HALOGEN SUBSTITUTION OF AMPHETAMINE BIOCHEMICAL AND PHARMACOLOGICAL CONSEQUENCES

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METABOLIC CONSEQUENCES OF HALOGEN SUBSTITUTION IN *p*-POSITION OF AMPHETAMINE

THE MAJOR pathways for the metabolism of amphetamine involve *p*-hydroxylation of the aromatic ring of amphetamine to *p*-hydroxyamphetamine (POH) followed by β-hydroxylation of the *d*-isomer to *p*-hydroxynorephedrine or oxidative deamination of the side chain. The relative extent of these two routes of metabolism varies markedly with the species (AXELROD, 1954; ELLISON *et al.*, 1966; DRING *et al.*, 1970). In the rat, where *p*-hydroxylation is the predominant pathway, one would expect that *p*-substituted derivatives of amphetamine such as *p*-chloroamphetamine (PCA) should be metabolised much more slowly than amphetamine (FULLER and HINES, 1967). MILLER *et al.* (1971) have demonstrated that the biological half-life of PCA in the rat was about seven times that of amphetamine, while in mice, where *p*-hydroxylation is less important, the rate of metabolism of PCA closely approximated that of amphetamine. Moreover, iprindole, an inhibitor of the aromatic hydroxylation of amphetamine (FREEMAN and SULSER, 1972) markedly enhances and prolongs the action of amphetamine, causes increased levels of the drug in brain and prolongs the half-life of amphetamine from 45 to 190 min. As expected, however, the pharmacological action and the half-life of PCA are not influenced by iprindole in the rat. Halogen substitution in *p*- or *m*-position changes the subcellular distribution of the amphetamine derivatives. Thus, PCA and *m*-chloroamphetamine (in DMI pretreated rats) are present mainly in the particulate fraction of brain homogenates whereas amphetamine is mainly localised in the supernatant fraction (FULLER *et al.*, 1972). Whether the localisation of PCA in the particulate fraction is due to uptake into synaptic vesicles has not been established but WONG *et al.* (1972) have recently demonstrated that PCA is associated with the synaptosomal fraction of brain homogenates. β,β-Difluoro substitution of PCA shortens the half-life of PCA and leads to a marked accumulation of the drug in fat tissue while in all other tissues, the difluoro compound is present in concentrations lower than those of PCA (FULLER *et al.*, 1973b). Interestingly, desmethylimipramine which enhances the levels of amphetamine in rat brain through inhibition of its metabolism by para-hydroxylation (SULSER *et al.*, 1966; CONSOLO *et al.*, 1967; LEWANDER, 1969) does not affect the levels of β,β-difluoroamphetamine (FULLER *et al.*, 1973a), suggesting that the metabolism of the β,β-difluoro derivative occurs by a pathway other than para-hydroxylation. PARLI and LEE (1972) have recently demonstrated the oxime of difluorophenylacetone in free and conjugated form in the urine of rats given β,β-difluoroamphetamine thus indicating oxidative deamination. The differences in the

half-life of PCA in rats and mice (MILLER *et al.*, 1971) and between PCA and β,β-difluoro-PCA in rats (FULLER *et al.*, 1973b) are reflected in differences in the duration of the effects of the drugs on brain 5-hydroxyindoles.

It is noteworthy that PCA like POH is a substrate for β-hydroxylase and that the rate of formation of *p*-chloronorephedrine from PCA approximates that of *p*-hydroxynorephedrine from POH (personal communication by Dr. F. C. Brown). Since it has been demonstrated that *d*-POH but not *l*-POH is a substrate for β-hydroxylase (GOLDSTEIN and ANAGNOSTE, 1965), it will be of interest to investigate whether or not the conversion of PCA to *p*-chloronorephedrine is also stereoselective for the *d*-isomer and to study the contributions, if any, of *p*-chloronorephedrine to the action of the parent drug on adrenergic and serotonergic mechanisms.

