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INFLUENCE OF CHLORPROMAZINE ON DECARBOXYLASES OF AROMATIC AMINO ACIDS

K. F. GEY, W. P. BURKARD and A. PLETSCHER

Medical Research Department, F. Hoffmann-La Roche & Co. Ltd., Basel, Switzerland

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Abstract—*In vitro* 10^{-4} M chlorpromazine did not influence decarboxylation of 5-hydroxytryptophan (5HTP) and 3 : 4-dihydroxyphenylalanine (dopa) in supernatant of rat brain and kidney. 10^{-3}M chlorpromazine only inhibited dopa decarboxylation in brain supernatant to which no pyridoxal-5′-phosphat (py-5-p) had been added.

Pretreatment of intact rats with high doses of chlorpromazine had no significant effect on decarboxylation of dopa or 5HTP in total homogenates or supernatant of brain.

In supernatant of kidney of rats pretreated with chlorpromazine there was, however, a 30–70 per cent rise in decarboxylation of dopa and 5HTP, provided that no py-5-p was added to the incubation medium.

The results are discussed with regard to the effects of chlorpromazine on the metabolism of py-5-p and aromatic monoamines.

IN RAT brain chlorpromazine counteracts the following changes in monoamine metabolism:[1]

(a) Release of 5-hydroxytryptamine (5HT), norepinephrine, dopamine (DA) caused by reserpine or benzoquinolizine derivatives.

(b) accumulation of norepinephrine and 5HT due to inhibition of monoamine oxidase (MAO) by iproniazid (N_2-*iso*propyl-*iso*nicotinic acid hydrazide, Marsilid*), isocarboxazid (N_2-benzyl-5′-methyl-3′ isoxazolylcarbonyl hydrazine, Marplan*).

(c) Increase of monoamines following administration of the monoamine precursor 5-hydroxytryptophan (5HTP).

The last two findings would be explained if it could be shown that chlorpromazine inhibits the decarboxylases responsible for the formation of aromatic monoamines. Inhibition of 5HTP decarboxylase in the kidney of chlorpromazine-pretreated rats has in fact been reported,[2] but in these experiments the newly formed 5HT was assayed by the isolated uterus of the oestrous rat. In biological tests of this kind, chlorpromazine might antagonize the pharmacological effect of 5HT.[3, 4] An antagonistic effect of chlorpromazine on 5HT has been observed in other isolated organs, e.g. the colon.[5] The effect of chlorpromazine on the decarboxylases of non-aromatic amino acids has not been clarified. Concerning glutamic acid decarboxylase of brain no effect[6, 7] as well as inhibition[8] was found *in vitro* and *in vivo*.

In the present study, the influence of chlorpromazine on decarboxylation of 5HTP and dopa was investigated *in vitro* and *in vivo* in brain and kidney of rats. Chemical

* Trade name.

or manometrical methods were used to determine the reaction products (5HT, DA and CO_2).

METHODS

Non-fasted female Wistar rats of 60–80 g received chlorpromazine i.p.. Untreated rats served as controls. Pools of brains (excluding the medulla oblongata) and of decapsulated kidneys from seven animals were homogenized in 3 or 4 ml per g organ of ice-cold phosphate buffer (0·067 M with pH 8·0 for 5HTP decarboxylase and pH 6·8 for dopa decarboxylase). A Potter–Elvehjem homogenizer (Teflon, clearance 0·004–0·006 in.) was used for brain and a Servall Omni-mixer (16·000 rev/min. for 45 sec) for kidney.

(a) *Enzyme activity in brain*

Dopa- and 5HTP decarboxylase were measured in 3 ml supernatant (29,000 **g** at 2°C for 30 min) by the slightly modified procedure of Davis and Awapara.[9] Incubations were carried out with 10 μmoles dopa (with or without 0·377 μmoles pyridoxal-5'-phosphate (py-5-p)) for 30 min, or with 12·5 μmoles 5HTP (with or without 0·038 μmoles py-5-p) for 60 min under N_2. The newly formed DA or 5HT was estimated spectrophotofluorometrically after adsorption of the monoamines on Amberlite CG-50 (final washing with 20 ml 0·67M phosphate buffer, pH 6·8). Non-incubated samples served as blank.

In additional experiments, 5HTP decarboxylase was estimated in total homogenates and in supernatant by spectrophotofluorometric measurement of the newly formed 5HT.[10] Incubations were carried out as above but on two occasions 1 ml each of homogenate or supernatant was used, adding 5·0 μmoles 5HTP, with or without 0·038 μmoles py-5-p.

