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EFFECT OF *d*-AMPHETAMINE ON THE TURNOVER, SYNTHESIS AND METABOLISM OF BRAIN PHOSPHATIDYLCHOLINE*

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Abstract—The effect of acute and chronic *d*-amphetamine treatment on the synthesis, metabolism and turnover of ^{14}C-choline labeled phosphatidylcholine was measured in discrete regions of the rat brain. Chronic *d*-amphetamine treatment was found to inhibit the turnover of ^{14}C-phosphatidylcholine in the cortex, cerebellum, hypothalamus, diencephalon, brain stem and caudate nucleus. Acute *d*-amphetamine treatment was found to inhibit the incorporation of ^{14}C-choline into phosphatidylcholine only in the cortex and cerebellum. Studies *in vitro* suggest that *d*-amphetamine inhibits cortical phosphatidylcholine synthesis at the cytidine diphosphorylcholine diglyceride transferase step. *d*-Amphetamine was not found to alter the base-exchange reaction or phospholipase C activity.

THE POSSIBILITY that indirectly acting sympathomimetic agents such as tyramine or amphetamine could alter brain phospholipid metabolism is suggested by the observation that dopamine or norepinephrine affects the synthesis of these complex lipids, Hokin,[1,2] using a brain tissue slice preparation, has found that norepinephrine increases the incorporation of ^{32}P into phosphatidic acid and in some brain regions phosphatidylinositol, but decreases ^{32}P incorporation into phosphatidylcholine and phosphatidylethanolamine plus phosphatidylserine. Similarly, dopamine decreased ^{32}P incorporation into phosphatidylcholine in slices prepared from the neostriatum. The inhibitory effect of catecholamines on phosphatidylcholine synthesis is of particular interest, as this phospholipid has been postulated to have an important role in certain membrane-related processes which can control neurotransmitter function. Phosphatidylcholine can protect Na^+, K^+-stimulated adenosine triphosphatase and mitochondrial adenosine triphosphatase against inhibition by oligomycin.[3] Treatment of a crude nerve ending preparation with phospholipase C or D, both of which primarily hydrolyze phosphatidylcholine, has been found to inhibit catecholamine release but not affect catecholamine uptake.† Lysophosphatidylcholine has been postulated to have an important role in the release of catecholamines from the adrenal medulla.[4] Finally, since phosphatidylcholine is the major complex lipid component of nerve ending and storage vesicle membranes,[5] any change in the synthesis or metabolism of this substance could be expected to alter nerve ending integrity, structure or function.

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In the present study, the effect of chronic *d*-amphetamine treatment on the turnover, synthesis and metabolism of ^{14}C-choline labeled phosphatidylcholine was investigated in discrete regions of the rat brain.

MATERIALS AND METHODS

Materials. Methyl-^{14}C-choline (specific activity, 54 mCi/m-mole) was obtained from Amersham-Searle Corp. Choline, phosphorylcholine, cytidine diphosphorylcholine and phospholipase C (*Clostridium welchii* 2·5 units/mg solid) were purchased from Sigma Chemical Company. Phosphatidylcholine, lysophosphatidylcholine and sphingomyelin were purchased from Supelco Inc. All other reagents and supplies were obtained locally.

Turnover of ^{14}C-phosphatidylcholine. The effect of chronic *d*-amphetamine administration on the turnover of ^{14}C-choline labeled phosphatidylcholine was examined as follows. Under light ether anesthesia, male Sprague–Dawley rats weighing 150 g (age 60–70 days) were given 5 μCi ^{14}C-choline intracisternally.[6] Five days later, twice daily injections (i.p.) of 1 mg/kg of *d*-amphetamine or saline were begun. The dose of *d*-amphetamine was doubled every 5 days with the experiment terminating 15 days, after the first *d*-amphetamine injection. Animals were sacrificed 12 hr after their last injection on days 10, 15 and 20. The control groups sacrificed on day 5 received no drug treatment. The brains were dissected into the following regions: hypothalamus, caudate nucleus, brain stem, cortex, cerebellum, and diencephalon-midbrain. The dissected tissue was immediately homogenized in a Teflon-glass homogenizer with 9 vol. of 0·32 M sucrose containing 1 m-mole $MgCl_2$ and 1 mM Tris buffer, pH = 6·8. The homogenate was centrifuged for 10 min at 1000 *g* and the pellet obtained was discarded. The supernatant was centrifuged for 20 min at 17,000 *g* and yielded the P_2 pellet containing nerve endings, mitochondria and myelin. The post-mitochondrial supernatant was centrifuged for 1 hr at 105,000 *g* to yield the microsomal pellet and the soluble fraction. All subcellular fractions were frozen at $-10°$ until analysis.