EFFECT OF HALOGEN SUBSTITUTION OF AMPHETAMINE ON ADRENERGIC MECHANISMS

p-Chlorinated derivatives of amphetamine exert pharmacological effects which are similar to those of the parent compounds (NIELSEN *et al.*, 1967; FREY and MAGNUSSEN, 1968). In contrast to amphetamine, PCA does not alter the concentration of either norepinephrine or dopamine in brain but markedly changes the metabolism of cerebral serotonin (PLETSCHER *et al.*, 1964; 1966; FULLER *et al.*, 1965). The initial central excitatory action of PCA is, like that of amphetamine, related to its effect on the metabolism of cerebral catecholamines (STRADA *et al.*, 1970). Like amphetamine, PCA causes a large increase in the concentration of normetanephrine and a reduction in the levels of the deaminated metabolites of ^{3}H-norepinephrine. Accordingly, similar mechanisms might be responsible for the effects: release of catecholamines, blockade of their reuptake and possibly either direct inhibition of MAO (GLOWINSKI and AXELROD, 1965; GLOWINSKI *et al.*, 1966; FULLER, 1966) or indirect inhibition of the enzyme resulting from the blockade of neuronal uptake of catecholamines (RUTLEDGE, 1970). The effects elicited by PCA on the metabolism of ^{3}H-norepinephrine are, however, more pronounced and longer lasting in accordance with its longer biological half-life (STRADA *et al.*, 1970; CARR and MOORE, 1970).

Studies with tyrosine hydroxylase inhibitors have lead to the view that the central action of amphetamine depends on an uninterrupted synthesis of catecholamines (WEISSMAN *et al.*, 1966; HANSEN, 1967; DINGELL *et al.*, 1967; SULSER *et al.*, 1968; SCHEEL-KRÜGER, 1971) whereas that of PCA appears to be mediated through the release of stored catecholamines (STRADA and SULSER, 1971). Recent results from our laboratory strengthen this view. Amphetamine and PCA were studied in animals whose noradrenergic terminals in brain had been destroyed by intraventricular 6-hydroxydopamine. This procedure reduces the level of norepinephrine to about 16 per cent of its control value while that of dopamine in the striatum is decreased only by about 40 per cent. The activity of tyrosine hydroxylase, measured by the coupled assay of WAYMIRE *et al.* (1972) and expressed as $nCi^{14}CO_2$/30 min/20mg tissue, decreases in the striatum from 22·8 ± 1·1 to 7·7 ± 0·9 and in the diencephalon from 3·5 ± 0·1 to 2·1 ± 0·1. It is of interest that such a procedure does not alter the central action of PCA and only slightly reduces that of amphetamine. Reserpinisation of animals (depletion of remaining stores) whose noradrenergic neurons were destroyed by 6-hydroxydopamine, completely blocks the action of PCA and either enhances or does not change that of amphetamine (Fig. 1). These data provide more

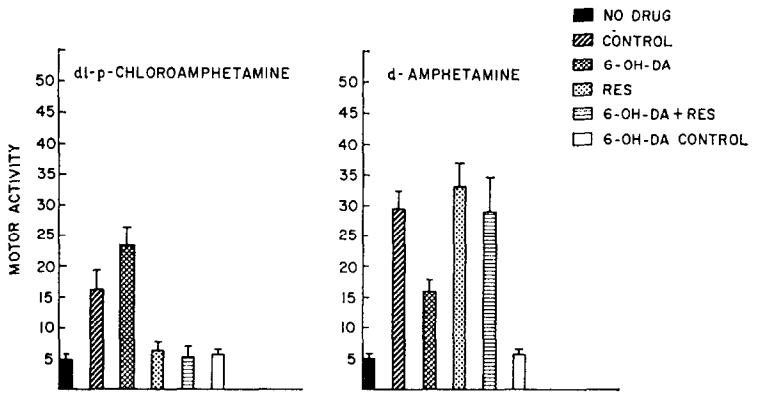

FIG. 1.—Modification by 6-hydroxydopamine (6-OH-DA) and reserpine (RES) of the psychomotor stimulation elicited by *d*-amphetamine (3mg/kg i.p.) and *dl-p*-chloroamphetamine (PCA; 5mg/kg i.p.). Two daily doses of 6-OH-DA (250μg in 10μl) were injected intraventricularly through a polyethylene cannula. The animals were used one week after the last dose of 6-OH-DA. Reserpine was administered intraperitoneally (5mg/kg) 4 hr prior to the injection of amphetamine or PCA. Psychomotor stimulation was measured in Williamson activity cages. The data are expressed as integrated counts ± S.E.M.

direct evidence that the central stimulatory action of amphetamine is mediated predominantly through newly synthesised catecholamines, whereas the action of PCA depends on the store of catecholamines, predominantly dopamine. These results are also compatible with data showing that motor activity of rats can be increased by doses of PCA which increase the turnover rate of dopamine but not necessarily that of norepinephrine in various parts of the brain (COSTA *et al.*, 1971). Since the steady state concentration of dopamine remains unchanged after PCA, the data might suggest that PCA alters the synthesis of catecholamines, particularly dopamine in nerve terminals and cell bodies in brain. However, PCA (5 mg/kg i.p.) does not change the activity of tyrosine hydroxylase in the striatum or diencephalon (Table 1). Moreover, the *in vitro* addition of PCA (10^{-9}–10^{-5} M) does not alter the activity of tyrosine hydroxylase in preparations from striatum or diencephalon. Since we measured total enzyme activity, we cannot rule out that the drug may cause a shift in the activity of tyrosine hydroxylase from the soluble to the particulate 'synaptosomal' fraction as has been reported to occur with methamphetamine (MANDELL *et al.*, 1972).

