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Increased Self-Administration of *d*-Amphetamine by Rats Pretreated with Metergoline[1]

W. H. LYNESS[2] AND K. E. MOORE

Department of Pharmacology and Toxicology, Michigan State University, East Lansing, MI 48824

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LYNESS, W. H. AND K. E. MOORE. *Increased self-administration of d-amphetamine by rats pretreated with metergoline*. PHARMACOL BIOCHEM BEHAV 18(5) 721–724, 1983.—Rats trained to self-administer *d*-amphetamine were pretreated with metergoline, a long-acting 5-hydroxytryptaminergic antagonist, and immediately placed in self-administration cages for 8 hours. During the first 3 hours after metergoline the normal pattern of *d*-amphetamine self-administration was unaltered, but thereafter the rate of self-injections was increased. Between 4 and 6 hr the self-injections in metergoline-treated rats were increased in a regular fashion and subsequently (7–8 hr) in a stereotyped manner, i.e., rapid bursts of lever presses with little space between injections. In amphetamine-naive rats the levels of striatal and nucleus accumbens dopamine metabolites, dihydroxyphenylacetic acid and homovanillic acid, rose with time after metergoline injection. Levels of 5-hydroxyindoleacetic acid remained unchanged in these brain areas. The neurochemical results suggest a correlation between the dopaminergic actions of metergoline and *d*-amphetamine self-administration.

d-Amphetamine Self-administration Metergoline Dopaminergic system

EXPERIMENTAL evidence supports the concept that the self-administration by rats of psychomotor stimulants, such as cocaine and *d*-amphetamine, is mediated by the activation of dopamine (DA) receptors. For example, following the administration of DA receptor antagonists the amount of *d*-amphetamine self-injected initially increases, but subsequently ceases (extinction response; [24]). In addition, systemic injections of DA agonists decrease amphetamine self-administration for periods consistent with the duration of action of the agonist [25]. One important site within the brain at which the psychomotor stimulants act appears to be the nucleus accumbens since selective lesions of DA neuronal terminals within this region abolish lever pressing behavior for both cocaine [17] and *d*-amphetamine [13].

Several lines of evidence suggest that 5-hydroxytryptamine (5HT)-containing neurons influence the behavioral effects of *d*-amphetamine, possibly by interacting with DA neurons within nucleus accumbens. Substantial amounts of 5HT are located within the nucleus accumbens [19] and depletion of this amine located in this nucleus potentiates amphetamine-stimulated motor activities [2,15]. In addition, generalized depletion of 5HT after intracerebroventricular (ICV) injection of 5,7-dihydroxytryptamine (5,7-DHT) enhances amphetamine-stimulated locomotor activity [12] and increases the rate of self-injection of *d*-amphetamine [14]. Inexplicably, localized destruction of 5HT neurons within nucleus accumbens does not alter the acquisition or maintenance of a stable rate of *d*-amphetamine self-administration [14] nor does it change biochemical indices of activity of DA neurons terminating in this region [15]. These data suggest the 5HT influence on amphetamine self-injection resides in an anatomical region distinct from nucleus accumbens.

The present report describes the effects of systemic injections of metergoline, a long-acting and at low doses (0.5–2 mg/kg) a relatively specific 5HT receptor antagonist [3–5, 20], on biochemical indices of ascending DA and 5HT neuronal activity, and on the characteristics of *d*-amphetamine self-administration. It was assumed that 5HT-receptor antagonism would produce behavioral effects similar to those seen after generalized destruction of 5HT neurons with 5,7-DHT, i.e., increased self-administration.

