Psychopharmacologia (Berl.) 18, 99—107 (1970)

An Analysis of Methylphenidate Induced Gnawing in Guinea Pigs*

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Received July 8, 1969
Final Version: May 4, 1970

Abstract. 20 compounds acting on the central nervous system and the autonomic nervous system were tested in guinea pigs for their ability to induce gnawing. Only methylphenidate induced vigorous gnawing similar to that produced by apomorphine and amphetamine. Methylphenidate differs in its mode of action from both apomorphine and amphetamine. In the guinea pig, phenylethyl configuration with OH groups at para and meta positions of the phenyl ring does not seem to be an essential criterion for inducing gnawing as suggested for the rat (Ernst, 1965). Catecholamines do not appear to play any significant role in the mediation of methylphenidate gnawing, or even in the gnawing response itself in guinea pigs, since increase in the level of dopamine and other catecholamines does not induce gnawing.

Key-Words: Chewing Response — Methylphenidate Gnawing — Dopamine in Gnawing — Guinea Pig Gnawing.

Gnawing is a chewing behaviour normally present in many rodents. Compulsive gnawing can be induced by apomorphine (Amsler, 1923), dopa (Ernst, 1965; Randrup and Munkvad, 1966) and dexamphetamine (Janssen, 1961; Randrup *et al.*, 1963) in the rat and by apomorphine in the rabbit (Brucke *et al.*, 1957) and guinea pig (Chandra *et al.*, 1966). Ernst (1965) has suggested that compounds possessing phenylethyl configuration with OH groups at para and meta positions of the phenyl ring act directly on the receptors, and compounds lacking this configuration act indirectly through the release of some endogenous substance possessing this structure.

The present study has been undertaken to find out the type of compounds which would induce gnawing in the guinea pig and to test whether the hypothesis of Ernst (1967) holds good in this species. A preliminary report of part of this work has appeared (Srimal and Dhawan, 1969).

* Communication No. 1394 of Central Drug Research Institute, Lucknow, India.

Methods

The study was conducted in adult guinea pigs (300 to 600 g) of either sex, in groups of five. The animals were placed on a wooden table (75 × 75 cm) with raised edges. A 22 cm high wire gauze mesh was fixed along the edge of the table to prevent the animals from falling off.

All the compounds were injected intraperitoneally and the animals were observed for four hours for signs of excitement and gnawing. Gnawing, if present, was marked by biting of the raised edges of the table. It was graded + if vigorous, ± if occasional and − if absent.

The compounds used in the study were 1-(O-allylphenoxy)-3-isopropylamino-2-propanol (H 56/28), amethocaine hydrochloride (Anethaine), amphetamine sulphate, amitriptyline hydrochloride, atropine sulphate, caffeine citrate, chlorpromazine hydrochloride (Largactil), cocaine hydrochloride, disulfiram (Antabuse), L-dopa, dopamine, ephedrine hydrochloride, eserine salicylate, haloperidol, imipramine hydrochloride (Tofranil), iproniazid phosphate, 4-(2-isopropylamino-1-hydroxyethyl) methanesulfonanilide (MJ 1999), lysergic acid diethylamide (LSD-25), mephenesin hydrochloride (Myanesin), meprobamate, mescaline hydrochloride, α-methyldopa (Aldomet), α-methyltyrosine, methylphenidate sulphate (Ritalin), morphine sulphate, nalorphine hydrochloride (Lethidrone), phenobarbitone sodium, perphenazine, procaine hydrochloride, protoveratrine maleate, pyrogallol, reserpine (Serpasil), tetrabenazine, tranylcypromine sulphate, tremorine dihydrochloride, triflumethazine dihydrochloride, triflupromazine hydrochloride, tyramine hydrochloride and yohimbine hydrochloride.

All the compounds were either dissolved in normal saline or used from ampoules and diluted with normal saline, except L-dopa which was dissolved in 0.1 N HCl, haloperidol which was dissolved in a minimal quantity of propylene glycol with a drop of lactic acid and diluted with normal saline and α-methyltyrosine which was dissolved in 0.1 N NaOH. Tetrabenzine and disulfiram were used as a suspension in gum acacia.

Results

Ernst (1967) has suggested dopamine to be the endogenous amine through which indirectly acting compounds exert their action in rats. This hypothesis has been tested in guinea pigs.

