The Effect of D-Amphetamine on the Concentration of Phospholipids in the Rat Hippocampus

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It is generally accepted that the behaviourally stimulant properties of D-amphetamine can be attributed to its ability to release catecholamines from intraneuronal storage sites and block their reuptake [1, 2]. Since lipids constitute an essential part of cellular membranes [3], and there is some evidence that they may be involved in ion transport processes in nerve membranes [4], it seems possible that amphetamine could be producing its effect on amine metabolism by a direct action on membrane phospholipid metabolism. The present investigation was designed to assess this possibility.

The fluorometric microdetermination of Schiefer and Neuhoff [5] was used to determine the concentration of phospholipids in the hippocampus. This brain region was studied because it could be clearly and reproducible identified, rapidly dissected from surrounding brain regions and contained sufficient tissue for multiple determinations to be made.

Groups of 5 adult rats (♂, 180–200 g) were injected with D-amphetamine (10 mg/kg i.p.) or physiological saline (control group) and killed by decapitation 1 hour later. At the time of killing, the amphetamine treated rats were hyperactive; there is evidence from our previous studies that the drug produces its maximal effect on brain amine metabolism after this period of treatment [6]. The brains were rapidly removed, placed on a cold dish, the hippocampi rapidly dissected and frozen on solid carbon dioxide. Small pieces of tissue, weighing approximately 1 mg, were removed by means of a metal punch [7] and the phospholipids extracted by chloroform : methanol (2 : 1). The extract was then submitted to two-dimensional chromatography on silica gel microplates and the phospholipids visualized with iodine vapour. The exact position of phosphatidyl choline, phosphatidyl ethanolamine, sphingomyelin, phosphatidyl serine and phosphatidyl inositides could then be located, removed with a micro spatula and estimated fluorometrically using rhodamine 6 GO. This procedure has be described in detail elsewhere [5, 7]. At least 4 separate estimations of the phospholipid content were made on each hippocampus.

The results are given in Table I. In this investigation it can be seen that, in the hippocampus, D-amphetamine produces a significant decrease in the concentration of phosphatidyl choline and an increase in that of phosphatidyl ethanolamine. The slight rise in the total phospholipid concentration can be partly attributed to the rise in phosphatidyl ethanolamine as the summation of the concentrations of the individual phospholipids as a percentage of the total phospholipid is the same in both groups (54%). The reason for the increase in phosphatidyl ethanolamine must await further investigation but preliminary results show that D-amphetamine significantly reduces (approx. 30%) the concentration of ethanolamine in the hippocampus [8]. It thus seems possible that the turnover of phosphatidyl ethanolamine is increased by D-amphetamine.

Hitzemann and Loh [9] have recently shown that the same dose of D-amphetamine inhibits the synthesis of phosphatidyl choline in the rat brain, probably by inhibiting the cytidine diphosphorylcholine diglyceride transferase activity. These investigators also found that the turnover of phosphatidyl choline was

Table I. Effect of D-amphetamine on the concentration of phospholipids in the rat hippocampus.

Group	Total P. lipid	P'tidyl choline	P'tidyl serine	P'tidyl innositides	P'tidyl ethanolamine
Controls (N=5)	2.93 ± 0.29	0.726 ± 0.064	0.032 ± 0.007	0.032 ± 0.006	0.758 ± 0.23
Amphetamine (N=5)	3.88 ± 0.32	0.468 ± 0.072	0.050 ± 0.008	0.028 ± 0.006	1.52 * ± 0.20

All values, as the Mean ± s.e.m., are expressed as mg phospholipid/g wet weight of tissue. * $P<0.05$.

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reduced in several regions of the rat brain but they did not study the effects of this drug on the metabolism of this phospholipid in the hippocampus.

The concentration of sphingomyelin in the hippocampus is very low in comparison to that of the other phospholipids which were estimated. Reliable and reproducible results for the concentration of this phospholipid in the hippocampus were not obtained.

In conclusion, these and the results of other investigators [9], suggest that the D-amphetamine has an effect on membrane phospholipid metabolism; this could play a contributory role in the effect of this drug release and reuptake of neurotransmitter substance in the brain.

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