METHOD

Male Sprague-Dawley rats (250–275 g; Spartan Farms, Haslett, MI) were anesthetized with Equithesin (3 ml/kg, IP) and surgically implanted with silastic jugular cannulae [22]. After a 14 day recovery period the self-administration studies were initiated. Rats were placed in cages (18×20×26 cm, width, length, height, respectively) equipped with an operant lever activating a pneumatic device which delivers predetermined volumes of drug intravenously (0.5 sec) via the jugular cannula (see [13, 14, 22, 23]). Initial training ses-

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[2]Current address to which requests for reprints should be sent: Department of Pharmacology and Therapeutics, Texas Tech University Health Sciences Center, Lubbock, TX 79430.

sions of 8 hr (0900–1700 hr) consisted of 1 lever press for 1 drug injection. At the beginning of each of the first 3 days of training rats were given 3 "free" injections to facilitate the acquisition of self-administration behavior. Each injection delivered 0.125 mg/kg *d*-amphetamine sulfate in 200 μl/kg of sterile saline. The total time from lever press to resetting of the pneumatic components was 2 sec. Within 7–10 days most rats achieved a stable rate of self-injection and maintained a relatively constant hourly rate of injection during the last 7 hours of an 8 hr test session [14]. On the day of testing, rats were injected with 1 mg/kg metergoline IP just prior to placement in the test apparatus. The sequence of lever presses was recorded on event recorders as they were on previous days (no metergoline). In both the self-administration and neurochemical studies the animals only received one injection of metergoline.

For the neurochemical studies, amphetamine-naive animals were sacrificed by decapitation at 1, 3, 5, 7 and 9 hr after an IP metergoline injection (1 mg/kg). The brains were frozen on dry ice and sectioned on a freezing microtome. Punches of nucleus accumbens and striatum were removed from 1 mm frozen slices at the level of A9650 to A8650 μ [8] using 1.0 and 1.5 mm i.d. stainless steel tubing, respectively. DA, dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), 5HT and 5-hydroxyindoleacetic acid (5HIAA) were analyzed concurrently by reverse phase liquid chromatography with electrochemical detection (LCEC; Model LC-40, Bioanalytical Systems, West Lafayette, IN) using a procedure similar to that described by Loullis *et al.* [10].

Tissues were homogenized in 100 μl of mobile phase buffer (0.1 M citrate-phosphate, pH 3.5, 12% methanol, 0.004% sodium octyl sulfate). The homogenates were centrifuged and the protein content of the pellet was determined by the method of Lowry *et al.* [11]. An 80 μl aliquot of the supernatant was filtered and injected directly onto a μBondapak C_{18} column (30 cm × 3.9 mm i.d., Waters Assoc., Milford, MA) at ambient temperature. Flow rate of the mobile phase was 1.8 ml/min and detector potential +0.75 V vs. an $Ag/AgCl_2$ reference electrode. Standards were prepared in the same citrate phosphate mobile phase; linearity of peak height to concentration was observed throughout the range of 1–20 ng.

Statistical comparisons were made using analysis of variance and Duncan's Multiple Range test. The alpha level was 0.05.

RESULTS

Behavioral Studies

During the first hour that the rats were placed in the self-administration cages they self-injected *d*-amphetamine at a relatively higher rate than they do during the subsequent periods (i.e., hours 2–8; see Controls in Fig. 1). This pattern of responding may be due to the fact that the animals initially build up blood and brain concentrations of *d*-amphetamine to a desired level, and thereafter respond at a rate necessary to maintain this level. Rats injected with metergoline (1 mg/kg) just prior to being placed in the self-administration cages responded in a manner similar to that of the controls of the first 3 hr, but thereafter the number of *d*-amphetamine injections made by the metergoline-treated animals increased. During hours 4–5 the increased rate of responding was of a non-stereotypic nature. That is, the injections were taken at relatively consistent intervals but at a higher frequency than

