Accumulation of amphetamine and *p*-chloroamphetamine into synaptosomes of rat brain

p-Chloroamphetamine, but not amphetamine, lowers brain 5-hydroxytryptamine concentrations (Pletscher, Bartholini & others, 1964; Fuller, Hines & Mills, 1965) by either inhibition of its synthesis (Sanders-Bush & Sulser, 1970) or by releasing bound 5-HT (Fuller, 1966; Pletscher, Da Prada & Burkard, 1970). On the other hand, both *p*-chloroamphetamine and amphetamine interfere with noradrenaline uptake into brain nerve endings (Strada, Sanders-Bush & Sulser, 1970). Perhaps, the distinct difference between the two drugs is their binding ability to the particulate matter of rat brain (Fuller & Hines, 1967). The present report is an extension of our observation in the localization of *p*-chloroamphetamine and amphetamine in subcellular particles of the rat brain.

Groups of four Sprague–Dawley rats (150–180 g) were separately treated with equimolar doses of amphetamine (18 mg/kg) and *p*-chloroamphetamine (24 mg/kg) and were decapitated at 1 h. A crude mitochondrial preparation was obtained from a 30% brain homogenate in 0·32M sucrose after stepwise differential centrifugation of 1085 *g* (10 min) and 17 300 *g* (20 min). It was further fractionated by centrifugation in a discontinuous Ficoll gradient (Kurokawa, Sakamoto & Kato, 1965). A microsomal fraction was isolated from the post-mitochondrial supernatant fraction after centrifugation at 100 000 *g* (60 min).

The subcellular distribution of the two drugs is shown in Table 1. Values in the first two columns show the relative amounts in percentage of drug in the 1085 *g* supernatant fraction. Most of the amphetamine (77%) was found in the post-mitochondrial supernatant fraction; 62·5% of the amphetamine was associated with the high-speed supernatant fraction. After the crude mitochondrial fraction was subjected to centrifugation in a discontinuous Ficoll gradient, four sub-fractions resulted including a supernatant fraction, myelin, synaptosomes and mitochondria. The particulate fractions, except mitochondria, contained 6 to 8% amphetamine. The particles sedimented further away from the soluble fraction contained less drug per mg protein. A total of 31·5% (7·9% + 23·6%) of the amphetamine was particle-bound.

Table 1. *Subcellular distribution of amphetamine and* p*-chloroamphetamine in rat brains.*

	Amphetamine	*p*-Chloro-amphetamine	Amphetamine	*p*-Chloro-amphetamine
	% of total drug in starting material*		nmol per mg protein	
17 300 *g* supernatant	77·2	43·0	2·56	2·38
100 000 *g* supernatant	62·5	14·6	2·47	1·47
100 000 *g* pellet (microsomal fraction)	7·9	18·4	0·94	3·28
17 300 *g* pellet	23·6	32·7	0·88	2·32
50 000 *g* supernatant	6·2	2·8	2·72	1·74
Myelin (0·32M sucrose—3% Ficoll interface)	6·3	8·8	1·36	3·5
Synaptosomes (3–13% Ficoll interface)	6·4	14·0	0·82	2·92
Mitochondria (pellet)	0·8	3·8	0·12	1·04

* Starting material was a 30% brain homogenate centrifuged at 1085 *g* to remove unbroken cells and cell nuclei. The recovery of drugs at this point was 53% for amphetamine and 51% for *p*-chloroamphetamine of the original homogenates. Each value represents the mean of three experiments.