TABLE 1. EFFECT OF *p*-CHLOROAMPHETAMINE (PCA) ON THE ACTIVITY OF TYROSINE HYDROXYLASE

Time after PCA*	Tyrosine hydroxylase activity nCi $^{14}CO_2$/30 min/20mg tissue			
	Striatum		Diencephalon	
(hr)	Control	PCA	Control	PCA
1	21·90 ± 0·78	19·28 ± 1·15	3·78 ± 0·20	3·48 ± 0·10
4	17·80 ± 1·31	17·89 ± 1·85	3·71 ± 0·20	3·69 ± 0·14

**dl*-PCA was administered intraperitoneally (5mg/kg).
Tyrosine hydroxylase activity was measured according to WAYMIRE *et al.* (1972).

EFFECT OF HALOGEN SUBSTITUTION OF AMPHETAMINE ON SEROTONERGIC MECHANISMS

With regard to the serotonergic system, striking differences in the biochemical effects of amphetamine and PCA are evident. Unlike amphetamine, the chloro derivative causes a simultaneous and prolonged decrease in the brain levels of 5HT and its principal metabolite 5HIAA (PLETSCHER *et al.*, 1964). A decrease in the turnover of 5HT also occurs as demonstrated by a decrease in the rise of brain 5HIAA after the administration of probenecid (SANDERS-BUSH and SULSER, 1970). COSTA and REVUELTA (1972a) have confirmed these results using an isotopic technique. Recent studies from our laboratory have suggested that an inhibition of brain tryptophan hydroxylase can explain the marked reduction in turnover of 5HT after the administration of PCA (SANDERS-BUSH *et al.*, 1972a).

The mechanism of the reduction of the activity of tryptophan hydroxylase after the administration of PCA is not yet understood. One day after the *in vivo* administration of PCA, a dose related reduction in tryptophan hydroxylase was found (Table 2). However, in agreement with data of PLETSCHER *et al.* (1970), PCA did not modify the activity of tryptophan hydroxylase when added *in vitro*. Kinetic studies of enzymes isolated from control rats and from rats treated with PCA showed that the drug did not change the apparent K_m for either tryptophan ($3{\cdot}0 \times 10^{-4}$ M) or $DMPH_4$ ($1{\cdot}5 \times 10^{-4}$ M). Moreover, the reduction of tryptophan hydroxylase in preparations from animals treated with PCA is not reversed by dialysis. These results suggest that the administration of PCA may reduce the amount of active enzyme without altering its properties. Therefore, it was important to examine the time course of the reduction in the activity of tryptophan hydroxylase and brain 5HT by PCA and to compare it to that caused by *p*-chlorophenylalanine, an irreversible inhibitor of tryptophan hydroxylase. Both drugs cause a marked reduction in the activity of tryptophan hydroxylase and the levels of 5HT in brain after one day. However, two weeks after injection, the effect of *p*-chlorophenylalanine has disappeared while both enzyme activity and levels of 5HT are still maximally reduced in rats treated with PCA (SANDERS-BUSH *et al.*, 1972b). Even 4 months after a single dose of PCA, although some recovery has occurred, the levels of 5HT and 5HIAA and the activity of tryptophan hydroxylase are still significantly reduced (Table 3). The parent compound

TABLE 2. EFFECT OF THE INTRAPERITONEAL ADMINISTRATION OF *p*-CHLOROAMPHETAMINE ON THE ACTIVITY OF TRYPTOPHAN HYDROXYLASE

Dose (mg/kg*)	Tryptophan hydroxylase activity	
	nCi^{14}5C-HT formed/g/hr ± S.E.	Per cent inhibition
0 (5)	31·4 ± 2·9	0
2 (4)	25·2 ± 0·4	20
5 (7)	21·0 ± 1·2	33
7·5 (7)	18·0 ± 2·2	43
10 (7)	12·5 ± 1·2	60

* *p*-Chloroamphetamine was administered, i.p., 16 hr prior to sacrifice. The number of animals is indicated in parentheses.
Tryptophan hydroxylase activity was measured according to the procedure described by SANDERS-BUSH *et al.* (1972a).