^{14}C-phosphatidylcholine synthesis. Animals were given *d*-amphetamine i.p. 0·5 hr prior to the injection i.c. of 5 μCi ^{14}C-choline. One-half hr later, the animals were sacrificed, brains removed and dissected and immediately homogenized in 10% cold TCA. The homogenate was centrifuged for 10 min at 5000 *g*. The pellet was washed twice with 5% trichloroacetic acid (TCA) containing 1% choline prior to analysis for phospholipids.

^{14}C-phosphatidylcholine synthesis in vitro. Animals were sacrificed, brains removed and the cortex was dissected from the rest of the brain. The cortical tissue was cooled for 2 min in ice-cold modified Krebs–Ringer bicarbonate (KRB) buffer[7] prior to preparing tissue slices (0·26 mm thickness) with a McIlwain tissue chopper. The slices were transferred to 50-ml round-bottom centrifuge tubes containing 10 ml KRB buffer bubbled with 95% O_2:5% CO_2. After 15 min, 1 μCi ^{14}C-choline was added and the incubation continued for 2 hr. The reaction was terminated by rapidly washing the slices with fresh portions of KRB buffer. Slices were then treated with TCA as previously described.

In some experiments, a 10% homogenate of cortical tissue was prepared with 0·32 M sucrose. After centrifugation for 10 min at 1000 *g*, 1 ml of the post-nuclear supernatant was incubated with 9 ml of the KRB buffer and 1 μCi ^{14}C-choline at 37° for 2 hr. The reaction was terminated by the addition of 2 ml of 50% TCA.

Incorporation of ^{14}C-choline into phosphatidylcholine via base-exchange. Cortical brain tissue was homogenized in 9 vol. of 0·32 M sucrose. The homogenate was centrifuged for 10 min at 1000 *g* and the supernatant was used as the enzyme source. One millilitre of enzyme was suspended in 9 ml buffer containing 50 mM Tris, pH 9, and 25 mM $CaCl_2$.[8] One μCi ^{14}C-choline was added and the incubation was continued for 2 hr at 37°. The reaction was terminated by adding 2 ml 50% TCA.

Phospholipid analysis. Fractions were extracted twice with 19 vol. of chloroform–methanol (2:1, v/v).[9] The lipid extract was washed with theoretical upper phase until no radioactivity appeared in the aqueous phase. The lipid extract was evaporated to dryness at 40° and the residue was dissolved in 100 μl chloroform–methanol (2:1). Twenty-five μl was spotted on Silica gel G TLC plates (Merck). The spots were over-spotted with a stock solution of phosphatidylcholine, lipophosphatidylcholine and sphingomyelin. Plates were developed in a solvent system of chloroform–methanol–acetic acid–water (50:25:9:4, v/v).[10] Spots were identified by spraying with a dilute solution of iodine, scraped into scintillation vials containing 1 ml methanol and extracted overnight. Ten ml dioxane phosphor was added and samples were counted in a Beckman LS-100 liquid scintillation counter. A portion of the chloroform–methanol extract was assayed for total lipid phosphorous.[11] Data are given as dis/min per micromole of total lipid phosphorus.

Choline metabolites. In some experiments, the aqueous phase from the phospholipid extraction was analyzed for ^{14}C-choline, ^{14}C-phosphorylcholine, ^{14}C-cytidine diphosphorylcholine and ^{14}C-acetylcholine as described by Gomez *et al.*[12] The sample was evaporated to dryness, resuspended in 100 μl methanol–HCl (20:1, v/v) and 50 μl was spotted with a stock solution containing choline metabolites. The solvent system was *n*-butanol–ethanol–acetic acid–water (8:2:1:3). Spots were visualized with a dilute iodine spray, scraped out and counted as previously described. Turnover data were analyzed by linear regression analysis to obtain the best line fit. When applicable, Student's *t*-test was used to determine levels of significance.