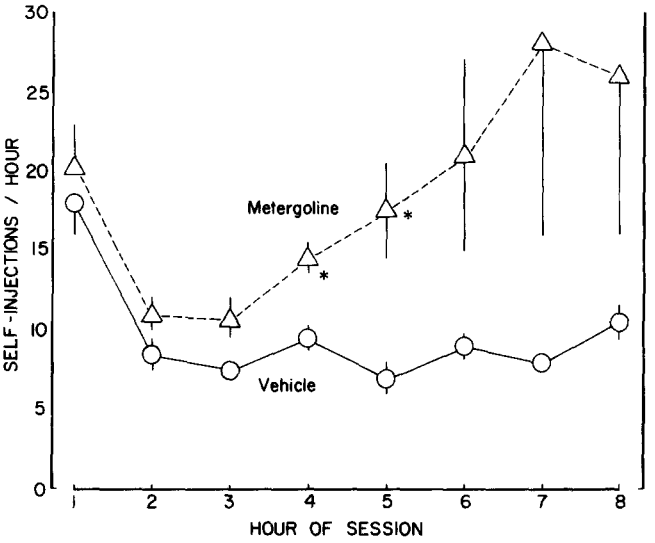

FIG. 1. *d*-Amphetamine self-administration by vehicle- and metergoline-pretreated rats. Animals were allowed to self-inject 0.125 mg *d*-amphetamine sulfate/kg/injection for 8 hr every second day. Data presented represents the number of self-injections/hr during an 8 hr test session on the 14th day of training which was preceded by an injection of vehicle (Control, ○), and on the 15th day of training which was preceded by an injection of metergoline (1 mg/kg, IP; Metergoline, △). Each symbol represents the mean and each vertical line ±SE as determined from 5 rats. *Values from metergoline pretreated rats that are significantly different from controls at corresponding times.

that of the controls (Fig. 2). During hours 7–8 the number of self-injections of *d*-amphetamine was increased, but in a more variable manner (note large standard errors in Fig. 1 and "bursting pattern" during the 8th hour in Fig. 2). This latter pattern of responding could be an "extinction response" or a reflection of stereotypic behaviors. The extinction response is seen when animals self-administering *d*-amphetamine are pretreated with a DA antagonist, such as haloperidol [24], or when *d*-amphetamine in the self-injection reservoir is replaced with saline [26]; it is characterized by an initial increase followed by a decrease and an eventual cessation of responding. Stereotypic responding is often observed when rats self-administer large doses of *d*-amphetamine or apomorphine. The repetitive stereotyped sniffing or gnawing leads to an absence of lever presses, while repetitive forepaw treading causes a large number of closely spaced presses. The metergoline-induced bursting pattern of responding during the last 2 hours of the test appears to be stereotypic since upon removal from the self-administration cages the metergoline-pretreated rats were observed to exhibit gnawing, head weaving and forepaw treading.

Biochemical Studies

In Table 1 the concentrations of 5HT, 5HIAA, DOPAC and HVA in nucleus accumbens and striatum are listed at various times after an injection of metergoline (1 mg/kg). The drug did not alter the 5HT content at any time and the small changes in the concentrations of 5HIAA may represent spurious values since there was no consistent trend. The lack of effect of metergoline on the concentrations of 5HT and its

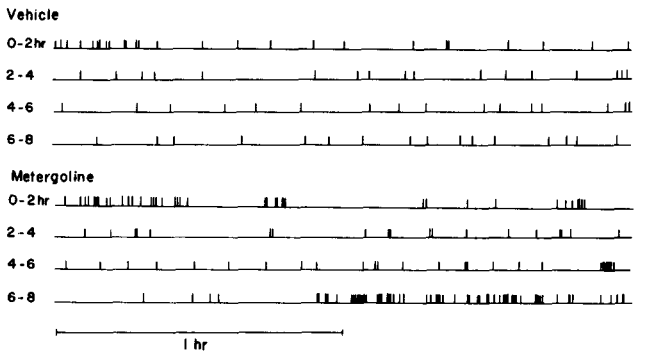

FIG. 2. Representative event recording of *d*-amphetamine self-injections by rat No. 3 for 8 hr on the 14th day of training (top) which was preceded by an IP injection of vehicle and on the 15th day of training which was preceded by an IP injection of metergoline (1 mg/kg). Each vertical lines represents 1 self-injection of 0.125 mg *d*-amphetamine sulfate/kg.

major metabolite is consistent with the report [6] that this dose did not alter 5HT turnover rates in rat brain, although higher doses (5–10 mg/kg) tended to increase the turnover. Metergoline caused a progressive but small increase in the concentration of both DA metabolites in nucleus accumbens and striatum, although there was some variation in the magnitude and time course of the effect in the 2 brain regions.