TABLE 3. CEREBRAL LEVELS OF 5HT AND 5HIAA AND ACTIVITY OF TRYPTOPHAN HYDROXYLASE AT VARIOUS TIMES AFTER A SINGLE DOSE OF *p*-CHLOROAMPHETAMINE

Time after injection	Percent of control ± S.E.M.		
	5HT	5HIAA	Tryptophan hydroxylase
16 hr	39·0 ± 3·6(4)*	39·6 ± 3·0(4)*	57·6 ± 1·3(4)*
4 days	39·6 ± 5·1(5)*	31·5 ± 2·7(8)*	52·3 ± 7·9(5)†
10 days	38·2 ± 3·3(5)*	—	49·6 ± 1·5(5)*
2 weeks	47·9 ± 3·2(9)*	25·8 ± 2·1(6)*	41·4 ± 7·6(9)*
4 weeks	62·6 ± 4·5(11)†	35·3 ± 5·7(5)*	57·7 ± 6·6(11)*
6 weeks	73·1 ± 2·3(4)*	49·2 ± 3·7(4)*	72·3 ± 7·7(4)†
2 months	73·2 ± 2·7(5)†	—	—
4 months	79·5 ± 8·5(4)‡	60·1 ± 3·9(7)*	60·5 ± 3·0(4)†

Rats were injected i.p. with a single dose of either saline or 10 mg/kg of *p*-chloroamphetamine. The animals were sacrificed at various times after the injection. Results are mean values and are expressed as percentage of the respective control values. The number of animals is shown in parentheses. Mean values for all control animals were: 5HT, 0·69 ± 0·02 μg/g ($n = 43$); 5HIAA, 0·29 ± 0·01 μg/g ($n = 33$); tryptophan hydroxylase, 78·1 ± 3·9 nCi ^{14}C-5HT formed/g/hr ($n = 39$). (From SANDERS-BUSH *et al.*, 1972b)
*$P < 0·001$. †$P < 0·01$. ‡$P < 0·05$.

amphetamine does not reduce the activity of tryptophan hydroxylase after a single acute dose or after chronic treatment with the drug.

Recently we have found another important difference between the action of *p*-chlorophenylalanine and PCA. Unlike *p*-chlorophenylalanine, the amphetamine derivative does not reduce the synthesis of 5HT in peripheral organs such as intestine. Thus, the mechanism of the decrease in the activity of tryptophan hydroxylase after these two drugs must certainly be different.

Other halogen substituted phenylethylamine derivatives which decrease brain 5HT have been described. In rats the *meta*-chloro derivative of amphetamine only lowers 5HT in animals treated with desmethylimipramine, which increases the brain levels of the drug presumably by inhibiting its *para*-hydroxylation (FULLER *et al.*, 1972). The ortho-derivative does not lower brain 5HT even after treatment with desmethylimipramine. Other derivatives of amphetamine with marked effects on brain 5HT are fenfluramine (DUHAULT and VERDAVAINNE, 1967) and norfenfluramine (MORGAN *et al.*, 1972). The mechanism for the decrease in 5HT after PCA and norfenfluramine is apparently different. Although both drugs cause a decrease of 5HT and 5HIAA in brain, PCA decreases the turnover of brain 5HT (SANDERS-BUSH and SULSER, 1970; COSTA and REVUELTA, 1972a) while norfenfluramine has been reported to increase it (COSTA and REVUELTA, 1972b).

OTHER DIFFERENTIAL EFFECTS BETWEEN AMPHETAMINE AND ITS *p*-CHLORINATED DERIVATIVES

The bulk of evidence indicates that the CNS stimulation elicited by both amphetamine and PCA is mediated through catecholamines. Since norepinephrine has been shown to increase the level of cyclic AMP in rat brain slices *in vitro* (KAKIUCHI and RALL, 1968; PALMER *et al.*, 1972) and a dopamine sensitive adenyl cyclase has been found in the cerebral cortex (MCCUNE *et al.*, 1971) and in the caudate nucleus (KEBABIAN *et al.*, 1972), an increase in the level of endogenous cyclic AMP would be expected following the administration of amphetamine or PCA. Studies from our

laboratory have shown, however, that amphetamine causes no detectable effects in the concentrations of cyclic AMP in any brain area at any time even though marked behavioural activation and increased sympathetic activity are evident (SCHMIDT *et al.*, 1972). Moreover, *dl*-PCA actually causes a decrease in the concentration of cyclic AMP in brain (PALMER *et al.*, 1972). These unexpected and puzzling findings remain a challenge for further investigation.

The clinical profile of *p*-chlorinated derivatives of amphetamine also differs from that of the parent drugs. Thus, while amphetamine has no value in the treatment of depressive illness, *p*-chloromethamphetamine has been reported to be a true anti-depressant without causing central motor stimulation and insomnia (VAN PRAAG *et al.*, 1969; 1971).

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