DIFFERENTIAL EFFECTS OF MORPHINE SULPHATE ON PHOSPHOLIPID SPECIES IN THE SYNAPTOSOMES OF THE RAT BRAIN

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SUMMARY: Studies were carried out to explore the effect of acute and chronic administration of morphine sulphate and drug withdrawal on the concentrations of synaptosomal phospholipid species [phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylinositol (PI) and phosphatidylserine (PS)] in brain areas of adult male albino rats.

It was found that acute administration of morphine (35 mg/kg body weight) decreased concentrations of synaptosomal PC and PE in cerebellum and increased their levels in striatum. Conversely, PS and PI showed significant increases in cerebellum and significant decreases in striatum.

However, chronic administration of increasing doses (15-75 mg/kg body weight) of the drug decreased the concentrations of synaptosomal PE, PS and PI of striatum and PC in cortex.

Two days after morphine withdrawal the synaptosomal PC, PE, PS and PI showed significant increases in striatum and thalamus-hypothalamus. Conversely, PC and PE concentrations were decreased significantly in synaptosomes of cerebellum after two days of drug withdrawal. Also, after four days of morphine withdrawal, the synaptosomal PC and PE were diminished in cerebellum and increased in cortex.

Overall, the present results suggest that the mechanisms of opioid action are closely associated with changes in the turnover of the brain phospholipid species.

Key Words: Phospholipids, morphine sulphate, drug withdrawal.

INTRODUCTION

Phospholipids play an integral part in the structure of neurons and may be directly involved in the function of membrane receptors (13).

There is a considerable controversy over the relationship of opiate addiction and tolerance, to alteration in the number of opiate receptors. Changes in the number of opiate receptors during the development of tolerance-dependence have been reported (6,36). Recent evidence suggests that phospholipids turnover change during acute drug treatment and also during sensitization and tolerance. Acute and chronic morphine treatment may influence the calcium-dependent exchange reaction, causing a change

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in the turnover and/or composition of membrane phospholipids (13). Chronic morphine treatment especially increases calcium stimulated (^{14}C) serine incorporation into phospholipids. Under identical condition, the incorporation of (^{14}C) ethanolamine is only slightly increased and the incorporation of (^{14}C) choline is significantly decreased.

Also, Natsuki (27) indicated that the morphine dependent mice had significantly less ^{14}C-choline incorporation into phosphatidylcholine, which was further decreased by naloxone.

The present study was designed to provide more information on the effect of morphine treatment on the turnover of phospholipids in synaptosomes. Synaptosomes are prepared from different brain regions of adult male albino rats.

MATERIALS AND METHODS

Drug

The drug used during the experiments of this study was morphine sulphate, which was obtained from Miser Company for pharmaceutical drugs and dissolved in 0.9% NaCI solution. The drug was injected intraperitoneally (i.p.).

Experimental animals

Adult male albino rats (*Rattus norvegicus*), weighing from 120-170 g were used throughout the experiments. Animals were put on standard diet and water was supplied *ad libitum*. Animals were divided into six main groups each of 5 rats, which were acute, chronic and withdrawal groups. In acute group, animals received acute dose of morphine sulphate (35 mg/kg body weight). Treated rats were sacrificed by decapitation 2 hours after injection. In chronic group, animals received two injections of morphine sulphate per day commencing with a dose of 15 mg/kg each injection on the first day and increasing each injection by 15 mg/kg/day until a dose of 75 mg/kg each injection was attained on fifth day. This dose was maintained for one additional day such that each rat received a total of 12 injections. Group of these treated animals was sacrificed by decapitation, 2 hours after the last maintenance dose of morphine (chronic group). While, the other four groups (withdrawal groups) were treated in the same way as the chronic group. After drug administration was terminated, animals of the four groups were then sacrificed at time intervals of 12, 24, 48 and 96 hours after the last chronic dose.

Control animals were injected with equivalent volume of 0.9% NaCl (saline). Rats of this group were sacrificed by decapitation 2 hours after injection. Immediately, after sacrifice, brains of all rats were excised from the skulls and placed on iced-glass for dissection into specific regions namely, cortex, cerebellum, striatum and thalamus-hypothalamus. All brain regions were weighed and kept in the deep freezer at -20°C until they were needed for analysis.

Preparation of a synaptosome-rich fraction

Synaptosome-rich fractions were prepared by standard centrifugation technique. Briefly, each brain area was homogenized in 10 volumes of 0.32-M sucrose with potter-elvehjier glass homogenizer and was centrifuged at 1.000 g for 10 min. The supernatant fraction was collected and centrifuged at 20.000 g for 20 min. This pellet contained the crude mitochondrial fraction (P_2 fraction). The P_2 fraction was subjected to osmotic shock by 10 volumes of double-distilled deionized water. The suspension was centrifuged at 12.000 g for 20 min. The resulting pellet was the synaptosome-rich fraction (27). Lipids were extracted from the resulting pellets according to the method of Folch *et al.* The pellet was homogenized in 19 volume chloroform: methanol (2:1) mixture. The organic layer was separated by centrifugation and washed three times with saline solution. This organic layer was re-dissolved in 1 ml chloroform methanol (2:1) and was used in separation of phospholipids species (phosphatidylcholine, phosphatidylinositol, phosphatidylserine, and phosphatidylethanolamine) by thin-layer chromatography (TLC).