TABLE 1

CONCENTRATIONS OF 5HT, 5HIAA, DOPAC AND HVA IN THE STRIATUM AND NUCLEUS ACCUMBENS OF RATS SACRIFICED AT VARIOUS TIMES AFTER METERGOLINE (1 mg/kg, IP)

Hours After Metergoline	5HT	5HIAA	DOPAC	HVA
Striatum				
0	6.0 ± 0.6	4.1 ± 0.6	16.6 ± 1.2	8.4 ± 0.4
1	7.0 ± 1.0	5.3 ± 0.9	16.6 ± 0.9	8.6 ± 0.2
3	7.7 ± 0.6	3.1 ± 0.2*	17.0 ± 1.3	9.7 ± 0.5
5	8.5 ± 0.6	4.2 ± 0.4	18.4 ± 1.3	13.7 ± 0.6*
7	7.2 ± 1.2	6.2 ± 0.9	25.4 ± 0.9*	15.9 ± 1.0*
9	5.9 ± 1.1	8.8 ± 1.1	31.7 ± 0.8*	16.1 ± 0.7*
N. Accumbens				
0	11.2 ± 1.1	9.3 ± 0.8	19.1 ± 0.3	10.2 ± 0.3
1	10.9 ± 0.8	13.8 ± 1.4*	20.6 ± 0.8	10.8 ± 0.6
3	11.7 ± 0.7	12.4 ± 1.3	20.3 ± 1.0	11.4 ± 0.7
5	12.7 ± 1.0	11.0 ± 0.7	26.8 ± 0.9*	15.1 ± 0.5*
7	10.3 ± 0.6	10.8 ± 1.3	28.4 ± 1.1*	14.9 ± 0.6*
9	12.7 ± 1.2	10.1 ± 0.7	28.6 ± 1.2*	17.3 ± 0.9*

Values (mean ± S.E.) represent concentrations (ng/mg protein) as determined from 6–9 animals.
*Values that are significantly different from "0 time" controls.

DISCUSSION

Destruction of 5HT neurons by intracerebroventricular injections of 5,7-DHT caused rats to increase the rate of *d*-amphetamine self-administration [14]. It was thought that metergoline, a long-acting 5HT antagonist [3, 5, 6, 20], might mimic the effects seen in the 5,7-DHT-pretreated rats. The results of the present study revealed that metergoline did indeed increase *d*-amphetamine self-administration, but the pattern of responding did not correspond exactly with that seen in the animals with 5HT neuronal lesions. This may be due, in part, to the peculiar time course for the actions of metergoline. For the first 3 hours after its administration metergoline did not influence the rate of *d*-amphetamine self-injections, but thereafter the rate of injections increased to such an extent that by hours 7–8 the animals exhibited stereotyped behaviors. In 5,7-DHT-pretreated animals a progressive increase in self-administration of *d*-amphetamine during a test session leading to stereotypies was generally not seen [14].

It is difficult to relate the effect of metergoline on *d*-amphetamine self-administration to the 5HT antagonistic properties of the former drug since it has been noted that metergoline blocked tryptamine-induced forepaw clonus and 5-hydroxytryptophan-induced head twitches within 60 min after its administration [3]. Thus, the delayed action of metergoline noted in the present study is difficult to understand unless the response results from the actions of an active metabolite and on neurons other than those containing 5HT.

