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Facilitation by α-Adrenolytics of Apomorphine Gnawing Behavior: Depression of Threshold Apomorphine Concentration in the Striatum of the Rat

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WISZNIOWSKA-SZAFRANIEC, G., L. DANEK, K. REICHENBERG AND J. VETULANI. *Facilitation by α-adrenolytics of apomorphine gnawing behavior: Depression of threshold apomorphine concentration in the striatum of the rat.* PHARMACOL BIOCHEM BEHAV **19**(1) 19–21, 1983.—Adrenolytics, aceperone and phenoxybenzamine, increased significantly the incidence of gnawing induced in the rat by a medium dose of apomorphine. The experiments in which the rats were killed at the onset of stereotyped gnawing to assess the threshold concentration of apomorphine in the striatum necessary to evoke this type of behavior have shown that these threshold concentrations were significantly reduced. It is concluded that the facilitation by adrenolytics of gnawing response to apomorphine is caused by an increase in the sensitivity of structures involved in this phenomenon to this dopaminergic stimulant.

Apomorphine stereotypy α-Adrenolytic—apomorphine interaction Apomorphine concentrations (striatum)

AN inhibitory effect of noradrenaline neurons on responses evoked by stimulation of dopamine receptors was postulated for the first time by Ahlenius and Engel [1], who noted that stereotypy evoked by levodopa in reserpine-pretreated rats was potentiated after inhibition of noradrenaline synthesis with a dopamine-β-hydroxylase inhibitor. Later, the inhibitory influence of noradrenergic system on stereotyped behavior resulting from dopaminergic stimulation was postulated by Żebrowska-Łupina and Kleinrok [14] and by Mogilnicka and Braestrup [9].

These and the following studies [5,13] based on the fact that α-adrenolytics or lesions of the noradrenergic system of locus coeruleus potentiated stereotyped behavior induced by apomorphine, amphetamine and other stimulants of the dopaminergic receptor. The potentiation was expressed either as prolongation of the period of display of stereotyped activity, or as an increase in its degree: higher incidence of stereotyped gnawing on the expense of licking, and increased incidence of licking on the expense of sniffing.

The mechanism of the potentiation by α-adrenolytics of stereotyped behavior has not been elucidated, and in this study we aimed at investigation if changes in apomorphine pharmacokinetics evoked by pretreatment with α-adrenolytics may explain the phenomenon.

Our earlier studies [8] have shown that the appearance of a given form of stereotyped behavior: sniffing, licking or gnawing, begins after apomorphine concentration in the brain areas involved in stereotypy reaches a threshold level. The appearance of stereotyped gnawing seemed to depend on the concentration of apomorphine in the striatum, which has been postulated to be the target organ for the gnawing-inducing action of apomorphine [3,4]. We investigated, therefore, how the threshold concentration of apomorphine in the striatum, causing the appearance of stereotyped gnawing, is altered in rats pretreated with α-adrenolytics.

METHOD

Animals

Male Wistar rats (160–220 g) were used throughout. They were kept under standard laboratory conditions, with free access to water till the beginning of experiment, and to granulated food (Murigran) till 24 hr before the experiment.

Drugs and drug treatment. Apomorphine hydrochloride (Sandoz, Basel) and phenoxybenzamine (Dibenyline®, Smith, Kline and French, Philadelphia, PA) were dissolved in saline; aceperone (Janssen, Beerse) was prepared as suspension in aqueous 3% solution of Tween 80. Apomorphine was injected in a volume of 2 ml/kg SC, other drugs were given in a volume of 4 ml/kg IP. The controls received equivalent volume of saline.

Stereotyped behavior. The rats received apomorphine, 5 mg/kg and were transferred to wire-mesh cages for observation of stereotyped behavior. The observation lasted for 45

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TABLE 1

EFFECT OF ALPHA-ADRENOLYTIC DRUGS ON THRESHOLD STRIATAL APOMORPHINE CONCENTRATION PRODUCING STEREOTYPED GNAWING

Pretreatment, dose (mg/kg)	Apomorphine concentration in the striatum (μg/g of tissue)	"Sensitization index"‡
Saline	1.41 ± 0.08 (15)	1.00
Aceperone, 2.5	1.17 ± 0.10 (7)*	1.21
Aceperone, 5	0.73 ± 0.05 (9)†	1.93
Aceperone, 10	0.80 ± 0.05 (9)†	1.76
Aceperone, 20	1.10 ± 0.04 (8)†	1.28
Phenoxybenzamine, 5	1.04 ± 0.04 (9)†	1.35
Phenoxybenzamine, 10	0.97 ± 0.03 (10)†	1.45
Phenoxybenzamine, 20	0.83 ± 0.04 (9)†	1.70
Phenoxybenzamine, 40	1.24 ± 0.06 (8)*	1.14

The data are means ± S.E.M. (n).

‡The ratio of threshold apomorphine concentration in the striatum of the controls to the concentration in the striatum of rats pretreated with α-adrenolytics.

*$p<0.05$, †$p<0.01$ (Duncan test).

min or till stereotyped gnawing appeared, whatever came first. The rats were pretreated with various doses of aceperone 1 hr before apomorphine, or phenoxybenzamine—2 hr before apomorphine. Saline was given to the controls at appropriate intervals.