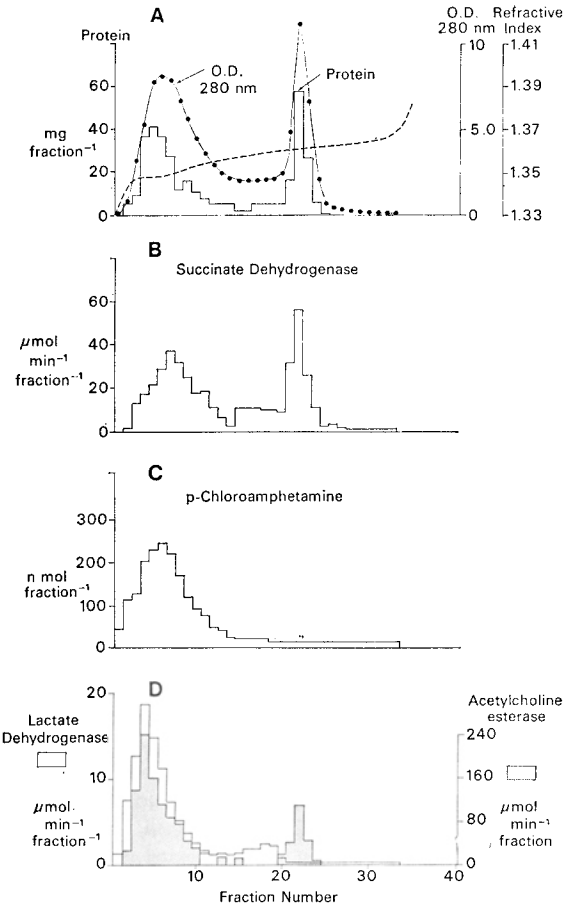

FIG. 1. Separation of synaptosomes and mitochondria by rate zonal centrifugation in a continuous Ficoll gradient. The zonal centrifuge B-XXIX rotor* was loaded with a medium of 8·5% sucrose and a gradient of 0 to 23% Ficoll. Mitochondrial pellets (4 mg protein/ml) in 90 ml of 0·25M sucrose, followed by 70 ml of 0·05M HEPE, pH 7·4 were centrifuged for 12 min at 10 000 rev/min ($\omega^2 t = 7{\cdot}8 \times 10^7$). Forty millilitre fractions were collected from the centre of the rotor (Wong, van Frank & Horng, 1970).

* B-XXIX rotor was supplied by Dr. N. G. Anderson through a co-operative agreement with the Molecular Anatomy Program of Oak Ridge National Laboratories.

In contrast, the high speed supernatant fraction contained only 14·6% of *p*-chloroamphetamine, whereas the crude mitochondria and microsomes had 33 and 18·4% of the drug for a total of 51% that was particulate bound. Among the three fractions resolved from the crude mitochondria, the synaptosomal fraction had the largest proportion of drug and myelin was next. Although microsomes, synaptosomes and myelin had about the same amount of drug per mg protein, the drug in myelin possibly arose from the contamination of some synaptosomal membrane-fragments; since the protein was as high as in microsomes and synaptosomes. Again, mitochondria had the least amount of *p*-chloroamphetamine.

Fig. 1 (A to D) shows that the crude mitochondrial preparation displayed a bi-modal distribution of protein and particulate matter as measured by optical density at 280 nm (A) and succinate dehydrogenase activity (B) across the density gradient of Ficoll at the end of 12 min of centrifugation. However, *p*-chloroamphetamine (C) was found in fractions associated with the peak of particles

sedimented at the less dense region of the gradient. Furthermore, the activities of lactate dehydrogenase and acetylcholinesterase (D) were mostly associated with the latter peak, although the enzyme activity was found more active on the lighter edge of the peak. Considering lactate dehydrogenase and acetylcholinesterase as marker enzymes for synaptosomes and succinate dehydrogenase as a marker for mitochondria, one concludes that the lighter particles were synaptosomes and the heavier particles were mitochondria. *p*-Chloroamphetamine was associated with the synaptosomes but not the mitochondrial fraction.

The association of *p*-chloroamphetamine with synaptosomes to a greater degree than occurred with amphetamine suggests a basis for differences in their pharmacological properties. Both compounds appear to affect noradrenergic and dopaminergic mechanisms (Strada & others, 1970; Costa, Naimzada & Revuelta, 1971), but only *p*-chloroamphetamine causes marked and prolonged depletion of 5-HT. Although there is no reason to think that *p*-chloroamphetamine associates exclusively with synaptosomes from serotoninergic neurons, it may be in such synaptosomes in higher concentrations than amphetamine and thus affect such neurons in a way that amphetamine does not. Carlsson (1970) has shown that *p*-chloromethamphetamine was as potent as any compound he studied in inhibiting the uptake of 5-HT into slices of rat brain. If competition for uptake was the mechanism for that effect, Carlsson's results and ours taken together would be consistent with impairment of 5-HT binding as one mechanism in the depletion of 5-HT by *p*-chloroamphetamine. Other evidence suggests that *p*-chloroamphetamine may inhibit tryptophan hydroxylation (Sanders-Bush & Bushing, 1971); again, the higher concentration of *p*-chloroamphetamine in serotoninergic neurons might account for its activity and the inactivity of amphetamine.

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