulfiram (3). U-14,624 can also be used to alter the

*in vivo* formation of certain false transmitters. For example, low doses of this drug have been used to enhance the accumulation of  $\alpha$ -methyldopamine and to prevent the formation of  $\alpha$ -methylnorepinephrine following the administration of methyldopa (Dominic and Moore, unpublished).

Preliminary studies indicate that rats are somewhat more susceptible to the behavioral depressant and toxic effects of U-14,624 than are mice (R. H. Rech, personal communication; Dominic and Moore, unpublished); studies on the effects of this compound in rats are currently in progress.

*Summary.* Intraperitoneal administration of U-14,624 to mice caused a dose-dependent increase in the brain content of dopamine and a decrease in the brain content of norepinephrine; the latter effect was temporally related to the depression of spontaneous locomotor activity. When administered by the oral route, U-14,624 depleted brain norepinephrine but was somewhat less effective in depressing motor activity and when added to the diet for 24 hr, it effectively lowered the brain norepinephrine content without altering motor activity. Thus, selective depletion of brain norepinephrine does not in itself decrease spontaneous locomotor activity.

U-14,624 was kindly supplied by Dr. G. A. Johnson, The Upjohn Co., Kalamazoo, Michigan.

1. Glowinski, J., and Baldessarini, R. J., *Pharmacol. Rev.* 18, 1201 (1966).
2. Creveling, C. R., Daly, J., Tokuyama, T., and Witkop, B., *Biochem. Pharmacol.*, 17, 65 (1968).
3. Moore, K. E., *Biochem. Pharmacol.*, 18, 1627 (1969).
4. Moore, K. E., and Rech, R. H., *Arch. Int. Pharmacodyn.*, in press.
5. Johnson, G. A., Boukma, S. J., and Kim, E. G., *J. Pharmacol. Exp. Ther.* 168, 229 (1969).
6. Moore, K. E., and Rech, R. H., *J. Pharmacol. Exp. Ther.* 156, 70 (1967).
7. Litchfield, J. T., Jr., and Wilcoxon, F., *J. Pharmacol. Exp. Ther.* 96, 99 (1949).
8. Goldstein, A., "Biostatistics, An Introductory Text." Macmillan, New York (1964).
9. Moore, K. E., *J. Pharm. Pharmacol.*, 20, 656 (1968).
10. Van Rossum, J., and Hurkmans, J., *Int. J. Neuropharmacol.* 3, 227 (1964).

Received Oct. 17, 1969. P.S.E.B.M., 1970, Vol. 133.