Assay of apomorphine in the striatum. The rats were observed for stereotypy, and as soon as stereotyped gnawing appeared they were removed from the cage and decapitated. The brains were rapidly excised and placed on an ice-chilled porcelaine plate, and the striata were dissected out, wrapped in aluminum foil, and stored overnight under solid carbon dioxide. On the next day they were weighed, and homogenized in 4 vol of 0.1 N HCl and ethyl acetate (3:1) mixture. Apomorphine was assayed according to the spectrofluorometric method of Van Tyle and Burkman [10] with modifications of Melzacka *et al.* [8].

Significance of results was evaluated with the Fisher exact probability test (incidence of gnawing) or analysis of variance followed by Duncan multiple comparison test [2] (apomorphine concentrations).

RESULTS

Apomorphine, 5 mg/kg SC, produced in all rats stereotyped sniffing and then licking. In about one half of the control rats the stereotyped gnawing developed; the mean latency was approximately 13 min. Aceperone, 2.5–20 mg/kg IP, increased the incidence of stereotyped gnawing: the results differed significantly from the control in groups receiving the doses of 5 and 10 mg/kg. A similar increase in the incidence of gnawing was observed in rats pretreated with 5–40 mg/kg of phenoxybenzamine (Fig. 1). The latency of gnawing remained unchanged.

The threshold level of apomorphine in the striatum of control rats was 1.41±0.08 μg per gram tissue. It was significantly lower in the striata of rats pretreated with α-adrenolytics; the highest doses of aceperone and phenoxybenzamine produced an effect significantly weaker ($p<0.05$) then the next lower dose (Table 1).

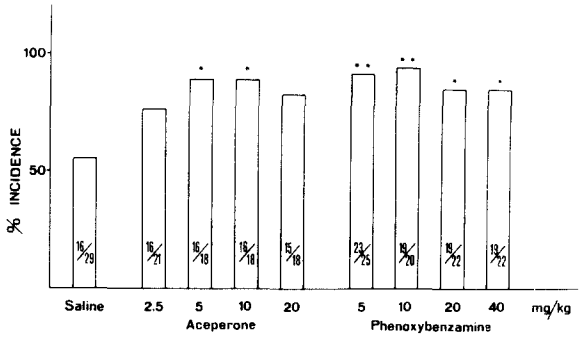

FIG. 1. The effect of aceperone and phenoxybenzamine on the incidence of apomorphine-induced gnawing stereotypy. The bars indicate the incidence of gnawing induced by 5 mg/kg apomorphine as percentage. The fractions within the bars denote absolute values of incidence (gnawing/total). *$p<0.05$, **$p<0.01$ (difference from saline control, Fisher exact probability test).

DISCUSSION

Apomorphine seems to exert its central action by acting directly on certain cerebral structures, and therefore it might be expected that certain threshold concentrations of the drug should be attained to trigger the behavioral effect. In fact, we have demonstrated that this assumption is correct for various forms of apomorphine stereotypy [8]. Our findings (Wiszniowska, in preparation) indicate that several drugs may affect the cerebral pharmacokinetics and concentration of apomorphine. We have reported that haloperidol depresses the striatal concentrations of apomorphine [11], and further studies have shown that it not only affects the penetration, but also changes pharmacokinetic parameters of apomorphine in some brain regions [7]. This effect may contribute to the known inhibitory effect of haloperidol on apomorphine stereotypy, particularly as the degree of inhibition of gnawing and of apomorphine concentration in the striatum are closely correlated [11].

In the present experiment an unexpected result has been found: the potentiation of the action of apomorphine observed as facilitation of compulsive gnawing was accompanied not by an increase, but a decrease of the threshold concentrations of apomorphine in the striatum. Therefore, the potentiation of the effect of apomorphine takes place against its pharmacokinetic interaction with α-adrenolytics. This suggests that there exists a pharmacodynamic interaction between the compounds tested, leading to enhanced response: α-adrenolytics increase the sensitivity of certain cerebral structures to apomorphine.

We suggest that the degree of sensitization of striatal structures involved in the action of apomorphine may be quantitatively expressed as the ratio of the threshold apomorphine concentration in the absence and the presence of pretreatment. This "sensitization index" approaches the value of 1.7 for the optimum dose of phenoxybenzamine, and almost 2 for aceperone.

The mechanism of this, almost two-fold potentiation of stereotyped gnawing by α-adrenolytics is obscure. It cannot reflect a general relationship between noradrenergic and dopaminergic systems, as another action of apomorphine, stimulation of locomotor activity, is effectively blocked by phenoxybenzamine [6]. It cannot be ascribed to an own, direct stimulatory action of α-adrenolytics on dopamine re-

ceptors, as it has been reported that phenoxybenzamine inhibits dopamine-stimulated adenylate cyclase activity [12]. The latter effect may explain why the highest doses of α-adrenolytics produced weaker facilitation of apomorphine gnawing than lower doses.

A tentative explanation is that adrenergic system exerts an inhibitory influence on a part of neuronal circuitry involved in expression of stereotyped behavior; the target of this influence may not necessarily be a dopaminergic neuron.

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