RESULTS

Turnover of ^{14}C-phosphatidylcholine in discrete brain regions. Thin-layer chromatographic analysis of the phospholipid extract demonstrated that more than 95 per cent of the total activity was located in ^{14}C-phosphatidylcholine. The small amount of radioactivity present as ^{14}C-lysophosphatidylcholine or ^{14}C-sphingomyelin prevented the accurate determination of the turnover of these compounds. Also, the amount of labeled ^{14}C-phosphatidylcholine in the soluble fraction was found in less than trace amounts.

Figures 1 and 2 illustrate that the control turnover of ^{14}C-phosphatidylcholine showed considerable variance in rank order between the P_2 and microsomal fractions (see Table 1). In the P_2 fraction, the order of decreasing rate of ^{14}C-phosphatidylcholine turnover was brain stem (14 days), cerebellum (14 days), diencephalon (16 days), hypothalamus (18 days), cortex (21 days) and caudate nucleus (23 days). The order of decreasing rate of ^{14}C-phosphatidylcholine turnover in the microsomal fraction was hypothalamus (6 days), caudate nucleus (7 days), cerebellum (7 days), brain stem (8 days), diencephalon (12 days) and cortex (17 days).

Effect of chronic d*-amphetamine treatment on ^{14}C-phosphatidylcholine turnover.* Beginning 5 days after ^{14}C-choline administration, animals were given *d*-amphetamine

using a progressively increasing dosage schedule. *d*-Amphetamine was given in doses of 1, 2 and 4 mg/kg with the dose being doubled every 5 days. Chronic *d*-amphetamine treatment generally caused a decrease in ^{14}C-phosphatidylcholine turnover in both the P_2 and microsomal fractions (Figs. 1 and 2 and Table 1). In the P_2 fraction, the caudate nucleus, cortex and brain stem showed the greatest inhibition of turnover, while the cerebellum was least affected. *d*-Amphetamine was found less effective in altering ^{14}C-phosphatidylcholine turnover in the microsomal fraction as compared