Since dopamine does not cross the blood-brain barrier, the concentration of catecholamines in the brain was raised by injecting dopa alone or after pretreatment with (i) a monoamine oxidase (MAO) inhibitor, (ii) a catechol-o-methyl transferase (COMT) inhibitor, (iii) a combination of both or (iv) a MAO inhibitor plus disulfiram. DOPA alone (upto 600 mg/kg) or after the above mentioned pretreatment failed to induce

Table 1. *The failure of raised brain catecholamine levels to induce gnawing in guinea pigs. In the case of pyrogallol two doses were injected at an interval of 1 h and dopa was given 1 h after the second dose. Figures in parentheses indicate the dose in mg/kg i.p.*

Pretreatment (mg/kg i.p.)	L-Dopa (200 mg/kg i.p.) administration after h	Gnawing response
1. Nil	0	—
2. Tranylcypromine (10)	3	—
3. Iproniazid (150)	2	—
4. Pyrogallol (200 + 200)	1	—
5. Pyrogallol + tranylcypromine	2	—
6. Iproniazid + disulfiram (200)	2	—

gnawing in guinea pigs (see Table 1). Weak gnawing could occasionally be seen after a very high dose (1200 mg/kg) of dopa but this was associated with a 60% mortality within 2 h.

In an effort to find out the type of compounds which are capable of inducing gnawing behaviour in guinea pigs, 20 drugs were tested. They have been listed in Table 2 and include among others, stimulants of the central nervous system and those affecting the autonomic nervous system. Out of 20 compounds only methylphenidate produced a good gnawing response. The response was dose dependent and 20.0 mg/kg i.p. produced vigorous gnawing in 100% of animals. Tranylcypromine, dopamine, tyramine and yohimbine made the guinea pigs hyperexcitable with occasional biting of the table but there was no well defined gnawing response (see Table 2).

Guinea pigs were challenged with methylphenidate (20.0 mg/kg i.p.) at the time of the expected peak affect of the catecholamine depletor or blocking agent (see Table 3). Only α-methyldopa (400.0 mg/kg) could completely block the effect of methylphenidate. The response was partially blocked (60%) by a high dose (320 mg/kg) of α-methyltyrosine (α-MT), the lower dose being ineffective (see Table 3). It was not possible to test a bigger dose of α-MT due to the mortality. Reserpine, tetrabenazine, β-adrenergic blocking agents MJ-1999 and H 56/28, and α-adrenergic blocking agents chlorpromazine and yohimbine pretreatment could not prevent methylphenidate gnawing in the doses tested.

Haloperidol (2.0 mg/kg) produced a slight inhibition of the gnawing response. Other tranquillizers like perphenazine (10.0 mg/kg), trifluomethazine (2.0 mg/kg), triflupromazine (5.0 mg/kg) and meprobamate (60.0 mg/kg) could not prevent methylphenidate induced gnawing. A centrally-acting muscle relaxant, mephenesin (100.0 mg/kg) was also

Table 2. *Effect of various agents on the gross behaviour of guinea pigs (groups of 5). + denotes vigorous gnawing ± hyperexcitability with occasional gnawing and — no gnawing*

No.	Compound	Dose (mg/kg i.p.)	Response of guinea pigs
1.	Amethocaine	5	—
2.	Amitriptyline	10	—
3.	Atropine	40	—
4.	Caffeine	30	—
5.	Cocaine	4	—
6.	L-Dopa	600	—
		1200	±
7.	Dopamine	4	±
8.	Ephedrine	30	—
9.	Eserine	0.2	—
10.	LSD-25	1.0	—
11.	Mescaline	25	—
12.	Methylphenidate	20	+
13.	Nalorphine	5	—
14.	Procaine	20	—
15.	Protoveratrine	0.1	—
16.	Pyrogallol	400	—
17.	Tranylcypromine	10	±
18.	Tremorine	2	—
19.	Tyramine	10	±
20.	Yohimbine	2	±

Table 3. *Effect of catecholamine depleting or blocking agents on the gnawing response to methylphenidate. The second dose of α-MT was given 3 h after the first dose. In the group receiving two doses of reserpine the interval between the two doses was 24 h. Time of methylphenidate challange has been reckoned from the first injection of α-MT and reserpine*

