CORRELATION BETWEEN HOMOVANILLIC ACID CONCENTRATION IN THE RAT BRAIN AND SENSITIVITY OF DOPAMINE RECEPTORS TO THE AGONIST AFTER NEUROLEPTIC ADMINISTRATION

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Experiments on male Wistar rats showed that 6-12 h after a single injection of haloperidol in a dose of 1 mg/kg the intensity of apomorphine stereotypy increased gradually, to exceed the control level after 24 h. After injection of this neuroleptic the intensity of apomorphine stereotypy showed negative correlation with the homovanillic acid concentration in the rats' forebrain. It is suggested that the homovanillic acid concentration reflects the sensitivity of dopamine receptors to agonists.

KEY WORDS: neuroleptics, haloperidol; homovanillic acid; apomorphine.

In the modern view neuroleptics cause catalepsy and are antagonists of the effect of the dopaminomimetics apomorphine, amphetamine, and L-dopa, by blocking dopamine receptors in the CNS [3, 6]. Receptor blockade, by a feedback mechanism, increases the synthesis and catabolism of dopamine [2, 3, 6] and increases the concentration of acid products of dopamine catabolism, notably of homovanillic acid (HVA) [7]. There is evidence that at various times after the ending of chronic administration of neuroleptics the effect of dopaminomimetics on animal behavior is potentiated [10, 11]; the suggestion has been made that this is due to the development of hypersensitivity of dopamine receptors to the agonist.

This paper describes the results of experiments showing that the HVA concentration after administration of a neuroleptic reflects the sensitivity of dopamine receptors to an agonist. The agonist chosen was apomorphine, a direct stimulator of dopamine receptors [7].

EXPERIMENTAL METHOD

Experiments were carried out on 128 male Wistar rats weighing 150-200 g, divided into groups each containing 8-10 animals. The neuroleptic haloperidol was injected intrapetitoneally in a dose of 1 mg/kg. Apomorphine was injected in a dose of 1 mg/kg subcutaneously 2, 6, 12, and 24 h after injection of haloperidol. The intensity of stereotypy was assessed in points of Costall's method [4] 15 min after injection of apomorphine. The duration of the apomorphine stereotypy, in minutes, also was determined. The intensity of catalepsy, in seconds, was determined by the method of Honma and Fuhushima [5] after the same time interval in other groups of animals receiving haloperidol. These animals were then killed and the concentrations of dopamine (DA), noradrenalin (NA), and HVA in the forebrain (thalamus, striatum, hippocampus, amygdala, and part of the cortex) was determined fluorometrically by a combined method [8, 9], using the MPF-2A (Hitachi) fluorescence spectrophotometer.

Groups of animals which received physiological saline instead of haloperidol served as the controls. Student's method was used for the statistical analysis. The coefficient of correlation was calculated by Pearson's formula [1].

EXPERIMENTAL RESULTS

After injection of haloperidol (1 mg/kg), the rats developed catalepsy, which reached a maximum 6 h after the injection. The catalepsy then gradually diminished, although it was still recorded 24 h after injection

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TABLE 1. Intensity and Duration of Apomorphine Stereotypy (apomorphine 1 mg/kg) and Intensity of Catalepsy 2-24 h after a Single Injection of Haloperidol (1 mg/kg)

Substance	Number of rats	Time after injection, h	Stereotypy		Catalepsy, sec
			intensity, points	duration, min	
Control (physiological saline)	32	2—24	2,3±0,2	67,5±3,4	0,3±0,2
Haloperidol	8	2	0,3±0,2*	11,0±2,0*	45±12*
	8	6	0,7±0,2*	20,2±3,4*	113±23*
	8	12	1,5±0,1*	27,3±2,2*	27±5*
	8	24	3,3±0,3*	47,3±1,9*	12±10*

*Here and in Table 2, difference from control significant at $P < 0.05$.

TABLE 2. Effect of Haloperidol in a Dose of 1 mg/kg on DA, NA, and HVA Concentrations in Rat Forebrain (in μg/g brain tissue; M ± m)

