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Pharmacokinetic Study of Apomorphine-Induced Stereotypy in Food Deprived Rats

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WATANABE, H., S. NAKANO AND N OGAWA. *Pharmacokinetic study of apomorphine-induced stereotypy in food deprived rats* PHARMAC. BIOCHEM BEHAV **14**(4)493–496, 1981 —The relationship between the effect of food deprivation on apomorphine-induced stereotypy and the plasma apomorphine concentration in rats was studied. Male Wistar rats, allowed to have free access to food and water or deprived of food for 48 hr, were injected subcutaneously with apomorphine hydrochloride (10 mg/kg) Food deprivation was liable to potentiate the apomorphine-induced stereotypy in the early stage after dosing. Higher plasma apomorphine concentrations were found in the food deprived rats at this observation period. The potentiation of apomorphine-induced stereotypy following food deprivation can in part be explained by the pharmacokinetic changes. The exact mechanism of the effect of food deprivation on apomorphine kinetics is not clear at present.

Apomorphine Stereotypy Food deprivation Pharmacokinetics

APOMORPHINE (APM), which is thought to act by direct stimulation of dopamine receptor in the central nervous system, induces stereotyped behavior in mammalian species; rodents, for example, compulsively sniff, lick, and gnaw [3].

It has been reported that the amount of locomotor activity following food deprivation depends on degree of deprivation experience, test situation, and sex [5,6]. Nevertheless, food deprivation has been generally considered to increase behavioral arousal [2]. It has been reported that starvation potentiates the locomotor response to amphetamine [2]. Another recent study indicates that food deprivation potentiates the stereotypy response to APM in rats [11].

Studies of pharmacokinetics of APM have been reported in rats [13,14]. However, no report has been published up to date concerning the pharmacokinetic explanation of the fasting effect on APM-induced stereotypy. Therefore, this study was performed to clarify the relationship between the effect of food deprivation on APM-induced stereotypy and the plasma APM concentration in rats.

EXPERIMENT 1

Method

Male Wistar rats, weighing 100–110 g (5 weeks old), were housed 4 per cage at a temperature of 24 ± 1°C, 12 hr light-dark cycle (light on 7:00–19:00), and humidity of 60 ± 10% for two weeks. The subjects were randomly divided into 2 groups of 10 rats. One group was allowed to have free access to food and water (control group). The other group was deprived of food for 48 hr prior to the experiment (food deprived group). The experiment was performed between 13:00 and 17:00 corresponding to the 6th to the 10th hr of the light-phase, the time during which the highest stereotypy score was obtained [9].

It was difficult to measure the plasma APM concentrations by mass fragmentography after 1 to 3 mg/kg of APM was administered in rats. Since 10 mg/kg dose of APM was suitable for studying the pharmacokinetics of APM, rats of each group were injected subcutaneously with 10 mg/kg of APM and each rat was randomly put into an observation cage. The ratings were made by an observer on a blind basis. The drug was prepared within 30 min prior to administration. Each rat was rated for the degree of stereotypy on 0-to-9 scale (modified from that used by Sahakian and colleagues [12]) based on 30-sec observation every 10 min for a period of 2.5 hr after injection. The ratings were as follows: 0=inactive or asleep; 1=active but the same as saline-treated rats; 2=predominantly locomotor activity with bursts of stereotyped sniffing or rearing; 3=stereotyped sniffing or rearing over a wide area; 4=stereotyped sniffing or rearing in one location; 5=stereotyped licking of the floor or walls of the cage at least once during the observation period;

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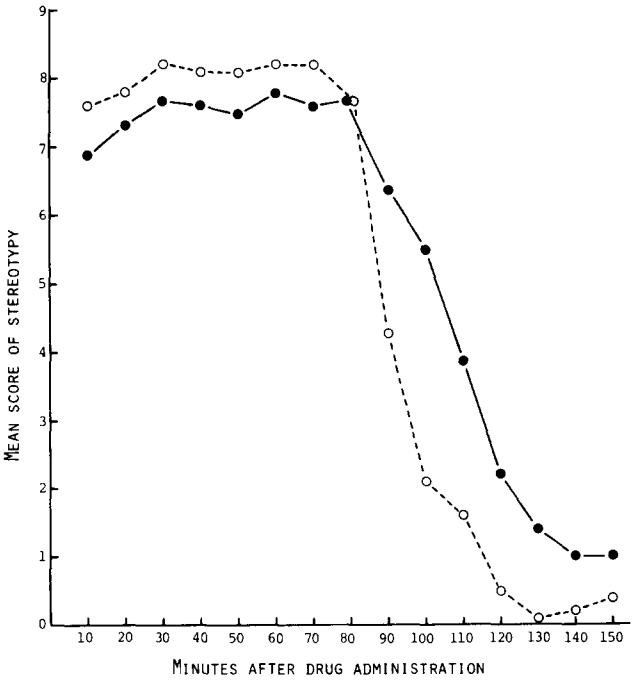

FIG 1. Influence of food deprivation on apomorphine-induced stereotypy in rats. Food deprived for 48 hr (○---○) and control (●——●) rats were injected with apomorphine hydrochloride (10 mg/kg subcutaneously) Each point represents the average of the stereotypy score of 10 rats.