Phospholipid species separation

The procedures for separation of phospholipids species by TLC were similar to that described by Helmy and Hack (12) using precoated TLC-plates Sil G-25 purchased from Macherey-Nagel GmbH and Co. KG. Postfach 101352 D-52313 Düren and solvent system (cyclohexane:isopropanol:water) (30:40:6). After solvent development, the TLC plates were exposed to iodine vapor for visualization of lipid spots. The flying spot Schimadzu 9000 scanner was used to estimate the concentration of migrated spots at 510 nm. The migration of PC, PE, PI and PS was verified by comparison with their standards purchased from the sigma chemical Co. Due to technical difficulties in consistently separating PS from PI by the thin-layer chromatography (TLC) techniques employed,

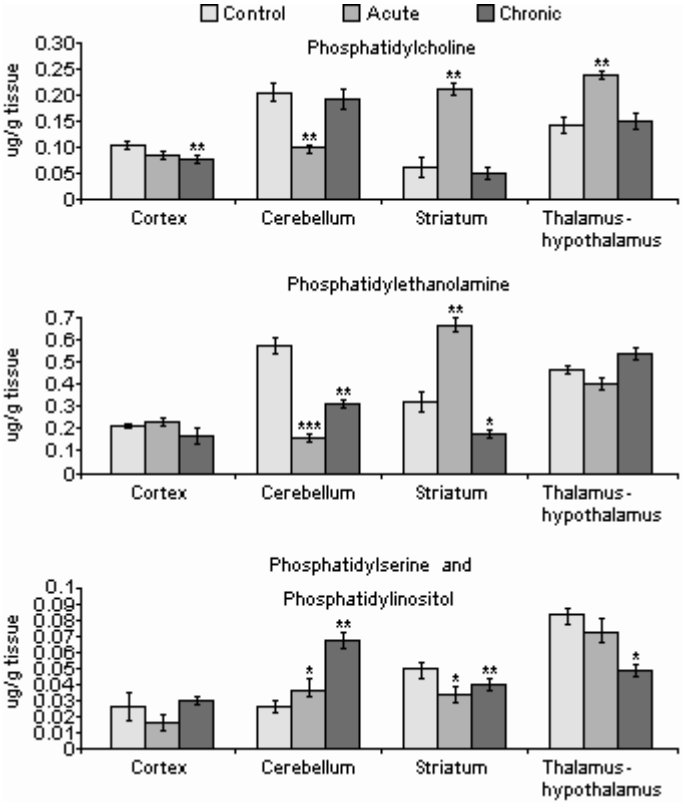
Table 1: Effect of acute and chronic administration of morphine sulphate on the levels of Phosphatidylcholine, Phosphatidylethanolamine, and Phosphatidylserine and Phosphatidylinositol in synaptosomal fraction of different brain areas of adult male albino rats.

Parameters	Brain areas	Animal groups	Mean ±S.E. as absolute values	Mean ±S.E. as % of control	% of control
Phosphatidylcholine	Cortex	Control	0.102 ± 0.001	100 ± 1.28	-
		Acute	0.081 ± 0.008	79.62 ± 8.08	79.62
		Chronic	0.076 ± 0.006	74.48 ± 5.99	74.48*
	Cerebellum	Control	0.203 ± 0.018	100 ± 9.13	-
		Acute	0.099 ± 0.004	48.57 ± 2.02	48.57**
		Chronic	0.194 ± 0.014	95.66 ± 6.81	95.66
	Striatum	Control	0.067 ± 0.015	100 ± 22.36	-
		Acute	0.213 ± 0.014	318.80 ± 20.38	318.80**
		Chronic	0.052 ± 0.017	77.64 ± 24.87	77.64
	Thalamus-hypothalamus	Control	0.140 ± 0.008	100 ± 6.25	-
		Acute	0.244 ± 0.006	174.27 ± 17.59	174.27**
		Chronic	0.158 ± 0.011	112.91 ± 8.35	112.91
Phosphatidylethanolamine	Cortex	Control	0.212 ± 0.010	100 ± 5.02	-
		Acute	0.230 ± 0.014	108.53 ± 6.91	108.53**
		Chronic	0.180 ± 0.027	84.91 ± 12.93	84.91
	Cerebellum	Control	0.560 ± 0.029	100 ± 5.22	-
		Acute	0.166 ± 0.012	29.75 ± 2.22	29.75**
		Chronic	0.307 ± 0.015	54.98 ± 2.79	54.98
	Striatum	Control	0.319 ± 0.037	100 ± 11.87	-
		Acute	0.636 ± 0.028	199.54 ± 8.83	199.54**
		Chronic	0.183 ± 0.022	57.62 ± 6.85	57.62
	Thalamus-hypothalamus	Control	0.451 ± 0.012	100 ± 2.74	-
		Acute	0.394 ± 0.036	87.50 ± 8.09	87.50**
		Chronic	0.515 ± 0.037	114.52 ± 8.32	114.52
Phosphatidylserine and phosphatidylinositol	Cortex	Control	0.027 ± 0.011	100 ± 42.58	-
		Acute	0.032 ± 0.004	58.74 ± 5.47	58.74**
		Chronic	0.016 ± 0.001	115.52 ± 15.97	115.52
	Cerebellum	Control	0.030 ± 0.002	100 ± 6.00	-
		Acute	0.071 ± 0.007	128.33 ± 6.10	128.33**
		Chronic	0.038 ± 0.001	235.77 ± 25.28	235.77
	Striatum	Control	0.053 ± 0.006	100 ± 11.85	-
		Acute	0.042 ± 0.004	70.60 ± 13.00	70.60**
		Chronic	0.037 ± 0.007	80.55 ± 6.82	80.55
	Thalamus-hypothalamus	Control	0.089 ± 0.005	100 ± 5.69	-
		Acute	0.054 ± 0.008	89.32 ± 5.34	89.32**
		Chronic	0.079 ± 0.004	61.14 ± 9.59	61.14