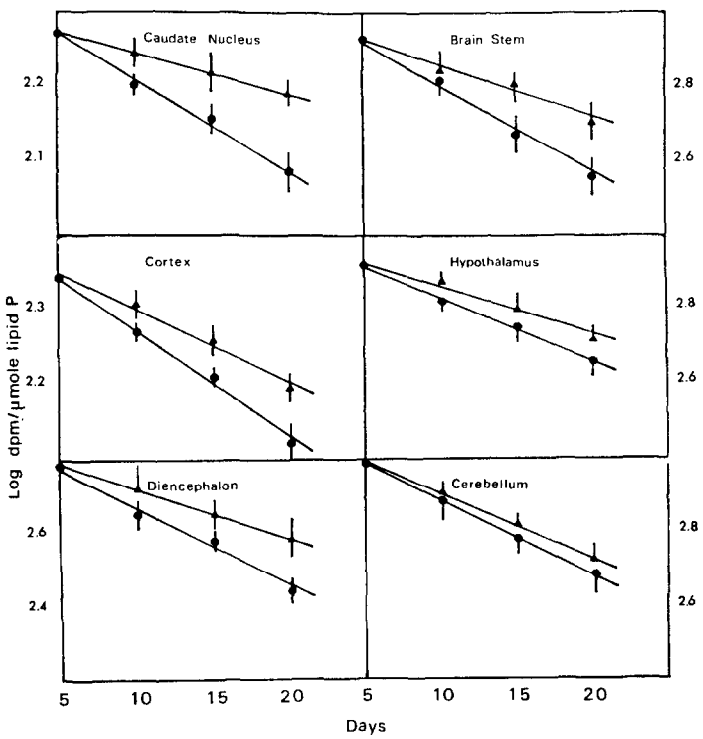

FIG. 1. Turnover of ^{14}C-phosphatidylcholine in the P_2 fraction of discrete regions of the rat brain in normal (●) and chronic amphetamine-treated (▲) animals. Animals were given 5 μCi ^{14}C-choline (spec. act., 54 mCi/m-mole) i.c. Beginning 5 days after ^{14}C-choline administration, animals were given 1 mg/kg of *d*-amphetamine i.p. twice daily. The dose of *d*-amphetamine was doubled 5 days later and doubled once again 10 days later. Control groups were sacrificed on days 5, 10, 15 and 20. Amphetamine-treated groups were sacrificed 12 hr after their last injection on days 10, 15 and 20. Data in Fig. 1 for the P_2 fraction are plotted as the log dis./min/micromole of lipid P ± S.E. vs time. Each point represents the mean of six determinations performed in duplicate.

to the P_2 fraction. In the microsomal fraction, the turnover rates of ^{14}C-phosphatidylcholine in the brain stem and diencephalon were most affected, while the turnover rates in the hypothalamus, caudate nucleus and the cerebellum were only slightly affected by *d*-amphetamine treatment.

Effect of acute d*-amphetamine treatment on* ^{14}*C-phosphatidylcholine synthesis.* Animals were given 1 or 10 mg/kg of *d*-amphetamine i.p. 0·5 hr prior to the injection of 5 μCi ^{14}C-choline i.c. After 0·5 hr, the animals were sacrificed and the levels of ^{14}C-choline and ^{14}C-phosphatidylcholine determined. The data in Table 2 show that

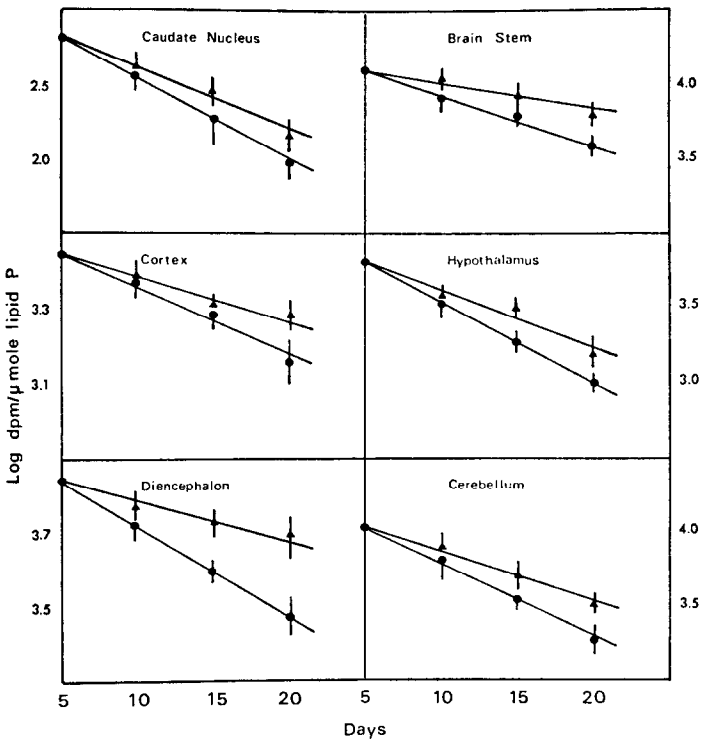

FIG. 2. Turnover of ^{14}C-phosphatidylcholine in the microsomal fraction of discrete regions of the rat brain in normal (●) and chronic amphetamine-treated (▲) animals. Details are described in the legend to Fig. 1.

TABLE 1. EFFECT OF CHRONIC *d*-AMPHETAMINE TREATMENT ON THE TURNOVER OF ^{14}C-PHOSPHATIDYLCHOLINE*

	$T_{\frac{1}{2}}$ (days)			
	P_2		Microsomes	
Brain region	Control	Amphetamine	Control	Amphetamine
Cortex	21	30	17	23
Cerebellum	14	17	7	10
Brain stem	14	29	8	15
Diencephalon	16	25	12	25
Caudate nucleus	23	46	6	8
Hypothalamus	18	24	6	8

* Animals were given 5 μCi ^{14}C-choline i.e. Twice daily injections of *d*-amphetamine were begun 5 days later and continued for 15 days. Injections were begun at 1 mg/kg, i.p., and doubled every 5 days. Animals were sacrificed at various times after beginning amphetamine injection and the levels of ^{14}C-phosphatidylcholine were determined in the P_2 and microsomal pellets prepared from the various brain regions. Half-life values ($T_{\frac{1}{2}}$) were determined by linear regression.

