J. Pharm. Pharmac., 1973, **25**, 101–103 *Received September* 11, 1972

Decreased conversion of tyrosine to catecholamines in the brain of rats treated with *p*-chlorophenylalanine

A. TAGLIAMONTE, P. TAGLIAMONTE, G. U. CORSINI, G. P. MEREU and G. L. GESSA

Istituto Di Farmacologia, Università Di Cagliari, Italy

Repeated treatment with *p*-chlorophenylalanine (100 mg kg^{-1}) daily for 5 days impairs the transport of amino-acids from plasma into the brain and decreases the rate of conversion of [^{3}H]tyrosine to [^{3}H] catecholamines in the rat brain. The possible mechanisms involved are discussed.

p-Chlorophenylalanine (PCPA) has been shown to selectively block the synthesis of 5-hydroxytryptamine (5-HT) (Koe & Weissman, 1966) by inhibiting tryptophan hydroxylase (Koe & Weissman, 1966; Jequier, Lovenberg & Sjoerdsma, 1967; McGeer & Peters, 1969). However, brain catecholamine levels are significantly reduced by PCPA in mice (Welch & Welch, 1968), rats (Koe & Weissman, 1966; McGeer, Peters & McGeer, 1968; Miller, Cox & others, 1970), rabbits (Perez-Cruet, Tagliamonte & others, 1971) and cats (Keller, 1972). Moreover, we have observed that the administration of pargyline, a monoamine oxidase inhibitor, to PCPA-treated rats, raised brain catecholamine levels significantly less than in rats receiving pargyline alone (Tagliamonte, Tagliamonte & Gessa, 1971).

These considerations prompted us to examine the effect of PCPA on the rate of conversion of L-[^{3}H]tyrosine to [^{3}H]dopamine and [^{3}H]noradrenaline in the rat brain.

MATERIALS AND METHODS

(±) *p*-Chlorophenylalanine (PCPA) was administered as the methyl-ester HCl dissolved in distilled water, at the dose of 100 mg kg^{-1} (i.p.) daily for 5 days to male Sprague-Dawley rats, 180–220 g. The experiments were carried out 18 h after the last injection.

Animals were stunned, their blood rapidly collected from the abdominal aorta into centrifuge tubes containing heparin. The blood was then centrifuged and the plasma stored at —20°. Brains were quickly removed, frozen in dry ice and stored at —20°.

Endogenous and labelled tyrosine in brain and plasma and brain catecholamines were analysed according to Costa, Spano & others (1968).

RESULTS

Table 1 shows that a repeated administration of PCPA did not modify plasma tyrosine, while it markedly decreased the levels of tyrosine, dopamine and noradrenaline in brain.

Table 1. *Effect of PCPA (100 mg kg^{-1} i.p. daily for 5 days) on the levels of plasma tyrosine and brain tyrosine, dopamine on noradrenaline, in rats.*

Treatment (No. of expt)	Plasma tyrosine μg ml^{-1}	Tyrosine μg g^{-1}	Brain Dopamine μg g^{-1}	Noradrenaline μg g^{-1}
none (8)	14·67 ± 0·36	11·97 ± 0·43	0·912 ± 0·061	0·471 ± 0·024
PCPA (8)	15·12 ± 0·41	6·56 ± 0·31**	0·606 ± 0·034*	0·345 ± 0·026*

Each value is the average ± s.e. of the number of experiments reported in parentheses.

* $P < 0{\cdot}025$
** $P < 0{\cdot}010$
} in respect to control values.

Values were analysed for statistically significant differences by the two-tailed Student's test (Snedecor, 1956).

Control rats and rats treated with PCPA were injected with L-tyrosine-[3,5-^{3}H] (25 Ci mmol^{-1}) 500 μCi kg^{-1} in the tail vein and 12 min later they were killed. At this time the specific activity of brain tyrosine was significantly lower in brain than in plasma in both groups of animals.

Table 2 shows the effect of PCPA on the disposition of labelled tyrosine. In plasma of PCPA-treated rats, both the number of counts of [^{3}H]tyrosine and the specific activity of tyrosine did not differ from the corresponding values of control rats. In brain, PCPA reduced the counts min^{-1} of [^{3}H]tyrosine by 59% and the tyrosine specific activity by 24·5%, indicating a marked decrease in the transport of tyrosine from plasma to brain.

Table 2. [^3H]*Tyrosine and tyrosine specific activity in plasma and brain of PCPA-treated rats.*

Treatment (No. of expt)	Counts min^{-1}*	Plasma tyrosine Specific activity** (A)	Counts min^{-1}*	Brain tyrosine Specific activity (B)	$\frac{B}{A}$
none (8)	16,374 ± 246	1,825 ± 13	19,259 ± 316	1,601 ± 42	0·877 ± 42
PCPA (8)	15,859 ± 293	1,826 ± 33	7,874 ± 133§	1,208 ± 67§	0·661 ± 34§

Each value is the average ± s.e. of the number of experiments reported in parentheses.