Absolute values measured by ug/g tissue.
Number of animals in each group=5
Non-significant (p>0.05)

* Significant (p<0.05),
** Highly significant (p<0.01),
*** Very highly significant (p<0.001)

Figure 1: Effect of acute and chronic administration of morphine sulphate on the levels of Phosphatidylcholine, Phosphatidylethanolamine, and Phosphatidylinositol and Phosphatidylserine in synaptosomal tissue of different brain areas of adult male albino rats. Each bar represents mean of absolute value ± SEM of five rats per group. Statistically significant from control * p<0.05, ** p<0.01, *** p<0.001.



the PS and PI data have been combined in all results.

Data were statistically analyzed by student's t-test to determine the statistical significance of difference between experimental and control groups. A one way analysis of variance followed by Dunnett's multiply comparison test was used to determine F values. Significant level was taken as $p \leq 0.05$.

RESULTS

Phosphatidylcholine (PC)

Examination of PC level after acute administration of morphine revealed the occurrence of highly significant increases in synaptosomal fraction of striatum and thalamus-hypothalamus ($F = 5.168$, $p < 0.01$ and $F = 4.447$, $p < 0.01$ respectively). On the other hand, the synaptosomal fraction of cortical and cerebral tissues showed different decreases ($F = 2.391$, $p > 0.05$ and $F = 6.711$, $p < 0.01$ respectively) in the levels of PC after acute administration of the drug (Figure 1 and Table 1).

However, the present results recorded that synaptosomal PC showed non-significant changes in most brain areas (cerebellum, striatum and thalamus-hypothalamus) ($F = 0.583$, $p > 0.05$; $F = 0.529$, $p > 0.05$ and $F = 0.773$, $p > 0.05$ respectively) after chronic administration of the morphine sulphate. In contrast, significant decrease ($F = 2.98$, $p < 0.01$) was recorded in synaptosomal fraction of cortical tissue (Figure 1 and Table 1).

The analysis of PC in synaptosomal fraction of different brain areas after morphine sulphate withdrawal,

Table 2: Effect of withdrawal of morphine sulphate on Phosp- hatidylcholine levels in synaptosomal tissue of different brain areas of adult male albino rats.