TABLE 2. EFFECT OF ACUTE *d*-AMPHETAMINE TREATMENT ON THE SYNTHESIS OF ^{14}C-PHOSPHATIDYLCHOLINE*

Brain region	Dose of *d*-amphetamine (mg/kg)	^{14}C-choline (dis/min/g ± S.E. × 10^{-5})	^{14}C-phosphatidylcholine (dis/min/μmole lipid P ± S.E. × 10^{-1})
Cortex	0	11·6 ± 3·7	25·8 ± 3·9
	1	12·7 ± 4·2	19·0 ± 1·6
	10	10·8 ± 3·1	15·0 ± 1·8†
Cerebellum	0	104·0 ± 20·2	187·0 ± 28·2
	1	103·0 ± 18·7	109·0 ± 7·3†
	10	91·8 ± 13·4	39·8 ± 1·7†
Hypothalamus	0	8·3 ± 2·2	39·2 ± 3·5
	1	4·5 ± 1·1	35·1 ± 2·4
	10	7·1 ± 1·8	48·6 ± 3·6
Caudate nucleus	0	7·1 ± 1·3	10·3 ± 2·6
	1	7·3 ± 1·4	11·3 ± 3·6
	10	8·4 ± 1·7	8·6 ± 1·4
Brain stem	0	114·0 ± 15·7	35·2 ± 4·1
	1	142·0 ± 14·1	38·0 ± 4·1
	10	85·3 ± 10·2	26·8 ± 6·8
Diencephalon	0	36·3 ± 5·2	6·3 ± 2·4
	1	36·6 ± 9·9	13·9 ± 4·2
	10	27·3 ± 8·2	7·2 ± 1·6

* Animals were given *d*-amphetamine i.p. 0·5 hr prior to the injection of 5 μCi ^{14}C-choline i.c.; 0·5 hr later, the animals were sacrificed and the levels of ^{14}C-choline and ^{14}C-phosphatidylcholine were determined in discrete brain regions. N = six animals per drug dose.

† Significantly different from control, $P < 0·05$.

d-amphetamine significantly inhibited the incorporation of ^{14}C-choline into phosphatidylcholine in the cortex and cerebellum. In other brain regions, notably the hypothalamus and the diencephalon, a slight stimulation of synthesis was observed.

Effect of d-*amphetamine on the incorporation of* ^{14}*C-choline into phosphatidylcholine* in vitro. The effect of *d*-amphetamine on the incorporation of ^{14}C-choline into phosphatidylcholine in cortical tissue slices was investigated. The data in Table 3 demonstrate that 10^{-3} M *d*-amphetamine had no effect on the incorporation of ^{14}C-choline into phosphatidylcholine and 10^{-2} M decreased incorporation 23 per cent ($P < 0·05$). Pretreatment of animals with 10 mg/kg of *d*-amphetamine i.p. 0·5 hr prior to sacrifice resulted in a 39 per cent decrease in ^{14}C-choline incorporation.

Different from the tissue slice experiments, *d*-amphetamine did not alter the formation of ^{14}C-phosphatidylcholine in a cortical brain homogenate. Additions *in vitro* of *d*-amphetamine (10^{-4}–10^{-2} M) or pretreatment with 10 mg/kg of drug i.p. had no apparent effect on the incorporation of ^{14}C-choline into phosphatidylcholine (Table 4).

Effect of d-*amphetamine on* ^{14}*C-choline metabolism.* The TCA-soluble radioactivity (Table 3) from the tissue slice experiments was analyzed for ^{14}C-choline metabolites. At 10^{-2} M *d*-amphetamine, the levels of ^{14}C-phosphorylcholine were unchanged but the levels of ^{14}C-cytidine diphosphorylcholine were more than doubled. In animals pretreated with 10 mg/kg of *d*-amphetamine, a small decrease in ^{14}C-phosphorylcholine was observed but a significant increase in ^{14}C-cytidine diphosphorylcholine levels was also found.

