Pharmacology Biochemistry & Behavior, Vol. 4, pp. 1–6.

Effects of Harmine and Brain Lesions on Apomorphine Induced Motor Activity

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(Received 19 June 1975)

WESTERMANN, K. H., K. FUNK AND L. PAWLOWSKI. *Effects of harmine and brain lesions on apomorphine-induced motor activity.* PHARMAC. BIOCHEM. BEHAV. 4(1) 1–6, 1976. – Application of harmine (10 mg/kg IP) 30 min before apomorphine decreased the motoric effects of the latter. Following harmine an increase in 5-HT and a decrease in 5-HIAA in different brain regions have been found. Injection of 5,6-DHT into nucleus medianus raphe 7 days before the experiment caused a significant increase of the apomorphine effect. Harmine pretreatment reduced this excessive motility as well as additional lesion of the substantia nigra with 6-OH-DA. Lesion induced by 6-OH-DA alone was without significant effect on the hypermotility following apomorphine. Application of PCPA 3 days before testing elicited an increase of apomorphine-induced hypermotolity which could be abolished by preceding harmine application. The experiments demonstrate the inhibitory effect of the central serotoninergic system on the apomorphine syndrom as well as the serotoninergic-dopaminergic interaction in hypermotility.

Harmine Apomorphine Substantia nigra Nucleus raphe

BESIDES the action on dopamine receptors apomorphine has been shown to influence noradrenergic and serotoninergic transmission in the central nervous system [15, 25, 29]. In that way the action on motility can be modified. Investigating the effects of apormorphine on 5-hydroxytryptamine (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA) levels Grabowska *et al.* [14,15] supposed an inhibitory action of 5-HT on apomorphine-induced hypermotility without effects on stereotypy. According to this hypothesis a direct effect of substances influencing serotonin content and turnover on this hypermotility can be expected.

Harmala alcaloids, especially harmaline and harmine, are known as tremorogenic substances with marked effects on serotonin content in the brain [20,31]. Therefore we investigated apomorphine-hypermotility following harmine pretreatment and the effects of lesions of the central serotoninergic and dopaminergic systems made with 5,6-dihydroxytryptamine (5,6 DHT) and 6-hydroxydopamine (6-OH-DA) on the motoric effects of apomorphine.

METHOD

Female Wistar rats (140–160 g body weight) were used in the experiments. Lesions of the central serotoninergic system were performed by injection of 5,6-DHT (20 μg/4 μl calculated as free base) into the nucleus medianus raphe, lesions of the dopaminergic system by injection of 6-OH-DA (8 μg/2 μl) into the substantia nigra of both sides. One group of rats with combined lesions got 6-OH-DA and 5,6-HT at 24 hr intervals. For injections the rats were narcotized and fixed in an operating table for stereotaxic injections according to a previously described method [37].

One and 2 weeks after lesion the rats were placed singly into photocell actometers with 2 crossed light beams and injected with saline or harmine (10 mg/kg) IP after habituation to the novel environment (60 min) and apomorphine (1 or 5 mg/kg IP) after 90 min. The activity counts were registered in 15 min intervals. As controls we used untreated rats.

In control experiments we tested changes of the 5-HT content in striatum (nucleus caudatoputamen and parts of globus pallidus), cerebral cortex and brain stem according to the method of [8].

After the end of experiments the site of intracerebral injection was controlled histologically.

RESULTS

In normal rats harmine injection (10 mg/kg) induced a short lasting tremor with slight increase of motility, similar to the increase after injection of salt solution. Harmine 30