TABLE 1

SCHEFFÉ ANALYSIS OF VARIANCE IN THE MEAN STEREOTYPY SCORES BETWEEN THE FOOD DEPRIVED GROUP AND THE CONTROL GROUP FOLLOWING SUBCUTANEOUS ADMINISTRATION OF APOMORPHINE HYDROCHLORIDE (10 mg/kg)

	Time after the drug administration (min)				
	90	100	110	120	130
F-value	10 42	27 32	12 50	6.83	3 99
p-value	<0 01	<0 001	<0 001	<0 01	<0 05

Only the results reaching statistical significance are shown

TABLE 2

ONSET AND DURATION OF GNAWING FOLLOWING SUBCUTANEOUS ADMINISTRATION OF APOMORPHINE HYDROCHLORIDE (10 mg/kg)

Experimental groups	Number of subjects	Minutes	
		Onset	Duration
Food deprived group (for 48 hr)	10	4.8 ± 0.7	80 2 ± 3 6
Control group	10	7.9 ± 1 2	95.5 ± 7 2
		*p<0 05	0.05<p<0 1

The results given represent the mean ± S E.M obtained from the values of 10 rats. *p, Student's t-test (two-tailed)

6=biting the cage wires at least once during the observation period; 7=compulsive continual biting, not in one location; 8=continual biting in one location; 9=continuous biting the same cage wire without any interruption and keeping this posture during the observation period. While the use of non-parametric statistics for the analysis of the rating data is more appropriate, the data were analyzed by analysis of variance, since this permits the determination of time course effects. The onset and duration of gnawing was measured as a definite component of APM-induced stereotypy by another observer. The data were analyzed by Student's t-test.

Results

The time-course data of APM-induced stereotypy after administration of 10 mg/kg is shown in Fig. 1. Analysis of variance revealed a significant interaction between feeding condition and time-course of APM-induced stereotypy, $F(14,252)=5.45$, $p<0.005$. Mean stereotypy scores of the food deprived group were inclined to be higher during the observation period between 10 to 70 min after administration as compared with those of the control group (Fig. 1). However, mean stereotypy scores of the food deprived group were significantly lower between 90 to 130 min after dosing (Table 1). The duration of gnawing was liable to be shorter in food deprived group than in control group (Table 2). The onset of gnawing in the food deprived group was significantly faster as compared to the control group (Table 2). Locomotor activity was not measured. However, all rats of these 2 groups showed quite intense gnawing represented by the rating scores of 7 to 9 between 10 to 80 min after dosing and rarely showed locomotor activity. It is likely that high levels of locomotor activity are incompatible with the intense gnawing induced by the relatively high dose of APM (10 mg/kg).

EXPERIMENT 2

Method

Male Wistar rats, weighing 100–120 g (5 weeks old), were housed 4 per cage in the same manner as described in experiment 1. Each rat in the 2 groups (nondeprived and deprived) was injected subcutaneously with 10 mg/kg of APM. The experiment was performed between 13:00 and 19:00 corresponding to the 6th to the 12th hr of the light-phase. Experiments on the 2 groups of animals were run concurrently and plasma samples were assayed at the same time.

Blood samples were obtained by cardiac puncture at 5, 10, 20, 60, and 120 min for each group after drug administration. Plasma was immediately separated by centrifugation at 2000 rpm and stored at −20°C until the time of assay. The amount of APM of each plasma sample was analyzed by mass fragmentography. The ions at m/e 411 of O,O-bis (trimethylsilyl) apomorphine (APM derivative) and m/e 439 of O,O-bis(trimethylsilyl)-N-n-propylnorapomorphine (PNAPM derivative) as an internal standard were monitored at an ionization energy of 20 eV. The concentrations of APM in these plasma samples were determined from the peak height ratio of the APM- and PNAPM-derivatives [15].