Substance	Number of rats	Time after injection, h	DA	NA	HVA
Control (physiological saline)	32	2—24	1,59±0,05	0,34±0,02	0,23±0,02
Haloperidol	8	2	1,57±0,11	0,41±0,03	0,59±0,10*
	8	6	1,83±0,18	0,41±0,04	0,48±0,06*
	8	12	1,73±0,10	0,38±0,02	0,42±0,08*
	8	24	2,06±0,03*	0,47±0,03*	0,11±0,01*

of the drug (Table 1). The intensity of apomorphine stereotypy changed in the opposite direction. It was minimal 2 h after the injection of haloperidol, after which it gradually recovered, to exceed the control level ($P < 0.05$) 24 h after the injection of haloperidol (Table 1). The HVA concentration changed parallel with the intensity of apomorphine stereotypy, but in the opposite direction (Table 2). Its concentration was highest 2 h after injection of haloperidol. It then fell gradually and was below the control level after 24 h ($P < 0.01$). An increase in the concentrations of DA and NA also was observed 24 h after the injection of haloperidol (Table 2). Comparison of the intensity of apomorphine stereotypy with the HVA concentration shows negative correlation between them. Analysis in fact revealed a high degree of negative correlation between the ability of apomorphine to induce stereotypy and the HVA level after injection of the neuroleptic. The coefficient of correlation was 0.92 ± 0.03 ($0.05 > P > 0.01$).

Consequently, the results of these experiments show that the HVA level after administration of haloperidol reflects the sensitivity of dopamine receptors to the action of the dopaminomimetic apomorphine, i.e., with an increase in sensitivity of the receptors, the outflow and catabolism of the mediator are reduced. In this case, it can tentatively be suggested, there is a compensatory increase in sensitivity of the receptors in response to the preceding blockade. This hypothesis is confirmed by the increase in the DA and NA concentrations, parallel to the decrease in the HVA concentration.

Hypersensitivity of dopamine receptors thus developed not only after chronic administration [11], but also after a single dose of neuroleptics. The results confirm the existence of a feedback mechanism regulating the rate of synthesis and catabolism of dopamine through appropriate dopamine receptors [3, 6, 7]. It can be concluded that apomorphine stereotypy is an expression of stimulation of postsynaptic and not of presynaptic receptors [2, 3]. Considering the high correlation between the intensity of stereotypy and the fall in the HVA concentration in the forebrain, it can be postulated that the activity of the dopaminergic system is controlled mainly through postsynaptic receptors.

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KINETICS OF [^{14}C]PHENAZEPAM EXCRETION IN ALBINO RATS AFTER SINGLE AND REPEATED INJECTIONS OF THE DRUG

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During the 5 days after intraperitoneal injection of [^{14}C]phenazepam into albino rats, both intact animals and animals previously receiving phenazepam injections for 15 days, about 77% of the total radioactivity was excreted with the urine and feces. The excretion processes can be described by a first-order equation. The rate of total excretion of phenazepam was identical after single or repeated injections of the drug. Meanwhile, after a single injection of phenazepam into the animals, it was excreted mainly with the urine, whereas after repeated injections it was excreted mainly with the feces. The process of excretion of phenazepam with the urine after repeated injection is biexponential in character.

KEY WORDS: phenazepam; repeated and single injection; excretion; excretion constants; half-elemination period.

The kinetics of excretion of phenazepam, a tranquilizer of the 1,4-benzodiazepine series [2, 3], with the urine and feces after a single injection or a course of 15 daily injections of the drug is examined in this paper.

EXPERIMENTAL METHOD

Experiments were carried out on two groups of male albino rats weighing 180-200 g. [^{14}C]Phenazepam (1 Ci/mole) in a dose of 14 mg/kg was injected into the animals as a Tween emulsion. Samples of urine collected after 12, 24, 48, 72, and 120 h and of feces collected after 24, 48, 72, and 120 h were investigated. Phenazepam was injected daily for 15 days in a dose of 14 mg/kg into the animals of the second group, and this was followed by a single injection of [^{14}C]phenazepam (1 Ci/mole) in a dose of 14 mg/kg. Samples of urine and feces were collected after 12, 24, 48, 72, 96, and 120 h. The samples of feces were dried in an incubator at 80°C, weighed, and then hydrolyzed, in the same way as the samples of urine, with formic acid for 1 h on boiling water bath. Radioactivity in the biological media was determined by means of an Intertechnique (France) SL-30 liquid scintillation photometer and expressed as percentages of the dose administered to each animal. The excretion data were analyzed by "rate" and "sigma-minus" methods [4] and by Mansgeldorf's method [1].

EXPERIMENTAL RESULTS

The results in Tables 1 and 2 indicate that during the 5 days after intraperitoneal injection of [^{14}C]phenazepam into both groups of experimental animals about 77% of the total radioactivity was excreted with the urine and feces. Calculations showed that the excretion of total radioactivity from the experimental animals can be described by the first-order equation:

$$B_t = B_\infty(1-e^{-kt}),$$

where B_t is the quantity of radioactivity excreted by time t; B_∞ is the quantity of radioactivity excreted in the course of an infinite exposure; and k is the excretion constant.

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