Drug	Dose (mg/kg i.p.)	Methylphenidate challange after h	Gnawing response
α-MT	80 + 80	7	+
	160 + 160	7	± (40%)
α-Methyldopa	400	3	—
Reserpine	5	6	+
	5 + 1	48	+
Tetrabenazine	40	4	+
	100	4	+ (Delayed)
MJ-1999	8	0.5	+
H 56/28	10	1.5	+
Chlorpromazine	30	1	+
Yohimbine	2	2	+

Table 4. *The effect of methylphenidate administration after pretreatment with various centrally acting drugs*

Agents	Dose (mg/kg i.p.)	Methylphenidate challange after min	Gnawing response
Imipramine	12.5	90	+
Mephenesin	100	15	+
Meprobamate	60	30	+
Mescaline	25	30	+
Morphine	40	30	−
Perphenazine	10	60	+
Phenobarbitone	15	60	+
Triflumethazine	2	30	+
Triflupromazine	5	30	+
Haloperidol	2	60	±

unable to antagonise this response. Morphine (40.0 mg/kg), however, prevented the gnawing response to methylphenidate. Another depressant of the central nervous system, phenobarbitone (15.0 mg/kg) was ineffective (see Table 4).

Comparison of Apomorphine, Amphetamine and Methylphenidate Induced Gnawing

In order to elucidate further the mechanism of action of methylphenidate, its response was compared with that of apomorphine and amphetamine. The guinea pigs were pretreated with various blocking and potentiating agents and at the time of their peak effect the animals were challenged with apomorphine (0.5 mg/kg), amphetamine (10.0 mg/kg) or methylphenidate (20.0 mg/kg). Phenothiazine derivatives, chlorpromazine and triflupromazine prevented the gnawing induced by apomorphine and amphetamine but not that induced by methylphenidate. Haloperidol completely blocked the gnawing induced by apomorphine and amphetamine but only partially (20% animals) inhibited that induced by methylphenidate. α-Methyldopa could block amphetamine and methylphenidate gnawing but not apomorphine gnawing. α-MT prevented amphetamine gnawing at lower doses but could also partially (40%) antagonise apomorphine and methylphenidate gnawing at the maximal tolerated dose level. Tranylcypromine increased the duration and intensity of the gnawing induced by amphetamine without altering methylphenidate gnawing. Apomorphine gnawing was, however, reduced by this agent. Similarly meprobamate reduced apomorphine gnawing

Table 5. *Groups of 5 guinea pigs were treated with the agents listed in the first column and then challanged with apomorphine (0.5 mg/kg), amphetamine (10.0 mg/kg) or methylphenidate (20.0 mg/kg) at the time of the peak effect of the premedicant. Potentiation of the gnawing response is indicated by ++*

Pretreatment by (mg/kg i.p.)	Gnawing challange after h	Apomorphine gnawing	Amphetamine gnawing	Methylphenidate gnawing
Chlorpromazine (30)	1	−	−	+
Triflupromazine (5)	0.5	−	−	+
Haloperidol (2)	1	−	−	±
α-Methyldopa (400)	3	+	−	−
α-MT (80 + 80)	7	+	−	+
(160 + 160)	7	±	−	±
Tranylcypromine (10)	3	±	++	+
Meprobamate (60)	0.5	±	+	+
Morphine (40)	0.5	−	−	−

without affecting the response to amphetamine and methylphenidate. Morphine was the only agent which could effectively block gnawing induced by all the three agents (see Table 5).

Discussion

The gnawing response is better discernable in the guinea pig than the rat since this is not a part of its normal behaviour. Moreover, agents inducing gnawing in rats, e.g. apomorphine and amphetamine are also effective in the guinea pig.

In the present study L-dopa failed to induce gnawing in guinea pigs even at 3 times the dose which is reported to induce gnawing in rats (Ernst, 1967). When the dose was raised 6 times (1200 mg/kg), it induced a very weak gnawing effect after 45 min, but 60% of the animals died within 2 h indicating that this dose was toxic. To raise the concentration still further the animals were pretreated with a MAO inhibitor (iproniazid) or a COMT inhibitor (pyrogallol) or both. Administration of 200 mg/kg L-dopa to animals pretreated with a MAO inhibitor (iproniazid) or a COMT inhibitor (pyrogallol) did not induce gnawing. Even concomitant administration of dopa and disulfiram, with or without addition of a MAO inhibitor did not induce gnawing in the guinea pig. As these procedures, according to the literature, cause an increase of catecholamines in the brain, it seems unlikely that the mediation of the gnawing response in this species is connected with the catecholamine levels.