(b) *Enzyme activity in kidney*

From 1 to 2 ml of supernatant of kidney homogenate were incubated in Warburg manometers with 25 μmoles dopa (with or without 0·18 μmoles py-5-p) or with 25 μmoles 5HTP (with or without 0·038 μmoles py-5-p). The final volume was 2·4 ml, with a pH the same as in the brain experiments. Incubation was carried out at 37·5 °C under N_2. It was stopped after 15 and 60 min, respectively, by tipping in 0·1 ml 20% H_2SO_4 from the second side arm. Calculation of enzyme activity from the initial velocity of CO_2-formation was done. Incubations without substrate served as blanks.

In some *in vitro* experiments chlorpromazine was added before incubation to aliquots of supernatant of brain and kidney of normal rats.

RESULTS AND DISCUSSION

Results of *in vitro* experiments are summarized in Table 1. Chlorpromazine in concentrations as high as 10^{-4} M does not inhibit decarboxylation of 5HTP or dopa significantly in enzyme preparations of rat brain and kidney ($P>0·05$). 10^{-3}M chlorpromazine only inhibits dopa decarboxylation in brain supernatant in the absence of py-5-p. This finding is probable without biological significance.

Results obtained in *intact animals* pretreated with chlorpromazine are shown in Table 2. Single or repeated doses of 20 or 25 mg/kg chlorpromazine i.p. have no significant effect on decarboxylation of 5HTP or dopa in *brain* ($P>0·05$), whether or not py-5-p is added to the incubation medium. In kidney there is a significant increase

TABLE 1. INFLUENCE OF CHLORPROMAZINE ON DECARBOXYLATION OF AROMATIC AMINO ACIDS *in vitro*

Supernatant of brain and kidney homogenates was used as enzyme source. The experimental conditions corresponded to the *in vivo* experiments. Absolute activity of the enzyme see Table 2(a). The figures represent mean values ± standard error in per cent of controls. Number of estimations in parenthesis.

Addition (final concentration)	Brain		Kidney	
	Without pyridoxal-5′-phosphate	With pyridoxal-5′-phosphate	Without pyridoxal-5′-phosphate	With pyridoxal-5′-phosphate
	Substrate dopa			
none	100 ± 3 (2)	100 ± 1 (2)	100 ± 9 (3)	100 ± 1 (2)
chlorpromazine 10^{-3}M	69 ± 4 (2)	95 ± 2 (2)	102 ± 4 (3)	85 ± 6 (2)
chlorpromazine 10^{-4}M	99 (1)	102 (1)	98 ± 6 (3)	92 ± 2 (2)
	Substrate 5HTP			
none	100 (1)	100 (1)	100 ± 11 (3)	100 ± 5 (4)
chlorpromazine 10^{-3}M	106 ± 11 (2)	113 ± 2 (2)	84 ± 9 (3)	91 ± 2 (4)
chlorpromazine 10^{-4}M	—	—	88 ± 4 (3)	98 ± 2 (4)

TABLE 2. DECARBOXYLATION OF DOPA AND 5HTP BY BRAIN AND KIDNEY OF CHLORPROMAZINE-PRETREATED RATS

The figures express mean values ± standard error of enzyme activity in per cent of controls. The figures are derived from two to five experiments with seven animals each.
The absolute activity of control groups is reported in Table 2(a).

Pretreatment prior to decapitation	Brain				Kidney	
	Total homogenate		Supernatant		Supernatant	
	Without pyridoxal-5′-phosphate	With pyridoxal-5′-phosphate	Without pyridoxal-5′-phosphate	With pyridoxal-5′-phosphate	Without pyridoxal-5′-phosphate	With pyridoxal-5′-phosphate
			Substrate dopa			
none	—	—	100 ± 4A	100 ± 1A	100 ± 3	100 ± 1
1 × 20 mg/kg chlorpromazine 1 hr	—	—	100 ± 5A	109 ± 3A	104 ± 3	97 ± 3
1 × 20 mg/kg chlorpromazine 3 hr	—	—	106 ± 4A	96 ± 2A	129 ± 2*	99 ± 3
3 × 25 mg/kg chlorpromazine within 3 days, last dose 16 hr	—	—	101 ± 8A	89 ± 7A	129 ± 1*	97 ± 1
			Substrate 5HTP			
none	100 ± 2B	100 ± 3B	100 ± 1B 100 ± 4A	100 ± 2B 100 ± 3A	100 ± 1	100 ± 1
1 × 20 mg/kg chlorpromazine 1 hr	103 ± 2B	97 ± 1B	105 ± 3B 100 ± 4A	106 ± 5B 93 ± 8A	141 ± 17	94 ± 12
1 × 20 mg/kg chlorpromazine 3 hr	102 ± 4B	99 ± 2B	114 ± 3B 115 ± 5A	107 ± 1B 109 ± 3A	170 ± 4*	108 ± 2
3 × 25 mg/kg chlorpromazine within 3 days, last dose 16 hr	—	—	109 ± 1B	85 ± 4B	170 ± 6§	117 ± 5