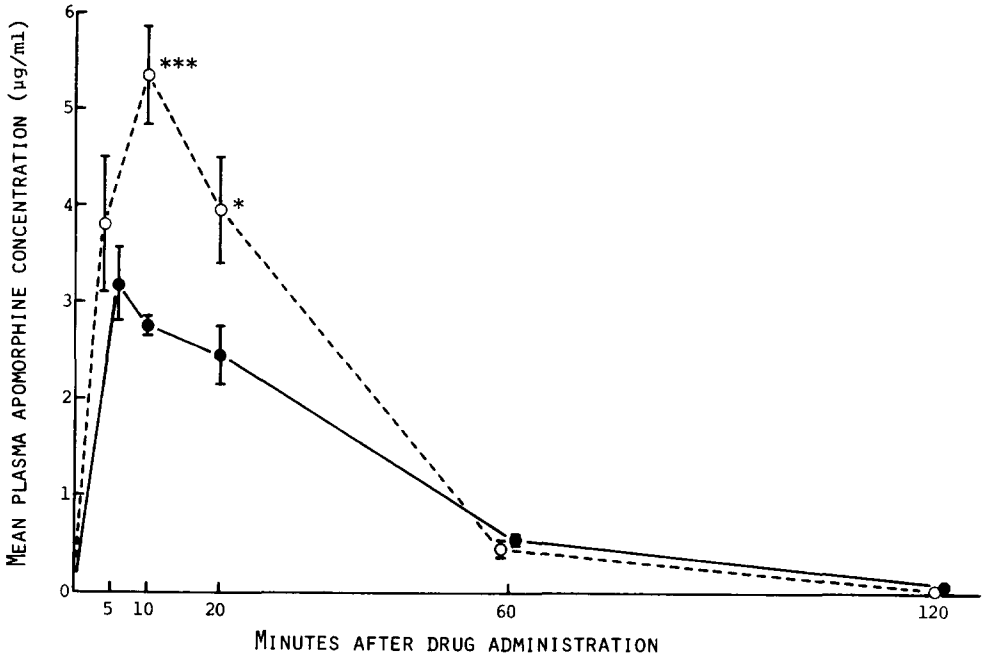

FIG 2 Time-course of apomorphine in plasma of food deprived for 48 hr (○---○) and control (●——●) rats following subcutaneous administration of apomorphine hydrochloride (10 mg/kg) Plasma samples were obtained by cardiac puncture at 5, 10, 20, 60, and 120 min after the injection and apomorphine concentrations were determined. The values given represent the mean ± S.E.M obtained from at least 7 determinations ***; $p<0.001$, *; $0.02<p<0.05$ by Student's t-test (two-tailed)

Results

Figure 2 shows mean plasma APM concentration curves for the food deprived group and the control group during the 120 min period after subcutaneous administration of 10 mg/kg of APM. The mean plasma APM concentrations were higher in the food deprived group than in the control group during the observation period between 5 to 20 min (Fig. 2). The rate of decline in plasma APM concentrations was more rapid in the food deprived group than in the control group. The peak concentration of APM occurred at 5 min (3.16 ± 0.42 μg/ml, Mean ± S.E.M.) after injection in the control group and at 10 min (5.36 ± 0.50 μg/ml) in the food deprived group (Fig. 2). The peak plasma APM concentration was significantly higher in the food deprived group (Student's t-test; $p<0.01$).

DISCUSSION

The mean stereotypy scores of the food deprived group were inclined to be higher during the observation period between 10 to 70 min after administration as compared to the control group. Therefore, food deprivation was liable to potentiate the 10 mg/kg APM-induced stereotypy in the early stage after dosing. In addition, food deprivation hastened the onset of gnawing. The present finding is consistent with the recent report which indicated that food deprivation enhanced stereotypy in the lower dose of APM (0.5 mg/kg) and modified it in the higher dose (1.5 mg/kg) [11]. The small difference observed at the present study in stereotypy score between the food deprived group and the control group might be due to a ceiling effect induced by 10 mg/kg dose of APM.

The effect of food deprivation on the plasma APM concentrations during the observation period between 5 to 120 min is given in Fig. 2. Higher plasma APM concentrations were found in the food deprived group during the period described above. Thus, the higher plasma APM concentrations in this observation period are correlated with the higher stereotypy scores in the food deprived group. The food deprived group showed higher stereotypy scores at 70 min after APM injection but no significant difference was seen in plasma APM concentrations at 60 min between these 2 groups. This discrepancy may be explained by the time-lag of the rate of distribution between the plasma and the brain. However, lower stereotypy scores in the food deprived group from 90 to 150 min can not be explained by the pharmacokinetics of APM. The reason is not clear at present. The faster appearance of stereotypy in the food deprived group may be due to the faster distribution of APM to the central nervous system (probably nigra-striatal regions) or due to increased sensitivity in the central dopaminergic receptor sites Thus, the effects of food deprivation on the APM-induced stereotypy may partly be interpreted by the pharmacokinetics.

Biochemical and physiological changes during food deprivation are not well known. However, it has been shown that free fatty acid levels in blood are considerably increased during food deprivation [1,10] and that this biochemical change affects pharmacokinetics of drugs. Free fatty acid has higher affinity constants for albumin than most drugs [8]. Thus, free fatty acid has caused increases in the free fraction of several highly plasma protein-bound drugs such as phenytoin, phenylbutazone, warfarin, sulfadiazine, and diazepam [4,16] The degree of variation in the amount of protein-

bound APM in plasma following food deprivation is not known at present. Nevertheless, these changes in free fraction of drugs as well as other physiological changes during food deprivation may cause significant changes in pharmacokinetics such as distribution, metabolism, and excretion of drugs. Influence of food deprivation on drug metabolism has been reported to be drug-dependent [7]. There are few reports studying the effect of food deprivation on absorption, distribution or excretion of drugs. Exact mechanisms of the APM pharmacokinetic changes in food deprived rats are not known at present. Further studies are needed to interpret more precisely the effect of food deprivation on the pharmacokinetics of APM

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