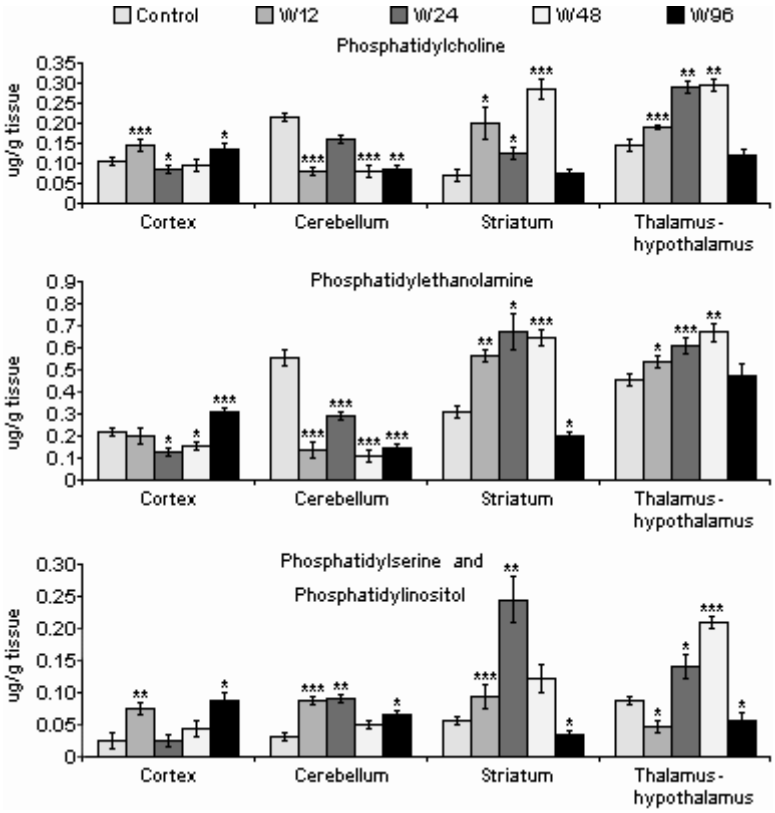
Parameters	Brain areas	Hours	Animal groups	Mean ±S.E. as absolute values	Mean ±S.E. as % of control	% of control
Phosphatidylcholine	Cortex	12	Control	0.102 ± 0.001	100.34 ± 1.29	-
			Treated	0.145 ± 0.00	142.56 ± 0.61	142.56***
		24	Control	0.102 ± 0.001	100.34 ± 1.29	-
			Treated	0.084 ± 0.006	82.60 ± 5.90	82.60*
		48	Control	0.102 ± 0.001	100.34 ± 1.29	-
			Treated	0.096 ± 0.003	94.63 ± 3.22	94.63
		96	Control	0.102 ± 0.001	100.34 ± 1.29	-
			Treated	0.137 ± 0.011	134.55 ± 10.93	134.55*
	Cerebellum	12	Control	0.204 ± 0.018	100 ± 9.13	-
			Treated	0.073 ± 0.008	35.66 ± 3.94	35.66***
		24	Control	0.204 ± 0.018	100 ± 9.13	-
			Treated	0.153 ± 0.007	74.97 ± 3.42	74.97
		48	Control	0.204 ± 0.018	100 ± 9.13	-
			Treated	0.077 ± 0.013	37.73 ± 6.53	37.73***
		96	Control	0.204 ± 0.018	100 ± 9.13	-
			Treated	0.082 ± 0.004	40.10 ± 1.95	40.10**
	Striatum	12	Control	0.067 ± 0.015	100.06 ± 22.36	-
			Treated	0.196 ± 0.039	293.03 ± 58.92	293.03*
		24	Control	0.067 ± 0.015	100.06 ± 22.36	-
			Treated	0.126 ± 0.008	188.62 ± 12.81	188.62*
		48	Control	0.067 ± 0.015	100.06 ± 22.36	-
			Treated	0.283 ± 0.022	424.88 ± 32.91	424.88***
		96	Control	0.067 ± 0.015	100.06 ± 22.36	-
			Treated	0.075 ± 0.003	112.12 ± 4.26	112.12
	Thalamus-hypothalamus	12	Control	0.140 ± 0.009	100 ± 6.25	-
			Treated	0.176 ± 0.010	125.43 ± 8.35	125.43***
		24	Control	0.140 ± 0.009	100 ± 6.25	-
			Treated	0.282 ± 0.018	200.80 ± 13.34	200.80**
		48	Control	0.140 ± 0.009	100 ± 6.25	-
			Treated	0.287 ± 0.017	204.88 ± 12.34	204.88**
		96	Control	0.140 ± 0.009	100 ± 6.25	-
			Treated	0.112 ± 0.019	79.96 ± 13.30	79.96

Absolute values measured by ug/g tissue.
Number of animals in each group=5
Non-significant (p>0.05)

* Significant (p<0.05)
** Highly significant (p<0.01)
*** Very highly significant (p<0.001)

showed that PC exhibited a significant increase (F = 4.805, p < 0.001 and F = 3.889, p < 0.05 respectively) in its content after 12 and 96 hours of drug withdrawal in the cortex. Conversely PC content showed highly significant decreases after 12, 48 and 96 hours of drug withdrawal in the synaptosomal fraction of cerebral tissue (F = 8.404, p

Figure 2: Effect of morphine sulphate withdrawal on the levels of Phosphatidylcholine, Phosphatidylethanolamine, and Phospha- tidylserine and Phosphatidylinositol in synaptosomal tissue of different brain areas of adult male albino rats. Each bar represents mean of absolute value ± SEM of five rats per group. Statistically significant from control * p<0.05, ** p<0.01, *** p<0.001.



< 0.001, F = 8.122, p < 0.001 and F = 7.813, p < 0.01 respectively). On the other hand, significant increases were recorded in the synaptosomal fraction of striatal (F = 4.55, p < 0.005, F = 2.092, p < 0.05 and F = 7.675, p < 0.001), thalamic and hypothalamic (F = 1.522, p < 0.001, F = 6.035, p < 0.01 and F = 6.279, p < 0.01) tissues after 12, 24 and 48 hours of drug withdrawal (Figure 2 and Table 2).

Phosphatidylethanolamine (PE)

The study of the effect of acute administration of morphine on the synaptosomal PE revealed that its level was significantly decreased (F = 15.55, p < 0.001) in cerebral tissue and significantly increased (F = 5.279, p < 0.01) in striatal tissue. In contrast, in synaptosomal fractions of cortical and thalamic-hypothalamic tissues, the PE levels showed non-significant changes (F = 0.810, p > 0.05 and F = 1.15, p > 0.05 respectively) (Figure 1 and Table 1).

The analysis of PE content after chronic administration of morphine sulphate showed significant decreases in its content in synaptosomal fraction of cerebellum and striatum (F = 9.963, p < 0.01 and F = 2.248, p < 0.05 respectively). However, non-significant changes were observed in synaptosomal fraction of cortical and thalamic-hypothalamic tissues (F = 1.432, p > 0.05 and F = 1.336, p > 0.05 respectively) (Figure 1 and Table 1).

The changes observed in PE level after morphine withdrawal revealed the occurrence of significant increases in synaptosomal fraction of striatum and thalamus-hypothalamus during almost the four periods of drug withdrawal,

Table 3: Effect of withdrawal of morphine sulphate on Phosp- hatidylethanolamine levels in synaptosomal tissue of different brain areas of adult male albino rats.