In the present study methylphenidate has been found to be as effective in inducing gnawing as amphetamine and apomorphine. Other CNS stimulant and depressant drugs tested did not induce gnawing (see Table 1) indicating the specificity of this effect. The mode of action of methylphenidate was studied by pretreating the guinea pigs with several catecholamine depletors (α-MT, α-methyl-dopa, reserpine, tetrabenazine), β-adrenergic blocking agents (MJ 1999, H 56/28), α-adrenergic blocking agents (chlorpromazine, yohimbine) and tranquillizers (haloperidol, perphenazine, chlorpromazine, triflumethazine, trifluprομazine) and an anti-depressant (imipramine)—agents known to modify the responses to catecholamines. Of all these agents only α-methyldopa completely blocked the gnawing response. The usual dose of α-MT (160 mg/kg) failed to block the response of methylphenidate. This dose is known to deplete catecholamines by more than 80% and to completely disrupt the conditioned avoidance response in guinea pigs (Moore, 1966). When the dose of α-MT was raised to 320 mg/kg there was blocking of methylphenidate induced gnawing in 60% of the animals. With this dose of α-MT there was complete blockade of amphetamine gnawing, and apomorphine gnawing was also blocked in 60% of animals. Further, this appeared to be a toxic dose of α-MT, since a mortality of 40% of the animals was noted in every experiment where this dose was administered. The blockade of gnawing response by this dose of α-MT, therefore, is probably a non-specific or toxic effect and not due to catecholamine depletion. It is unlikely, therefore, that methylphenidate is acting indirectly via the catecholamines to induce gnawing. The hypothesis proposed by Ernst does not seem to be applicable to guinea pigs.

A comparison of methylphenidate gnawing with apomorphine and amphetamine induced gnawing clearly indicates that differences exist in gnawing induced by these 3 agents (see Table 5). Haloperidol only partially (20%) antagonises methylphenidate gnawing but completely prevents apomorphine as well as amphetamine induced gnawing. This demonstrates the comparative resistance of methylphenidate to haloperidol. The phenothiazine tranquillizers are also unable to prevent methylphenidate gnawing whereas they effectively block apomorphine as well as amphetamine gnawing. Apomorphine gnawing is resistant to block by α-methyldopa as well as by usual doses of α-MT; methylphenidate gnawing is blocked by α-methyldopa and partially by high doses of α-MT whereas amphetamine gnawing is blocked by both these agents. α-Methyldopa lowers the concentration of catecholamines through the displacement of normally present catecholamines by its metabolites (Smith, 1960; Oates *et al.*, 1960) but α-MT depletes catecholamines directly (Moore, 1966). The dissimilar effects of these two agents may be due to the difference in their mechanism of action. Pretreatment of

the animals with a MAO inhibitor (tranylcypromine) markedly potentiates amphetamine gnawing, does not affect the methylphenidate response and somewhat antagonises the apomorphine gnawing. Similarly, meprobamate reduces the apomorphine gnawing without affecting amphetamine and methylphenidate responses. Morphine blocks the gnawing response of all the three agents completely, and may act directly on the receptors preventing direct or indirect action of other agonists. The non-uniform behaviour of the three gnawing inducing agents points to their different mode of action. It is not possible, however, with the presently available data to explain their precise mode of action.

Methylphenidate produces psychic stimulation which can be antagonised by hypnotics as well as tranquillizers. In the present experiments on guinea pigs no such antagonism has been observed. It appears, therefore, that induction of gnawing in guinea pigs by methylphenidate is not related to its psychic stimulant property. This is further supported by the fact that other similar stimulants like caffeine, ephedrine, cocaine and nalorphine etc. cannot evoke the gnawing response.

Acknowledgements. The authors are grateful to Dr. A. B. Buske of Ayerst Laboratories, Canada, for Antabuse; Miss A. Framme of A. B. Hassle, Sweden for H 56/28; Merck, Sharp and Dohme, U.S.A., for amitriptyline (Elavil) and α-methyltyrosine; Mead Johnson and Co., U.S.A., for MJ 1999; Dr. H. M. Lal of Searle (India) Ltd., Bombay, for haloperidol; Dr. J. David of Ciba Research Center, Bombay, for methylphenidate (Ritalin); Ciba Pharmaceutical Co., Basle, Switzerland, for pyrogallol; and Hoffman-La Roche, Switzerland, for Mescaline.

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