* $P = < 0{\cdot}01$ in comparison to controls (Student's test).
A = method according Davis and Awapara[9] with cation exchanger and photometry.
B = spectrophotofluorometric determination of 5HT according to Bogdanski *et al.*[10]
§ two experiments only.

in decarboxylation of both amino acids ($P<0{\cdot}01$). This marked increase of enzyme activity can be observed regularly in experiments carried out without addition of py-5-p. The results with kidney are in disagreement with a previous observation that the activity of 5HTP decarboxylase in rat kidney fell by 64 per cent after repeated administration of chlorpromazine.[2] This discrepancy can possibly be explained by interference of chlorpromazine in the biological test (see above).

The observation that chlorpromazine activates 5HTP and dopa decarboxylase in kidney is interesting in the following two respects:

(a) The activation takes place only when there is no additional py-5-p in the incubation medium. If optimal amounts of the coenzyme are added, results do not differ from those obtained in control animals (Table 2).

(b) Chlorpromazine-pretreatment increases the decarboxylation less than addition of py-5-p to the incubation medium. Thus, in supernatant of chlorpromazine-treated animals the activity of dopa and 5HTP decarboxylase is 129 per cent and 170 per cent, respectively compared with supernatant from untreated controls not supplemented with py-5-p (Table 2). Addition of py-5-p to supernatant of controls, however, stimulates the activity up to 422 per cent and 213 per cent, respectively (Table 2(a)).

TABLE 2(a). ABSOLUTE ENZYME ACTIVITY

Organ	Preparation		Mono-amine	μmoles/g_{fr}/hr	pyridoxal-5'-phosphate	number of experiments
brain	total homogenate	B	5HT	0·26 ± 0·006	—	12
			5HT	0·27 ± 0·007	+	12
	supernatant	A	DA	1·40 ± 0·06	—	65
			DA	2·00 ± 0·02	+	64
	supernatant	B	5HT	0·14 ± 0·002	—	45
			5HT	0·16 ± 0·003	+	44
	supernatant	A	5HT	0·21 ± 0·008	—	13
			5HT	0·22 ± 0·006	+	13
kidney	supernatant		DA	23·3 ± 0·7	—	51
			DA	98·3 ± 0·8	+	51
			5HT	3·9 ± 0·02	—	27
			5HT	8·3 ± 0·02	+	26

A = method according to Davis and Awapara[9] with cation exchanger and photometry.
B = spectrophotofluorometric determination of 5HT according to Bogdanski *et al.*[10]

These results suggest that chlorpromazine facilitates the formation of the complex apoenzyme/py-5-p in kidney, possibly by interfering with the metabolism of the coenzyme. It has, indeed, been found that chlorpromazine relieves some symptoms of vitamin B_6-deficiency in rats.[11] Furthermore, there is experimental evidence suggesting that chlorpromazine may interfere with pyridoxal-kinase which catalyses the formation of py-5-p from pyridoxal and adenosine-5'-triphosphate (ATP). *In vitro* the phenothiazine stimulates pyridoxalkinase in rat brain,[12] whereas *in vivo* excessive doses of this drug have an inhibitory action.[6] This divergency may be due to the fact that chlorpromazine either inhibits or stimulates the utilization of ATP depending on the concentration ratio of Mg^{2+}/ATP in the incubation system.[13]

As seen in preliminary experiments the effect of chlorpromazine on decarboxylase activity in rat brain and kidney is not appreciably changed by additional pretreatment with iproniazid, 16 hr before chlorpromazine injection.

Since according to the present experiments chlorpromazine does not inhibit decarboxylation of dopa or 5HTP in rats other mechanisms might be responsible for the interference of the drug with monoamine metabolism as outlined above. For instance, chlorpromazine could act by changing the permeability of membrances for monoamines[1] and amino acids.[14]

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