Parameters	Brain areas	Hours	Animal groups	Mean ±S.E. as absolute values	Mean ±S.E. as % of control	% of control
Phosphatidylethanolamine	Cortex	12	Control	0.213 ± 0.011	100 ± 5.02	-
			Treated	0.205 ± 0.014	96.75 ± 6.41	96.75
		24	Control	0.213 ± 0.011	100 ± 5.02	-
			Treated	0.126 ± 0.017	59.12 ± 7.94	59.12*
		48	Control	0.213 ± 0.011	100 ± 5.02	-
			Treated	0.154 ± 0.011	72.57 ± 5.27	72.57*
		96	Control	0.213 ± 0.011	100 ± 5.02	-
			Treated	0.304 ± 0.009	142.72 ± 4.03	142.72***
	Cerebellum	12	Control	0.560 ± 0.029	100 ± 5.22	-
			Treated	0.146 ± 0.008	26.06 ± 1.53	26.06**
		24	Control	0.560 ± 0.029	100 ± 5.22	-
			Treated	0.167 ± 0.015	29.81 ± 2.60	29.81***
		48	Control	0.560 ± 0.029	100 ± 5.22	-
			Treated	0.120 ± 0.020	21.49 ± 3.65	21.49***
		96	Control	0.560 ± 0.029	100 ± 5.22	-
			Treated	0.155 ± 0.017	27.76 ± 2.99	27.76***
	Striatum	12	Control	0.319 ± 0.037	100 ± 11.87	-
			Treated	0.583 ± 0.016	182.54 ± 5.14	182.54**
		24	Control	0.319 ± 0.037	100 ± 11.87	-
			Treated	0.692 ± 0.084	216.85 ± 26.49	216.85*
		48	Control	0.319 ± 0.037	100 ± 11.87	-
			Treated	0.656 ± 0.049	205.61 ± 15.34	205.61***
		96	Control	0.319 ± 0.037	100 ± 11.87	-
			Treated	0.206 ± 0.012	64.54 ± 3.86	64.54*
	Thalamus-hypothalamus	12	Control	0.450 ± 0.012	100 ± 2.75	-
			Treated	0.537 ± 0.025	119.28 ± 5.58	119.28*
		24	Control	0.450 ± 0.012	100 ± 2.75	-
			Treated	0.616 ± 0.028	136.63 ± 6.24	136.63***
		48	Control	0.450 ± 0.012	100 ± 2.75	-
			Treated	0.700 ± 0.023	155.44 ± 5.23	155.44**
		96	Control	0.450 ± 0.012	100 ± 2.75	-
			Treated	0.488 ± 0.059	108.26 ± 13.17	108.26

Absolute values measured by ug/g tissue.
Number of animals in each group=5
Non-significant (p>0.05)

* Significant (p<0.05)
** Highly significant (p<0.01)
*** Very highly significant (p<0.001)

whereas this increase was recorded only after 96 hours of drug withdrawal in synaptosomal fraction of cortical tissue ($F = 4.054$, $p < 0.001$). Conversely, the PE content showed highly significant decreases after 12, 24, 48 and 96 hours of drug withdrawal in the synaptosomal fraction of cerebral tissue ($F = 16.36$, $p < 0.001$, $F = 15.53$, $p < 0.001$, $F =$

17.37, $p < 0.001$ and $F = 15.99$, $p < 0.001$ respectively) (Figure 2 and Table 3).

Phosphatidylserine and phosphatidylinositol (PS and PI)

Examination of PS and PI levels in rats treated with acute dose of morphine, revealed that the drug induced declines in PS and PI levels in synaptosomal fraction of cortex, striatum and thalamus-hypothalamus ($F = 0.966$, $p > 0.05$; $F = 0.623$, $p < 0.05$ and $F = 0.681$, $p > 0.05$ respectively). Conversely, a significant increase ($F = 0.809$, $p < 0.05$) were recorded in PS and PI levels of synaptosomal fraction of cerebral tissue after acute administration of morphine (Figure 1 and Table 1).

Similarly, after chronic administration of morphine, PS and PI showed significant decreases in synaptosomal fraction of cortical, striatal and thalamic-hypothalamic tissues ($F = 0.367$, $p > 0.05$; $F = 0.417$, $p < 0.01$ and $F = 2.512$, $p < 0.05$ respectively). In contrast, PS and PI levels showed a significant increases ($F = 3.882$, $p < 0.01$) after chronic administration of morphine in the synaptosomal fraction of the cerebral tissue (Figure 1 and Table 1).

The analysis of PS and PI levels after morphine withdrawal, showed that marked and significant increases were recorded in their levels of synaptosomal fraction in different studied brain areas during the four periods of drug withdrawal except after 96 hours in striatum and 12 and 96 hours in thalamus-hypothalamus whereas PS and PI levels of synaptosomal fraction showed significant decreases ($F = 0.745$, $p < 0.05$; $F = 2.864$, $p < 0.05$ and $F = 2.136$, $p < 0.05$ respectively) (Figure 2 and Table 4).