§ $P < 0{\cdot}001$.

* Counts min^{-1} present in 2 ml of the final eluate from Dowex 50 × 4 column.

** Specific activity = d min^{-1} mM^{-1}.

As Table 3 shows, in the animals treated with PCPA the specific activities of dopamine and noradrenaline were reduced by 44·6 and 41·7%, respectively.

The rate of conversion of [^{3}H]tyrosine to [^{3}H]catecholamines was estimated from the ratio of their specific activity and that of brain tyrosine.

Although the specific activity of the tyrosine in adrenergic neurons may not be the same as that in the whole brain, it was assumed that the latter at least reflected the degree of labelling of the tyrosine in the neurons.

As Table 3 shows, PCPA decreased the rate of conversion of [^{3}H]tyrosine to [^{3}H] dopamine by 32% and to [^{3}H] noradrenaline by 26·5%.

Table 3. *Effect of PCPA on the rate of conversion of [^{3}H]tyrosine to [^{3}H]dopamine and [^{3}H]noradrenaline in the rat brain.*

Treatment (No. of expt)	Dopamine specific activity	Dopamine specific activity: Brain tyrosine specific activity	Noradrenaline specific activity	Noradrenaline specific activity: Brain tyrosine specific activity
none (8)	907 ± 32	0·566 ± 0·023	352 ± 26	0·219 ± 0·013
PCPA (8)	502 ± 23*	0·385 ± 0·014*	205 ± 18*	0·163 ± 0·000*

Each value is the average ± s.e. of the number of experiments reported in parentheses.

* $P < 0{\cdot}001$.

† Specific activity = d min^{-1} mM^{-1}.

DISCUSSION

Our results show that PCPA, considered a selective inhibitor of 5-HT synthesis (Koe & Weissman, 1966), also decreases the synthesis of dopamine and noradrenaline in brain. The decrease in catecholamine synthesis may be either secondary to a functional imbalance between the 5-HT and adrenergic systems (Tagliamonte, Tagliamonte & others, 1969) created by the inhibition of 5-HT synthesis or to a partial direct inhibition of tyrosine hydroxylase by PCPA.

The finding that PCPA reduced both the levels of endogenous tyrosine in brain and the specific activity of brain tyrosine indicates that PCPA impairs the transport of the amino-acid from plasma to brain. Since PCPA decreases brain tryptophan levels also (Tagliamonte, Tagliamonte & others, 1971), it is possible that it interferes with the transport of tryptophan as well.

Whether the inhibition of the amino-acid transport contributes to the decreased catecholamine, and/or 5-HT synthesis, is not known.

REFERENCES

COSTA, E., SPANO, P., GROPPETTI, A., ALGERI, S. & NEFF, N. H. (1968). *Atti Accad. Med. Lombarda*, **23**, 1100–1104.

JEQUIER, E., LOVENBERG, W. & SJOERDSMA, A. (1967). *Mol. Pharmac.*, **3**, 274–278.

KELLER, H. H. (1972). *Experientia*, **28**, 177–178.

KOE, B. K. & WEISSMAN, A. (1966). *J. Pharmac. exp. Ther.*, **154**, 499–516.

MCGEER, E. G. & PETERS, D. A. V. (1969). *Can. J. Biochem. Physiol.*, **47**, 501–508.

MCGEER, E. G., PETERS, D. A. V. & MCGEER, P. L. (1968). *Life Sci.*, **7**, 605–615.

MILLER, F. P., COX, R. H., SNODGRASS, W. R. & MAICKEL, R. P. (1970). *Biochem. Pharmac.*, **19**, 435–442.

PEREZ-CRUET, J., TAGLIAMONTE, A., TAGLIAMONTE, P. & GESSA, G. L. (1971). *Riv. Farmac. Ter.*, **11**, 27–34.

SNEDECOR, G. W. (1956). *Statistical Methods*, 5th Edn, Iowa State College Press, Ames.

TAGLIAMONTE, A., TAGLIAMONTE, P. & GESSA, G. L. (1971). *Nature*, **230**, 244–245.

TAGLIAMONTE, A., TAGLIAMONTE, P., GESSA, G. L. & BRODIE, B. B. (1969). *Science*, **166**, 1433–1435.

TAGLIAMONTE, A., TAGLIAMONTE, P., PEREZ-CRUET, J., STERN, S. & GESSA, G. L. (1971). *J. Pharmac. exp. Ther.*, **177**, 475–480.

WELCH, A. S. & WELCH, B. L. (1968). *Biochem. Pharmac.*, **17**, 699–708.