One must consider the possibility that the ability of metergoline to increase *d*-amphetamine self-administration is the result of some action of the drug which is unrelated to its 5HT antagonistic properties. At doses higher than those employed in the present study metergoline does have some antidopaminergic properties. For example, the drug is reported to block DA-stimulated adenylate cyclase in the striatum and to increase concentrations of DOPAC and HVA in mouse and rat brain [21]. Similarly, metergoline increases DOPAC concentrations in nucleus accumbens and striatum and enhances the ability of sulpiride to increase brain concentrations of this metabolite of DA [7]. In both of these studies, however, the doses of metergoline employed were 2.5–20 times greater than that used in the present study (1 mg/kg). On the other hand, pretreatment of rats with metergoline, 1 mg/kg, produced a block 3 hr later in the ability of both indolealkylamine-(LSD) and phenethylamine-type (mescaline) hallucinogens to disrupt an FR-40 operant schedule of food reinforcement, but did not alter the behaviorally disruptive effects of *d*-amphetamine on the same schedule [4].

It would be informative if the time course of the behavioral actions of metergoline could be related to its neurochemical actions. 5HT and 5HIAA concentrations in the brain were not altered by metergoline (Table 1). This is not surprising since earlier studies have noted that only much higher doses of metergoline increased 5HT turnover in whole brain [6]. Furthermore, it is known [1] that the synthesis and release of 5HT are not as tightly coupled as they are in catecholaminergic neurons.

In contrast to the lack of effect of metergoline on 5HT metabolites, the drug did slightly increase the concentration of DA metabolites (a possible reflection of increased DA neuronal activity) in both striatum and nucleus accumbens. Furthermore, there was a delay of several hours before this effect was observed. In this respect there appears to be some relationship between the behavioral and neurochemical actions of metergoline. The mechanism by which metergoline alters the brain concentration of the DA metabolites is not known. DA antagonists increase the concentrations of these

metabolites, and there is some indication that metergoline has DA antagonist properties, but only at doses higher than those employed in the present study ([7,21]; see above). An alternative explanation for the apparent metergoline-induced increase in DA neuronal activity relates to the ability of 5HT neurons to inhibit DA neurons. By blocking 5HT receptors metergoline would remove this inhibitory control mechanism and consequently activate the major ascending DA neurons resulting in increased concentrations of DA metabolites in brain regions containing terminals of these neurons [18]. Furthermore, if metergoline, at the dose used in the present studies, exerted DA antagonist properties it would be expected to block not predispose animals to the stereotypic behavior produced by amphetamine (Fig. 2).

There is behavioral evidence suggesting that DA neuronal activity increases following disruption of 5HT neurons with lesions or drugs (see discussion in [12]). However, recent reports suggest that the 5HT neuronal interaction responsible for the enhancement of DA agonist-induced behaviors occurs further down the polyneuronal circuitry and is not mediated by a direct effect on DA neurons [15]. Neither electrical stimulation of 5HT neurons nor electrolytic lesions of these neurons altered the activity of tyrosine hydroxylase in the DA neurons which terminate in the striatum [4, 5, 9, 16]. Furthermore, although 5,7-DHT-induced destruction of 5HT neurons enhances the locomotor stimulant actions of *d*-amphetamine, it does not alter basal or *d*-amphetamine-stimulated DA synthesis in terminals of nitrostriatal or mesolimbic neurons [12]. Thus, an understanding of the sites and mechanisms by which 5HT neurons influence the activity of ascending DA neurons and the action of DA drugs must await results of further investigations. Although it is tempting to speculate that the increased rate of self-administration of *d*-amphetamine in metergoline-pretreated animals results from 5HT antagonistic properties of the latter drug, our limited current knowledge of the mechanism of action of metergoline makes it difficult to confirm such speculation. The time course of changes in DA metabolites correlates better with the behavioral changes in these experiments.

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