DISCUSSION

The occurrence of marked elevations and declines in synaptosomal phosphatidylcholine in the present work after acute injection of morphine are concordant with the findings of Loh and Hitzemann (18) in discrete regions of the rat brain. Also, studies performed by Gaiti *et al.* (10) indicated that morphine can markedly affect the incorporation of $^{32}P_i$ (^{3}H) or (^{14}C) glycerol, and ^{14}C-choline into brain phospholipids.

Loh and Hitzemann (18) reported that while the synthesis of ^{14}C-phosphatidylcholine was decreased in the cortex and cerebellum, marked increases were observed in the brain stem, hypothalamus and diencephalon. The mechanism by which morphine increase phosphatidylcholine synthesis in the diencephalon involve the final enzyme in the biosynthetic sequence, cytidine phosphorylcholine diglyceride transferase. Also, the same authors indicated that, although acute morphine treatment increased ^{3}H-protein synthesis in the caudate nucleus, no effect on ^{14}C-phosphatidylcholine synthesis was observed. The heterogeneous changes in phosphatidylcholine content in different brain areas in the study of Loh and Hitzemann (18) after acute administration of morphine run in agreement with the results of the present work.

Also, in the present work drug withdrawal induced increases in the synaptosomal phosphatidylcholine level after 12 and 96 hours in the cortex and after all periods of withdrawal of the drug in striatum and thalamus-hypothalamus. In support of our observations, Natsuki (27) reported that in withdrawn mice injection of morphine significantly increase ^{14}C-choline incorporation into phosphatidylcholine, while naloxone had no effect.

In view of the above considerations, the increase in the levels of synaptosomal phosphatidylcholine after acute administration of morphine sulphate and during periods of drug withdrawal in the striatum and thalamus-hypothalamus may be related to increase in the phosphatidylcholine synthesis in these brain areas, and this involves the final enzyme in the biosynthetic sequence, cytidine phosphorylcholine diglyceride transferase (18).

However, the present results recorded that phosphatidylcholine showed non-significant changes in different brain areas after chronic administration of morphine indicating that; during a phase of chronic morphine tolerance and dependence development, there are no significant changes in synaptosomal phosphatidylcholine content in the brain.

Loh and Hitzemann (18) indicated that chronic morphine treatment decrease the turnover of ^{14}C-phosphatidylcholine in both the mitochondrial and microsomal fractions of the cortex. In other brain regions, the turnover of phosphatidylcholine was increased in the mitochondrial

Table 4: Effect of withdrawal of morphine sulphate on Phosp- hatidylinositol and Phosphatidylserine levels in synaptosomal tissue of different brain areas of adult male albino rats.

Parameters	Brain areas	Hours	Animal groups	Mean ±S.E. as absolute values	Mean ±S.E. as % of control	% of control
Phosphatidylserine and Phosphatidylinositol	Cortex	12	Control	0.027 ± 0.012	100 ± 42.58	-
			Treated	0.079 ± 0.008	287.67 ± 29.03	287.67**
		24	Control	0.027 ± 0.012	100 ± 42.58	-
			Treated	0.029 ± 0.004	106.48 ± 14.81	106.48
		48	Control	0.027 ± 0.012	100 ± 42.58	-
			Treated	0.049 ± 0.007	180.89 ± 26.51	180.89
		96	Control	0.027 ± 0.012	100 ± 42.58	-
			Treated	0.090 ± 0.013	328.02 ± 47.71	328.02*
	Cerebellum	12	Control	0.030 ± 0.002	100 ± 6.00	-
			Treated	0.093 ± 0.007	307.85 ± 23.42	307.85***
		24	Control	0.030 ± 0.002	100 ± 6.00	-
			Treated	0.093 ± 0.011	309.17 ± 36.29	309.17**
		48	Control	0.030 ± 0.002	100 ± 6.00	-
			Treated	0.054 ± 0.008	178.73 ± 28.27	178.73
		96	Control	0.030 ± 0.002	100 ± 6.00	-
			Treated	0.068 ± 0.009	226.13 ± 29.57	226.13*
	Striatum	12	Control	0.053 ± 0.006	100 ± 11.85	-
			Treated	0.095 ± 0.017	80.74 ± 32.88	80.74***
		24	Control	0.053 ± 0.006	100 ± 11.85	-
			Treated	0.249 ± 0.034	471.13 ± 65.74	471.13**
		48	Control	0.053 ± 0.006	100 ± 11.85	-
			Treated	0.121 ± 0.023	229.17 ± 44.32	229.17
		96	Control	0.053 ± 0.006	100 ± 11.85	-
			Treated	0.034 ± 0.002	64.47 ± 3.69	64.47*
	Thalamus-hypothalamus	12	Control	0.089 ± 0.005	100 ± 5.69	-
			Treated	0.049 ± 0.008	55.60 ± 9.45	55.60*
		24	Control	0.089 ± 0.005	100 ± 5.69	-
			Treated	0.148 ± 0.017	166.59 ± 19.55	166.59*
		48	Control	0.089 ± 0.005	100 ± 5.69	-
			Treated	0.212 ± 0.010	238.41 ± 11.56	238.41***
		96	Control	0.089 ± 0.005	100 ± 5.69	-
			Treated	0.059 ± 0.008	66.63 ± 9.07	66.63*

Absolute values measured by ug/g tissue.
Number of animals in each group=5
Non-significant (p>0.05)

* Significant (p<0.05)
** Highly significant (p<0.01)
*** Very highly significant (p<0.001)

fraction of the brain stem, diencephalon and hypothalamus, decreased in the microsomal fraction of caudate nucleus and brain stem, and increased in microsomal fraction of diencephalon. These results are in agreement with our results recorded in the present study. Also, Hitzemann and Loh (13) found that morphine pellet implantation either

decrease or has no effect on the rates of $^{32}P_i$ and (^3H) choline incorporation into microsomal phosphatidylcholine. However, Hula *et al.* (16) indicated that significant decrease of absolute amounts of phosphatidylcholine was shown in brain of morphine dependent rats.