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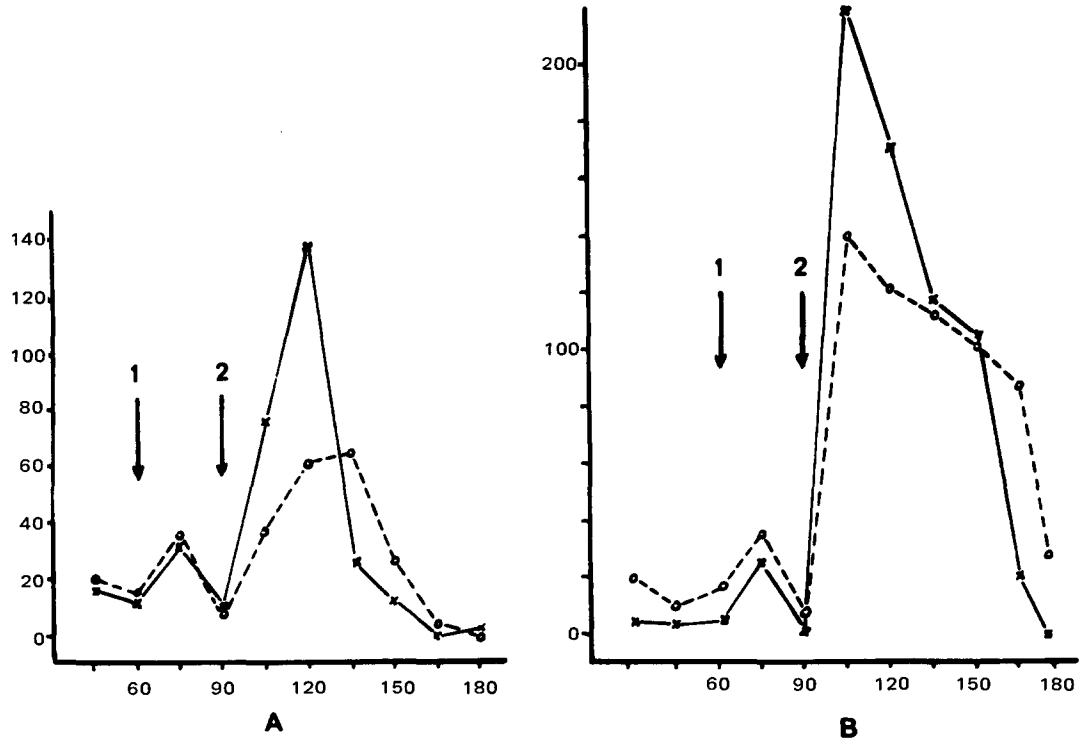

FIG. 1. Effect of harmine pretreatment on locomotor stimulation following apomorphine, 1 mg/kg (A) or 5 mg/kg (B). Each point represents a mean of 6–9 rats. 1 → : injection of harmine or saline; 2 → : injection of apomorphine; o– – –o: motility after harmine (10 mg/kg IP); x—— x: motility after saline (control). Ordinate: Activity (counts/15 min). Abcissa: Time after placing animals into motility boxes.

min before apomorphine caused a significant decrease of locomotor stimulation 15 and 30 min after the low dose of apomorphine. Following the high dose the changes were not significant, but the shift to the right remained (Fig. 1). Harmine application either 60 min before or simultaneously with apomorphine was without effect (Fig. 2).

The 5-HT content in striatum following harmine was found to be highest 15 min after injection. The content then diminished, reaching the normal value 75 min later (Table 1).

Changes in 5-HT with following changes in 5-HIAA levels were not limited to the nucleus caudatoputamen (Table 2).

One week after lesion of the central serotoninergic system by 5,6-DHT we observed an increase of apomorphine-hypermotility, which was significant 15 and 30 min after injection of apomorphine ($p<0.1\%$ and $p<5\%$). The harmine action on motility was increased too. Two weeks after lesion the apomorphine effect was higher only after 15 min ($p<5\%$) in comparison with control animals. Harmine pretreatment of these animals yielded values which were comparable with the effect of apomorphine in controls (Fig. 3).

Additional lesion of the central dopaminergic system by 6-OH-DA injection into the substantia nigra was observed to have the same result as harmine in 5,6-DHT animals: reduction of the excessive locomotor activity after apomorphine, while the lesion by 6-OH-DA alone was without clearcut effect on hypermotility (Fig. 4).

TABLE 1

TIME COURSE OF CHANGES IN 5-HT CONTENT IN THE STRIATUM FOLLOWING HARMINE (10 MG/KG IP) IN PERCENT OF CONTROL VALUES ($\bar{x} \pm s_{\bar{x}}$)

	Time after Harmine Injection (min)			
	15	30	60	90
5-HT	132 ± 21*	165 ± 13*	130 ± 10†	102 ± 12

*$p<0.1\%$ †$p<1\%$ (calculated by Student's t-test)

p-Chlorophenylalanine (PCPA 320 mg/kg IP) applied 3 days before testing elicited an increase of motility after apomorphine which could be abolished by preceding harmine application (Table 3).

DISCUSSION

Apomorphine-induced stereotypy and hypermotility in rats and mice are considered to be the result of stimulation of dopamine receptors in the striatum [3, 11, 34, 39] with consequences for dopamine synthesis and release [3, 19, 28]. The increase of motility was blocked by haloperidol and spiroperidol but could not be completely blocked by substances which decreased brain catecholamine levels [25,34]. Thus catecholamines are involved in some of the stimulatory effects of apomorphine.

