J. Pharm. Pharmac., 1969, **21**, 687

LETTERS TO THE EDITOR

The subcellular distribution of (+)-amphetamine and (±)-*p*-chloroamphetamine in the rat brain as influenced by reserpine

Amphetamine and its *p*-chloro derivative, *p*-chloroamphetamine have some common pharmacological actions. Both produce hypermotility in mice (Pfeifer, György & Fodor, 1968); *p*-chloroamphetamine is somewhat less effective than amphetamine. Amphetamine increases the basal metabolism of rats and mice and this effect has a central origin (Issekutz & Gyermek, 1949; Pfeifer, Vizi & others, 1964). *p*-Chloroamphetamine also increases metabolism in rats and mice (Pfeifer, unpublished). Amphetamine hypermotility occurs in the presence of reserpine, while that of *p*-chloroamphetamine is inhibited by reserpine (Pfeifer & others, 1968). Amphetamine slightly decreases brain noradrenaline (McLean & McCartney, 1961; Sanan & Vogt, 1962) and does not influence the brain level of 5-hydroxytryptamine (5-HT) whereas *p*-chloroamphetamine decreases brain 5-HT levels considerably without influencing the noradrenaline content of the brain (Pletscher, Bartholini & others, 1964; Fuller, Hines & Mills, 1965). It is supposed that amphetamine may act by releasing extra-granular catecholamines (Carlsson, Lindqvist & others, 1966) and in large doses it has a direct releasing effect on the amine storing granules (Lundborg, 1969).

The aim of the present work was to see if studies on subcellular distribution of amphetamine and its *p*-chloro-derivative in the brain could further elucidate their mode of action.

C.F.E. albino rats (120–150 g) were given 5 mg/kg of (+)-amphetamine sulphate together with 10 nCi[^{3}H]-(+)-amphetamine or 15 mg/kg of (±)-*p*-chloroamphetamine intraperitoneally 30 or 60 min, respectively before decapitation. Reserpine was administered $4\frac{1}{2}$ h before. Amphetamine was determined by the liquid scintillation method with a four channel Packard instrument after extraction according to Axelrod (1954). *p*-Chloroamphetamine was determined spectrophotometrically (Axelrod, 1954).

The subcellular fractions were prepared (Kataoka & De Robertis, 1967) with a MSE ultracentrifuge. Nuclear (N), crude mitochondrial (M), microsomal (Ms) and soluble (S) fractions were prepared. From the crude mitochondrial fraction three subfractions were isolated after hyposmotic shock: mitochondrial (Mi), vesicular (Ves) and the soluble axoplasm (A). The identification of the fractions was made by electron microscopy. The protein content was determined as N_2 by a Coleman N_2-analyser after precipitation with trichloroacetic acid.

The subcellular distribution of amphetamine and *p*-chloroamphetamine is very different (Fig. 1). While the greatest amount of amphetamine is in the soluble fraction of the primary fractions and in sub-mitochondrial fractions, *p*-chloroamphetamine is bound chiefly to the particulate fraction both in the primary and in the submitochondrial fractions. The *p*-chloroamphetamine content is 1·87 μg/g and the amphetamine content is 0·356 μg/g, in the vesicular fraction, while the physiological noradrenaline content is 0·034 μg/g according to De Robertis (1966). Reserpine does not influence the absolute content of amphetamine in the whole brain or in the subcellular fractions and has no effect on the subcellular distribution either. On the other hand, *p*-chloroamphetamine, given after reserpine, accumulates to a lesser degree in the whole brain, and significantly lower drug levels are found in the

