pattern of metabolism has been found in peripheral nerve tissues (e.g. bovine splenic nerve,[5] guinea pig was deferens[7] and guinea pig atria[8]) in which NE is the sympathetic neurotransmitter. On the other hand, radioactive DA was the only measurable catecholamine that accumulated in tissue in which DA rather than NE is thought to be the major neurotransmitter (e.g. the caudate nucleus and molluscan ganglia[9-11]). Thus, the present results are in accordance with the notion that NE is the neurotransmitter in the cells of the LC in the rat, although this may not be the case in other species.[4]

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Effect of *para*-chlorophenylalanine on catecholamine synthesis in rat brain, heart and adrenals

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PARA-CHLOROPHENYLALANINE (PCPA) has been used extensively as a serotonin depleter during investigations of the involvement of brain serotonin in temperature control,[1] seizures,[2-5] sleep,[6,7] mental disorders,[8] sexual activity[8,9] and other functions. However, PCPA also has actions on catecholamine biosynthesis. Several authors have reported significant lowering of brain catecholamines following PCPA treatment[10-12] and in mice depletion of noradrenaline was as pronounced as that of serotonin.[10] *In vitro*, PCPA inhibits tyrosine hydroxylase to a slight extent[11] and is a potent inhibitor of phenylalanine hydroxylase.[12] Date on the role of serotonin in seizures obtained by the use of PCPA have been questioned.[13,14] These observations suggest that the effect of PCPA may not be as specific as is generally accepted. Since changes in biogenic amine turnover rates tend to be more relevant to changed neuronal activity than changes in content,[15,16] we investigated the effect of PCPA on the turnover rates of the catecholamines in several tissues including brain.

The catecholamine synthesis rate was measured by a slight modification of the method of Neff *et al.*[17] Catecholamine content was assayed by the method of McGeer *et al.*[18] Male Sprague–Dawley rats, 250–270 g, were injected with PCPA methyl ester (CalBiochem) in aqueous propylene glycol[19] at a dose of 300 mg/kg intraperitoneally. Control animals received only the injection vehicle. The catecholamine synthesis rates and noradrenaline and adrenaline content were measured in brain, heart and adrenals at 2 and 24 hr after the PCPA injection. L-tyrosine-3,5-H^3 58 c/mM, 200 μc was infused into the tail veins of restrained rats over a period of 1 hr. At the end of the infusion, the

animals were sacrificed by decapitation, the tissues immediately removed and assayed for radioactive catecholamines. The catecholamines were isolated on an alumina column by the method used for the unlabeled amines.[18] No attempt was made to isolate the individual catecholamines; the synthesis data therefore refer to the total catecholamine content. All radioactivity measurements were made in a Nuclear Chicago Mark 1 liquid scintillation spectrometer. Counting efficiencies were determined by the use of internal standards and were around 37 per cent for tritium.

The results of acute PCPA treatment on the catecholamine content and rate of synthesis in heart and brain are summarized in Table 1. There were no significant differences in brain, heart or plasma tyrosine specific activities or in tissue weights between the control and treated groups of rats.

TABLE 1. NORADRENALINE CONTENT AND CATECHOLAMINE SYNTHESIS IN RAT BRAIN AND HEART 2 AND 24 HR AFTER TREATMENT WITH 300 mg/kg PCPA METHYL ESTER

	Brain		Heart	
Treatment	Noradrenaline (μg/g tissue)	Catecholamine synthesis (dis./min/g/hr)	Noradrenaline (μg/g tissue)	Catecholamine synthesis (dis./min/g/hr)
Control	0·280 ± 0·017*	1303 ± 34	0·761 ± 0·037	934 ± 76
2 hr	0·227 ± 0·008†	910 ± 43†	0·599 ± 0·022†	877 ± 20
24 hr	0·236 ± 0·017	926 ± 73†	0·547 ± 0·042†	811 ± 72

* Mean and standard error for a minimum of four animals.
† Significantly different from control values at $P < 0·05$.

PCPA lowered the noradrenaline content of rat brain to 81 per cent of control after 2 hr and to 84 per cent of control after 24 hr. The rate of incorporation of the radioactive label into brain catecholamines was reduced to approximately 70 per cent of normal at both 2 and 24 hr after the PCPA administration. This is consistent with other reports that PCPA depletes brain catecholamines[10–12] and inhibits brain tyrosine hydroxylase.[11]

The action of PCPA on brain serotonin biosynthesis *in vivo* is complex and not restricted to simple tryptophan hydroxylase inhibition by PCPA itself.[12,20] Several authors have suggested that PCPA is converted into one or more active metabolites which are at least partly responsible for the prolonged serotonin-depleting action and perhaps the catecholamine-lowering effect also.[12,19] It is possible that a PCPA metabolite is responsible for the action of this drug on brain catecholamine synthesis. As reported by others,[12,19] at least two probable PCPA metabolites are likely to inhibit tyrosine hydroxylase as well as trypophan hydroxylase.

Heart catecholamine content was reduced to 79 and 72 per cent of the control level at 2 and 24 hr, respectively, after the PCPA injection. However, the synthesis of labeled catecholamines from L-tyrosine-^{3}H was not significantly reduced at either time. It is possible that catecholamine release by a PCPA metabolite rather than tyrosine hydroxylase inhibition is the main factor in lowering heart catecholamine content. It does not appear to have been reported whether PCPA inhibits heart tyrosine hydroxylase.

TABLE 2. CATECHOLAMINE CONTENT AND SYNTHESIS IN RAT ADRENALS 2 AND 24 HR AFTER TREATMENT WITH 300 mg/kg PCPA METHYL ESTER

		Noradrenaline + adrenaline		Catecholamine synthesis	
Treatment	Weight per adrenal pair (mg)	(μg/adrenal pair)	(μg/g tissue)	(dis./min/ adrenal pair)	(dis./min/ g tissue)
Control	38·3 ± 1·7*	14·0 ± 0·4	369 ± 27	1,628 ± 168	42,500 ± 4400
2 hr	50·2 ± 2·6†	14·3 ± 1·0	302 ± 19	1,539 ± 102	30,700 ± 2000†
24 hr	53·8 ± 2·6†	13·6 ± 0·6	254 ± 14†	2,673 ± 415†	49,700 ± 7700

* Mean and standard error for a minimum of four animals.
† Significantly different from control values at $P < 0·05$.

The synthesis of total labeled catecholamines from the infused L-tyrosine-^{3}H in adrenals was unchanged after 2 hr treatment but was markedly increased (to 164 per cent of control) after 24 hr. The results are summarised in Table 2. The observed increase in adrenal catecholamine synthesis found after PCPA treatment could also be due to catecholamine release by PCPA or a metabolite. Many compounds, including serotonin,[21] have been known to cause release of adrenal catecholamines. Induction of tyrosine hydroxylase may be involved in the increased rate of catecholamine synthesis. Several drugs which release adrenal catecholamines are known to cause an increased activity of adrenal tyrosine hydroxylase.[22,23]

Our results which show a significantly decreased catecholamine synthesis from labeled tyrosine in rat brain after PCPA administration demonstrate further the inadvisability of relating the pharmacological effects of PCPA solely to serotonin depletion. Other possibilities should be taken into account when studying the actions of PCPA.

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