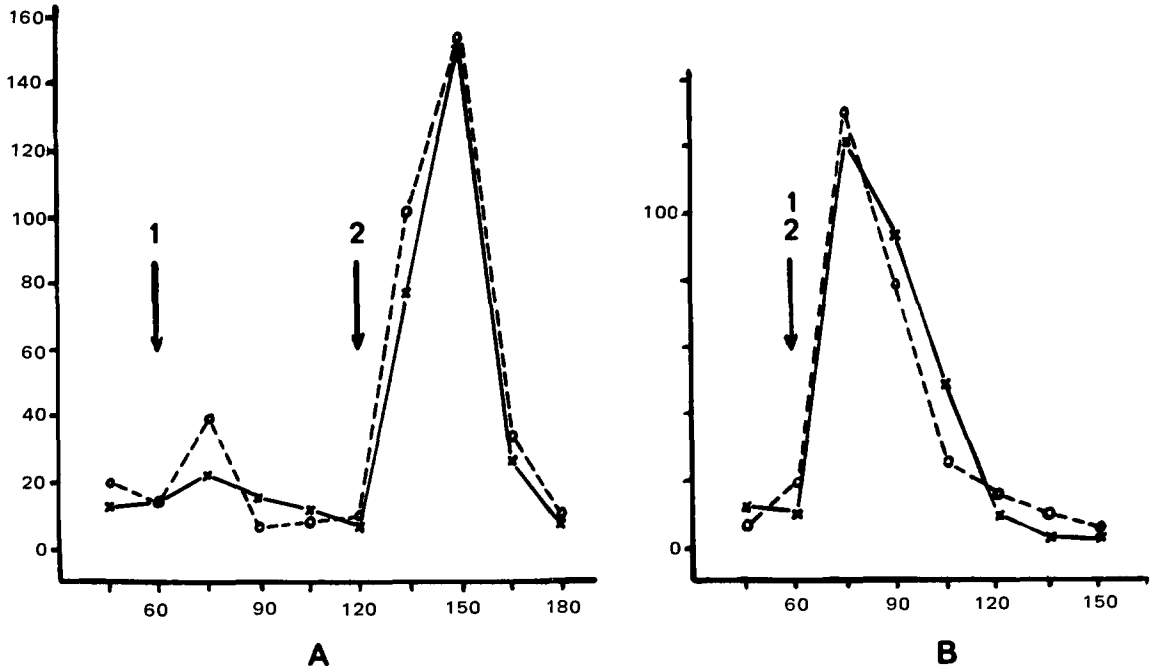

FIG. 2. Hypermotility following 1 mg/kg apomorphine given 60 min after (A) oder simultaneously with (B) harmine or saline. Legend see Fig. 1.

TABLE 2

CHANGES IN 5-HT AND 5-HIAA CONTENT OF 3 BRAIN REGIONS FOLLOWING HARMINE (10 MG/KG IP)

Brain Region	Control	1 Hr after Harmine	
	$\bar{x} \pm s_{\bar{x}}$	$\bar{x} \pm s_{\bar{x}}$	Change (%)
Striatum			
5-HT	0.71 ± 0.09	1.00 ± 0.40	41 ↑
5-HIAA	0.97 ± 0.42	0.44 ± 0.23	55 ↓ †
Cortex cerebri			
5-HT	0.42 ± 0.04	0.56 ± 0.15	33 ↑
5-HIAA	0.51 ± 0.07	0.23 ± 0.10	55 ↓ *
Brain stem			
5-HT	0.81 ± 0.14	0.93 ± 0.26	14 ↑
5-HIAA	1.09 ± 0.07	0.69 ± 0.20	37 ↓ *

*$p<1\%$ †$p<2\%$

Newer studies have shown that effects of apomorphine as well as effects of amphetamine are antagonized or modulated by influencing central 5-HT mechanisms [14, 15, 22]. Grabowska *et al.* [15] provided some evidence that the apomorphine effects on 5-HT turnover are secondary to the dopaminergic effects. In a similar way the interaction between dopaminergic and noradrenergic systems in the central nervous system following apomorphine can be brought about [29,30].