TABLE 3. EFFECT OF *d*-AMPHETAMINE ON THE INCORPORATION OF ^{14}C-CHOLINE INTO PHOSPHATIDYLCHOLINE IN CORTICAL TISSUE SLICES

Group	^{14}C-phosphatidylcholine (dis/min/μmole lipid P $\pm$ S.E.)	^{14}C-Ch (dis/min/g $\pm$ S.E. $\times 10^{-5}$)	^{14}C-PCh (dis/min/g $\pm$ S.E. $\times 10^{-5}$)	^{14}C-CDPCh (dis/min/g $\pm$ S.E. $\times 10^{-5}$)
Control	6100 $\pm$ 470	23·79 $\pm$ 1·82	2·23 $\pm$ 0·15	0·97 $\pm$ 0·07
Amphetamine (10^{-3} M)	6350 $\pm$ 520	22·57 $\pm$ 2·13	2·17 $\pm$ 0·20	1·05 $\pm$ 0·10
Amphetamine (10^{-2} M)	4440 $\pm$ 330†	20·61 $\pm$ 2·24	2·28 $\pm$ 0·38	1·93 $\pm$ 0·14†
Amphetamine (10 mg/kg, i.p.)	3930 $\pm$ 410†	23·10 $\pm$ 2·89	1·95 $\pm$ 0·24	3·49 $\pm$ 0·26†

* Cortical tissue slices (0·26 mm thickness) were incubated in a modified Krebs–Ringer buffer in the presence of 3 μCi ^{14}C-choline (spec. act. 54 mCi/m-mole) for 2 hr. *d*-Amphetamine was added to the incubation mixture or animals were pretreated with the drug 0·5 hr prior to sacrifice. After incubation, the levels of ^{14}C-phosphatidylcholine, ^{14}C-choline (Ch), ^{14}C-phosphorylcholine (PCh) and ^{14}C-cytidine diphosphorylcholine (CDPCh) were determined. ^{14}C-phosphatidylcholine data are expressed as dis/min/μmole of lipid P. The levels of ^{14}C-Ch, ^{14}C-PCh and ^{14}C-CDPCh are given as dis/min/g tissue wet weight. N = five to six determinations per group.

† Significantly different from control, $P < 0·05$.

TABLE 4. EFFECT OF *d*-AMPHETAMINE ON THE INCORPORATION OF ^{14}C-CHOLINE INTO PHOSPHATIDYLCHOLINE IN A CORTICAL BRAIN HOMOGENATE*

Group	^{14}C-phosphatidylcholine (dis/min/μmole lipid P)
Control	4160
Amphetamine (10^{-2} M)	3940
Amphetamine (10^{-3} M)	4060
Amphetamine (10^{-4} M)	4250
Amphetamine (10 mg/kg, i.p.)	3980

* A 10% cortical homogenate was prepared in 0·32 M sucrose and centrifuged at 1000 *g* for 10 min. One ml of the supernatant was suspended in 9 ml KRB buffer and 1 μCi ^{14}C-choline was added. The incubation was performed for 2 hr in the presence of added *d*-amphetamine. In some experiments animals were pretreated with 10 mg/kg of *d*-amphetamine 0·5 hr prior to sacrifice. The amount of ^{14}C-phosphatidylcholine formed was determined and the data are expressed as mean dis/min/μmole of lipid P. N = three experiments per group.

Effect of d-*amphetamine on base-exchange nonenzymatic incorporation.* Several workers have demonstrated that serine, ethanolamine and choline may be incorporated into phospholipids via a Ca^{2+}-stimulated base-exchange reaction which has an alkaline pH optimum. The data in Table 5 demonstrate that *d*-amphetamine in concentrations from 10^{-2} to 10^{-4} did not alter the rate of base-exchange. The experimental conditions used tend to minimize the cytidine dependent pathway of biosynthesis.