It is not possible to determine whether or not the increase in ^{14}C-choline incorporation into synaptosomal phospholipid occurs via a homologous or via heterogenous mechanism. The functional consequences of changes in homologous choline exchange are also not known. However, the specificity of the decrease in choline exchange observed in the morphine-dependent mice suggests that this decrease may play a role in central adaptive mechanisms (27). In addition to the effect of dependence on Ca^{2+} stimulated exchange, the rate of exchange without Ca^{2+} was affected. Morphine treatment inhibited the basal rates of exchange. It also inhibited the rate of exchange at high concentration of Ca^{2+}, while low concentrations of Ca^{2+} stimulated the exchange.

In a previous study, it was found that chronic d-amphetamine treatment inhibited the turnover of ^{14}C-phosphatidylcholine in all brain areas. It was concluded that this effect was due to either to a direct influence on phosphatidylcholine biosynthesis or to a secondary effect resulting from the inhibition of brain protein biosynthesis. Also, Loh and Hitzemann (18) demonstrated that chronic morphine treatment inhibited the turnover of cortical ^3H-protein in different subcellular fraction. These data would suggest a general inhibitory action of morphine on protein synthesis in this brain region.

Similar considerations can be made for interpreting the results of the present study after chronic administration of morphine. For example, the inhibition of synaptosomal phosphatidylcholine turnover in the cortex, cerebellum and striatum may result from an accumulative inhibitory effect on the synthesis of this compound and also from the marked inhibition of protein synthesis observed in these brain areas (18).

The present work realizes that the acute and chronic effects of morphine and withdrawal of the drug on synaptosomal phosphatidylcholine synthesis and turnover can not be interpreted only in general terms. However, in the present study, acute morphine treatment and withdrawal of the drug increased phosphatidylcholine synthesis in striatum and thalamus-hypothalamus. The present data demonstrate that enhanced synthesis and turnover of phosphatidylcholine occurs in the different brain regions, under the experimental conditions of the present work, associated with tolerance and dependence development.

Supporting evidence in favor of the decreases recorded in the phosphatidylethanolamine concentrations under the present experimental conditions in the synaptosomes of some brain areas, which might reflect morphine physical dependence, are supplied by Hula *et al.* (16), who demonstrated that the quantity of phosphatidylethanolamine plasmalogen lowered in rats with morphine physical dependence. It was also shown that, under morphine physical dependence in the study of Hula *et al.* (16), the acyl residues of phosphatidylcholine and phosphatidylethanolamine changed.

Mulé (23) reported that the effects of subcutaneously administered morphine on the turnover of phospholipids in non-tolerant guinea pigs were primarily evident 1 hr after drug administration. The last author also demonstrated that a stimulation was found with phosphatidylinositol, phosphatidylcholine, phosphatidylethanolamine and phosphatidylserine at 4 hours and no effect was found 10 hours after drug administration.

These results are in agreement with our results recorded in the present work where increases were recorded in the phosphatidylethanolamine synaptosomal fraction in striatum after acute administration of morphine and in striatum and thalamus-hypothalamus after drug withdrawal and decreases were recorded in its content in different brain areas after chronic administration of morphine and in cortex and cerebellum after withdrawal of the drug. Furthermore, these data suggest the existence of a correlation between the phospholipid effect of morphine and the pharmacologic action of this drug, since the analgesic response was maximal between the first and second hours and subsequently declined after morphine administration in the guinea pig (9,39).

Plausible theories (7,34) to explain the phenomena of tolerance to narcotic analgesics have been directed

toward either: biotransformation or disposition of the analgesic drug (changes in analgesic drugs) or biochemical changes induced by the analgesic drug at a cellular or molecullar level in neuronal tissue (changes in neuronal cells).

No direct evidence has been obtained to support the metabolic or altered distribution theories (26,38) of tolerance development. The studies of Mulé (24) provided experimental evidence in support of the biochemical alteration theory of tolerance development.

Therefore, the decrease in synaptosomal content of phosphatidylethanolamine in some brain areas in this study might reflect a tendency that phosphatidylethanolamine plays a significant role in the development of morphine physical dependence.

The increase in the levels of phosphatidylethanolamine in synaptosomes of striatum and thalamus-hypothalamus after some periods of drug withdrawal may be interpreted as a direct interaction with the phosphatidylethanolamine or enzymes and precursors involved in the biosynthesis of these molecules.