In our investigations harmine induced a relatively short-lasting increase in 5-HT and a decrease in 5-HIAA content in all tested brain regions (see Tables 1 and 2). Later the 5-HT content normalized but 5-HIAA levels increased slightly. These effects are obviously elicited by inhibition of monoamine oxidase [5,35]. Inhibition of monoamine oxidase and tremor elicited by harmine are independent of each other [5,7]. Tremor and rigidity described following harmine injection [5, 7, 20, 38] do not seem to be causally related to the decrease of apomorphine-induced locomotor activity since both former effects are observable only during 15 min after injection while the influence on hypermotility has been found by a time interval of 30 min between harmine and apomorphine injections.

Changes in 5-HT content could be shown for a longer time period (15–60 min after injection of harmine) and were parallel to the influence of harmine on the locomotor stimulation after apomorphine.

Intracerebral injections of 5,6-DHT have been found to produce lesions of central serotonin axons and terminals and long lasting decrease in 5-HT content in the brain [4, 6, 9, 18]. Similar to the reported potentiation of amphetamine-induced stimulation by raphe lesions or by treatment with PCPA [23,26] we observed an increase of apomorphine-hypermotility especially 1 week after injection of 5,6-DHT into median raphe nucleus confirming earlier results [16]. According to several other authors showing the inhibitory role of serotonin in different forms of behavior [10, 12, 13, 21, 23] the cross movements after apomorphine were inhibited by 5-HT. Inhibition of the 5-HT biosynthesis by PCPA as well as chemical lesion of the raphe system were able to eliminate this brake of motility [12, 22, 25].

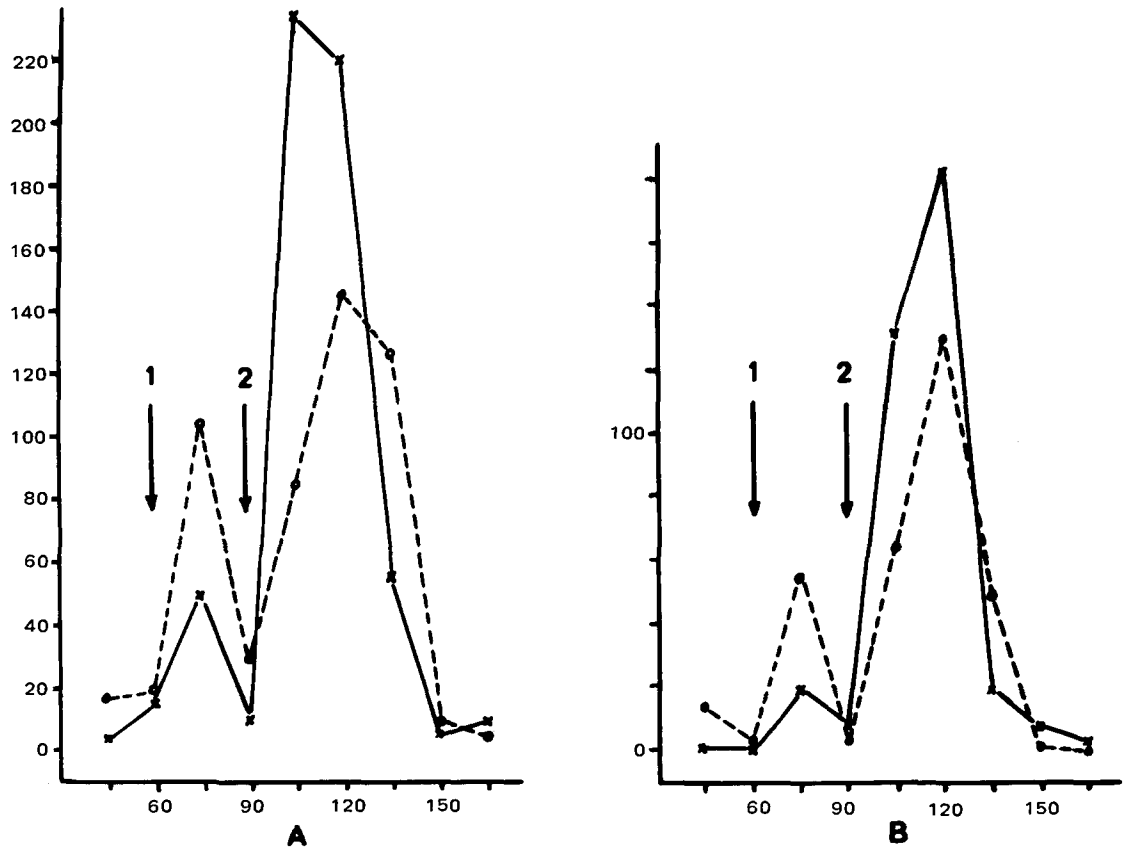

FIG. 3. Hypermotility following 1 mg/kg apomorphine 1 week (A) or 2 weeks (B) after injection of 5,6-DHT into the raphe region with or without harmine pretreatment. Legend see Fig. 1.