Effect of d-*amphetamine on phospholipase C activity.* A portion of the cortical post 1000 *g* supernatant from animals pretreated 1 day with ^{14}C-choline was incubated in

TABLE 5. EFFECT OF *d*-AMPHETAMINE ON THE INCORPORATION OF ^{14}C-CHOLINE INTO PHOSPHATIDYLCHOLINE VIA BASE-EXCHANGE*

Group	^{14}C-phosphatidylcholine (dis/min/μmole lipid P)
Control	1200
Amphetamine (10^{-2} M)	1110
Amphetamine (10^{-3} M)	1350

* A 10% cortical homogenate was prepared in 0·32 M sucrose and centrifuged at 1000 *g* for 10 min. A portion of the supernatant was suspended in 50 mM Tris buffer pH 9, containing 25 mM $CaCl_2$ and 1 μCi ^{14}C-choline (spec. act., 54 mCi/m-mole). The incubation was performed for 2 hr in the presence of added *d*-amphetamine. The amount of ^{14}C-phosphatidylcholine formed was determined and the data are expressed as mean dis/min/μmole of lipid P. N = three experiments.

TABLE 6. EFFECT OF *d*-AMPHETAMINE ON PHOSPHOLIPASE C ACTIVITY*

Group	^{14}C-phosphatidylcholine (cpm/μmole lipid P)
Control	2630
Phospholipase C (20 μg/ml)	740
(50 μg/ml)	570
Phospholipase C (20 μg/ml) + *d*-amphetamine (10^{-3} M)	800
(10^{-2} M)	765
(10 mg/kg, i.p.)	690

* Animals were given 5 μCi ^{14}C-choline i.c. One day later, the animals were sacrificed, a 10% cortical homogenate was made, and the homogenate was centrifuged 10 min at 1000 *g*. One ml of the supernatant was incubated with 9 ml KRB buffer and various additions of phospholipase C (*Cl. welchii*) and *d*-amphetamine. In some experiments, animals were treated with 10 mg/kg of *d*-amphetamine 30 min prior to sacrifice. The incubation was terminated after 30 min by adding 2 ml of 50% TCA. After suitable washing procedures, the level of ^{14}C-phosphotidylcholine in the TCA precipitate was determined. N = three experiments per group.

KRB buffer in the presence of 20 μg/ml of phospholipase C. This concentration of enzyme produced a 75 per cent reduction in ^{14}C-phosphatidylcholine levels (Table 6). *d*-Amphetamine in concentrations from 10^{-4} to 10^{-2} M and pretreatment with 10 mg/kg 0·5 hr prior to sacrifice had no effect on phospholipase C activity.

DISCUSSION

In the present experiments, the effect of various treatments with *d*-amphetamine on the synthesis, turnover and metabolism of ^{14}C-choline labeled phosphatidylcholine was investigated in discrete regions of the rat brain. The data demonstrate that

(1) chronic *d*-amphetamine treatment inhibits ^{14}C-phosphatidylcholine turnover in most brain regions and subcellular fractions, (2) acute *d*-amphetamine treatment inhibits the incorporation of ^{14}C-choline into phosphatidylcholine in the cortex and cerebellum *in vivo*, (3) *d*-amphetamine inhibits the incorporation of ^{14}C-choline into phosphatidylcholine in cortical slices and *d*-amphetamine does not appear to alter the metabolism of phosphatidylcholine. Possible mechanisms underlying these effects of *d*-amphetamine will be examined in some detail.

The turnover of brain phospholipids is a complex process involving, at least, synthesis, metabolism, substrate availability, utilization and the metabolic activity of the structure(s) which contains the phospholipid. The experimental design of the present study is such that only the turnover of those pools of phosphatidylcholine with a $T_{\frac{1}{2}}$ value in the range 4–28 days would be accurately measured. Burton[13] has demonstrated that phospholipids labeled in the fatty acid moieties will show varying turnover rates depending on the structure in which they are contained. Phospholipids associated with the myelin sheath exhibited $T_{\frac{1}{2}}$ values in the range of 50–100 days, while those associated with cellular membranes exhibited turnover rates in the range of 7–28 days. Extrapolating this information to our study, it would appear that a single pulse label of ^{14}C-choline primarily labels cellular membranes. The observation that little ^{14}C-sphingomyelin could be detected confirms the relatively slow turnover and, consequently, low incorporative ability of myelin in the adult rat.