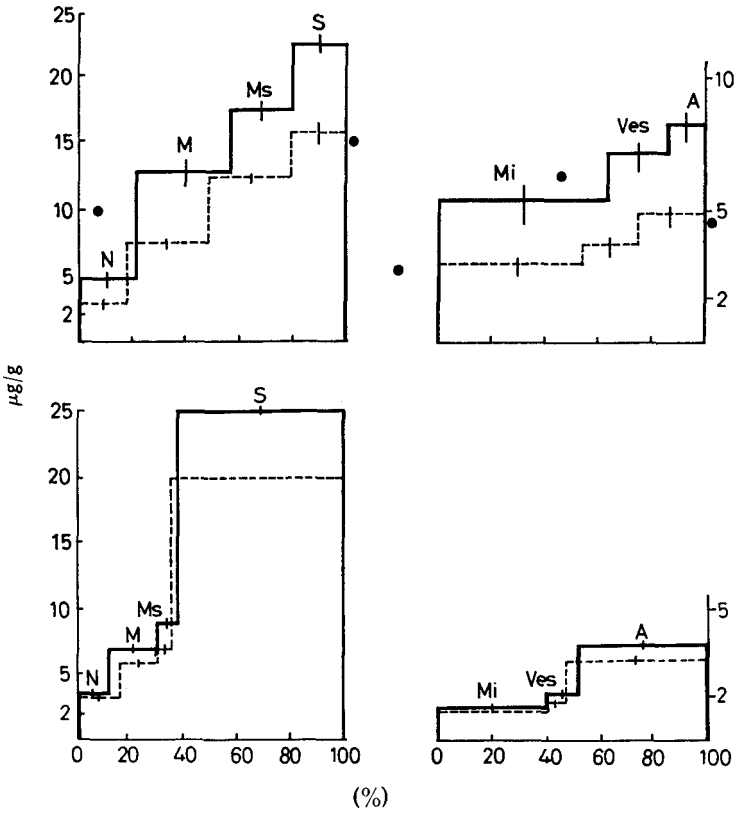

FIG. 1. Diagram showing the concentration in μg/g wet tissue and % distribution of (+)-amphetamine and (±)-*p*-chloroamphetamine in nuclear (N), crude mitochondrial (M), microsomal (Ms) and soluble (S) fractions of the primary fractions and in the submitochondrial fractions: mitochondrial (Mi), vesicular (Ves) and soluble axoplasm (A). Upper: ——— amphetamine - - - - - reserpine + amphetamine. Lower: ——— *p*-chloroamphetamine, - - - - - reserpine + *p*-chloroamphetamine. ● $P < 0.05$.

crude mitochondrial fraction, in the mitochondrial, vesicular and soluble axoplasm fractions after hyposmotic shock. There are also alterations in the percentage distribution (Fig. 1).

Table 1 shows the relative specific concentrations (RSC) of amphetamine and *p*-chloroamphetamine in the presence and absence of reserpine treatment in the primary subfractions and in the submitochondrial fractions after hyposmotic shock. For amphetamine the RSC is low in the particulate fractions and very high in the supernatant both in the primary and in the submitochondrial fractions. Reserpine has no influence on the RSC of amphetamine. On the other hand, *p*-chloroamphetamine has a high RSC in the particulate fractions. Reserpine increases the RSC in the microsomal fraction and in the soluble axoplasm.

The results presented suggest that *p*-chloroamphetamine and to a lesser degree amphetamine can be taken up into the catecholamine or 5-HT storage sites. As the absolute quantity of the drug detected in these fractions is several times more than the amount of the endogenous catecholamine or 5-HT, it may be assumed that the capacity of storage sites is not entirely utilized in normal conditions. This idea is supported by the work of Richards & Tranzer (1969) who demonstrated by electron microscopy that the pineal gland of rat can take up so much 5-hydroxydopamine that practically all the vesicles became electron dense. Furthermore, it is also possible

Table 1. *Subcellular distribution of* (+)*-amphetamine and* (±)-p-*chloroamphetamine in rat brain. Effect of reserpine.* Relative specific concentrations = % of drug in the fraction/% of protein in the fraction

Fractions	Protein %	Relative specific concentrations: Amphetamine Control	Amphetamine After reserpine	*p*-Cl-amphetamine Control	*p*-Cl-amphetamine After reserpine
Nuclear	34·8	0·41	0·48	0·58	0·52
Crude mitochondrial	35·0	0·45	0·39	1·04	0·85
Microsomal	14·6	0·50	0·40	1·50	2·17
Soluble	15·6	4·22	4·09	1·40	1·34
Submitochondrial fractions:					
Mitochondrial	79·6	0·51	0·50	0·79	0·68
Synaptic vesicle	12·6	0·85	0·60	1·83	1·65
Soluble (axoplasm)	7·7	6·31	6·81	1·91	3·27

that the excess catecholamine or 5-HT stores can take up substances without loosing their endogenous content. Perhaps that is why amphetamine depletes noradrenaline only in large (nearly toxic) doses. On the other hand, the 5-HT depleting effect of *p*-chloroamphetamine can be explained by its greater ability to be bound to the vesicular fractions.

Whether or not the difference in subcellular distribution of amphetamine and *p*-chloroamphetamine and the lack of influence of reserpine on the amphetamine and its definite influence on *p*-chloroamphetamine distribution is casual remains to be solved.

Institute for Experimental Medicine of the Hungarian Academy of Sciences, Budapest 9, *P.O.B.* 67, *Hungary.*

A. K. PFEIFER
L. CSÁKI
M. FODOR
L. GYÖRGY
I. ÖKRÖS

May 31, 1969

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