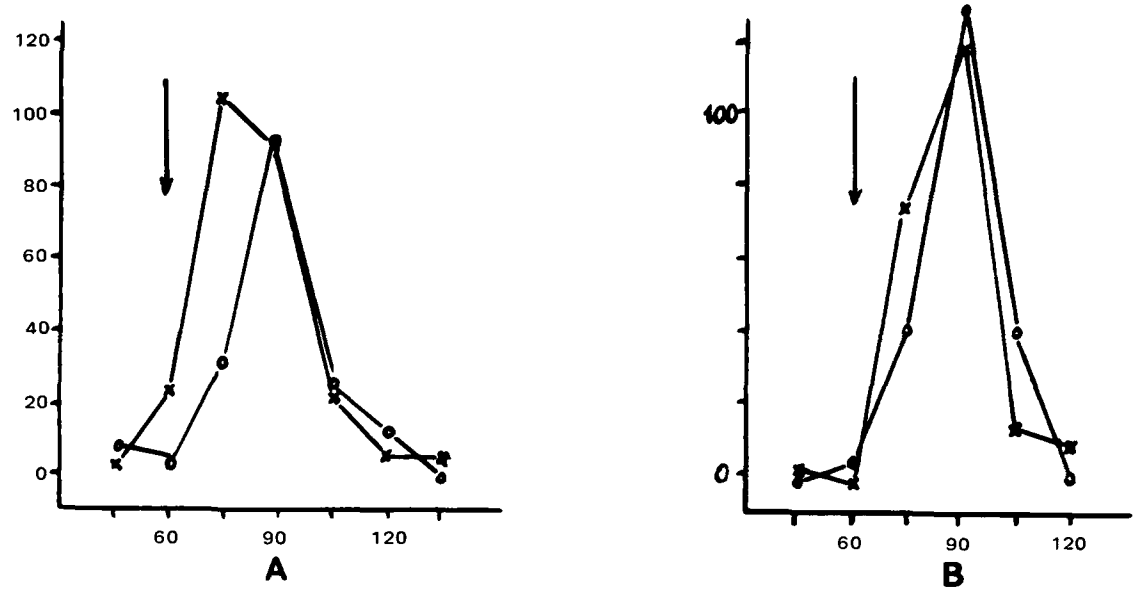

FIG. 4. Effect of 6-OH-DA pretreatment (o——o) or combined pretreatment with 6-OH-DA and 5,6-DHT (x——x) on motility after 1 mg/kg apomorphine, 1 week (A) or 2 weeks (B) after lesioning.

TABLE 3

EFFECT OF P-CHLOROPHENYLALANINE PRETREATMENT ON MOTILITY AFTER APOMORPHINE (5 MG/KG IP)

Group	Pretreatment	Harmine 30 min before Apomorphine	Motility Counts ($\bar{x} \pm s_{\bar{x}}$) 30 min after Apomorphine	Significance p (%)
I	–	–	170 ± 21	
II	–	10 mg/kg	121 ± 25	
III	PCPA	–	267 ± 34	<5 (I/III)
IV	PCPA	10 mg/kg	125 ± 22	<1 (III/IV)

*Statistical significance was calculated by Student's t-test

Lesions of the nigro-neostriatal system by 6-OH-DA with clearcut effects on DA content and dopaminergic transmission [1, 2, 38] in the striatum remained without consequence on apomorphine motility but eliminated effects of 5,6-HDT lesion. This is a further example of dopaminergic-serotoninergic interaction in the brain. At the present time it cannot be said whether this interaction is only of functional nature or has an anatomical basis in the neuronal network, e.g. in the postulated pathway nucleus raphe-substantia nigra-nucleus caudatoputamen [27]. The site of the possible synaptic interaction between dopaminergic and serotoninergic terminals might be the nucleus caudatoputamen too, since median raphe nucleus as well as substantia nigra possess fibre connections to the forebrain and striatum [2, 17, 21, 24, 36]. Further investigations are needed to enlighten the true site of interaction.

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