The present finding that chronic *d*-amphetamine treatment inhibited ^{14}C-choline labeled phosphatidylcholine turnover in most brain regions and in the P_2 and microsomal fractions suggests a common mechanism of inhibition. The possibility that *d*-amphetamine could directly inhibit the incorporation of ^{14}C-choline into phosphatidylcholine was investigated. Data on the effect of acute *d*-amphetamine treatment on the incorporation of ^{14}C-choline into phosphatidylcholine *in vivo* show that *d*-amphetamine significantly inhibited incorporation, but only in the cortex and cerebellum. Some brain regions such as the hypothalamus and diencephalon showed a slightly increased incorporation. Interestingly, the large decrease in ^{14}C-choline incorporation into phosphatidylcholine in the cerebellum after acute *d*-amphetamine treatment was not complemented by a large decrease in turnover in this brain region. These data suggest either that tolerance rapidly develops to the effects of *d*-amphetamine in the cerebellum or the drug affects phosphatidylcholine metabolism in this brain region or that the inhibition is an artifact of the experimental procedures employed. The magnitude of the *d*-amphetamine-induced decrease on the initial synthesis of ^{14}C-phosphatidylcholine in the cortex is similar to the decrease in ^{14}C-phosphatidylcholine turnover and suggests that the decrease in turnover may result from an accumulated inhibitory effect on synthesis. However, different mechanisms are probably involved in the other brain regions. For example, the decreased turnover of ^{14}C-phosphatidylcholine in noncortical regions may result secondarily from the fact that *d*-amphetamine inhibits brain protein synthesis.[14]

The mechanism by which acute *d*-amphetamine treatment inhibits ^{14}C-choline incorporation into phosphatidylcholine in the cortex was further investigated. The addition of high concentrations of *d*-amphetamine (10^{-2} M) or pretreatment with 10 mg/kg of drug inhibited the synthesis of ^{14}C-phosphatidylcholine in cortical slices. Examination of the formation of ^{14}C-choline metabolites in these experiments indicated that *d*-amphetamine caused ^{14}C-cytidine diphosphorylcholine to accumulate in

the tissue, suggesting that the drug interferes with the formation of phosphatidylcholine at the level of cytidine diphosphorylcholine diglyceride transferase. However, *d*-amphetamine was not found to alter the formation of ^{14}C-phosphatidylcholine in a cortical homogenate. These data indicate that the mechanism by which *d*-amphetamine alters phosphatidylcholine formation at the level of the cytidine diphosphorylcholine diglyceride transferase or perhaps other biosynthetic steps is dependent on an intact tissue preparation.

The mechanism by which *d*-amphetamine inhibits the turnover of ^{14}C-phosphatidylcholine in the cortex and other brain regions may involve not only a drug effect on synthesis but also an effect on metabolism. The non enzymatic base-exchange reaction may be considered both a metabolic and a biosynthetic pathway. Base-exchange has been shown to be an important mechanism for the turnover of the base components of phosphatidylcholine, phosphatidylethanolamine and phosphatidylserine in brain homogenate and nerve ending preparation.[8,15] In the present study, *d*-amphetamine was not found to alter the incorporation of ^{14}C-choline into phosphatidylcholine under experimental conditions which maximize the base-exchange reaction.

In addition to base-exchange, choline can be removed from phosphatidylcholine by the activity of phospholipase C. To examine the effect of *d*-amphetamine on this enzyme, animals were pretreated with ^{14}C-choline, and the effect of a purified phospholipase C preparation on the content of cortical ^{14}C-phosphatidycholine was measured. Under these conditions, *d*-amphetamine was not found to alter enzyme activity. However, since the drug effect on the brain enzyme was not measured, the possibility cannot be exluded that *d*-amphetamine has some action on brain phospholipase C activity.

The present study clearly demonstrates that *d*-amphetamine in small doses inhibits the synthesis and turnover of phosphotidylcholine, a major component of brain membranes. One important aspect of these findings may be related to the adverse psychological effects[16] caused by chronic *d*-amphetamine treatment. Temporary changes in phosphotidylcholine function may alter the synthesis, metabolism and utilization of critical brain substances such as neurotransmitters, which are intimately involved in behavioural patterns.

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