The occurrence of marked and significant decreases in phosphatidylserine and phosphatidylinositol of synaptosomal fraction of cortex, striatum and thalamus-hypothalamus after acute morphine administration in the present study might reflect that acute morphine treatment stimulates phosphatidylserine and phosphatidylinositol hydrolysis in these rat brain areas. These results are in concordance with the findings of Rafaa and Martinez (32) who indicated that there is evidence that acute morphine treatment stimulates phosphatidylinositol hydrolysis, while chronic morphine treatment inhibits phosphatidylinositol hydrolysis in rat cerebral cortex (5).

Natsuki *et al.* (28) found that acute morphine administration inhibits the basal rate of serine exchange. This inhibition of the basal rate of exchange apparently is not caused by small amount of residual morphine present in the washed microsomes, since the addition of morphine in vitro was found to stimulate rather than inhibit the basal exchange of serine.

The principal finding reported by Periyasamy and Hoss (30) is that -opioid agonists stimulate phosphatidylinositol turnover in various regions of the rat brain. Among opioid receptor types the effect appears to be specific to receptors. The density of -receptors, binding sites in rat brain- is the highest in the hippocampus, amygdala, cortex, hypothalamus and thalamus, and the lowest in pons-medulla and cerebellum (19,20,31). The magnitude of the -receptors- stimulated phosphatidylinositol response is generally correlated with the distribution of -receptors.

However, the significant decrease recorded in the phosphatidylserine and phosphatidylinositol in synaptosomal fraction especially in striatum and thalamus-hypothalamus after chronic administration of morphine in the present work might suggest morphine tolerance development with respect to striatal and thalamic-hypothalamic phosphatidylserine and phosphatidylinositol contents and production. These results run with agreement of the data of Hula et al. (16) who indicated that rats with morphine physical dependence showed significant decrease of absolute amounts of phosphatidylserine and phosphatidylinositol in rat brain.

Activity at specific metabotropic receptor subtypes stimulates phosphatidylinositol hydrolysis and leads to the production of the intracellular messengers inositol 1, 4, 5-triphosphate and diacylglycerol (3,21,33). Chronic opioid use may alter production of these intracellular messengers and thus elicit long-term changes, which contribute to opioid tolerance and dependence.

There may be stimulation of biosynthetic pathways leading to tissue depletion of D-1, 2-diglyceride, an essential intermediate in the biosynthesis of glycerophosphatides (17).

The occurrence of marked elevation in phosphatidylserine and phosphatidylinositol in the synaptosomal fraction of different brain areas under the experimental condition of the present especially after drug withdrawal study are concordant with findings of Natsuki *et al.* (28) in rats; Mulé (25); Natsuki *et al.* (29) and Sun *et al.* (37) in guinea pig.

Abood and Hoss (2) concluded that phosphatidylserine has high affinity for morphine. Other acidic phospholipids; such as the inositides and phosphatidic acid, also bind morphine, however, phosphatidylserine comprises by far the major portion of the total acidic lipids of neural tissue

and may play an important role in regulating the association of the opiates and many cationic drugs with their biological receptors.

Both morphine and -endorphin significantly increased [^{3}H]-phosphatidylinositol levels, a similar change was not seen in the levels of [^{3}H]-Diphosphatidylinositol ([^{3}H] - DPI) or [^{3}H] - Triphosphatidylinositol ([^{3}H] - TPI) (29). These data may suggest that only a small, unique and compartmentalized pool of phosphatidylinositol is used for the synthesis of diphosphatidylinositol and triphosphatidylinositol. The second reason, recorded by Natsuki *et al.* (29) who examined changes in phosphatidylinositol labeling was a result of the numerous reports suggesting that there is a relationship between phosphatidylinositol turnover and synaptic transmission (1,22).

Also, Natsuki *et al.* (29) observed that morphine and -endorphin but not naloxone increased the incorporation of [^{3}H]-glycerol into phosphatidylinositol (PI). This agonist-specific effect is what could be expected from cationic drugs, like the opiates. However, it was observed that morphine and naloxone but not -endorphin markedly increased the accumulation of [$^{32}P_i$]-PI such as the ratio of [$^{32}P_i$] PI/[^{3}H] PI was increased; this effect is analogous to the changes seen with various neurotransmitters. Thus, opiates have multiple effects of phosphatidylinositol turnover.

Cationic drugs, despite their structural similarities to neurotransmitters, affect phosphatidylinositol (PI) turnover in a somewhat different fashion in that both $^{32}P_i$ and labeled glycerol (or glucose) incorporation are increased (1). The mechanism underlying this effect thought to be the inhibition of phosphidate phosphohydrolase (4,11).

It is difficult to ascertain the physiologic significance of the phospholipid effect of these drugs because of the high level of drug required *in vitro* to produce dramatic changes in phospholipid metabolism. However, it is interesting to entertain the possibility that the phospholipid effect may be related to transport of organic molecules or ions within neuronal tissue. In this regard, Hokin and Hokin (14) have shown that an increased incorporation of $^{32}P_i$ into phosphatides occurred in salivary glands when protein secretion was stimulated by acetylcholine or epinephrine; in pigeon esophagus on stimulation of pepsin secretion with acetylcholine; in adenohypophysis on stimulation of adrenocorticotropin (ACTH) secretion; in avian salt gland on stimulation of sodium chloride secretion with acetylcholine, and in guinea pig adrenal medulla on stimulation of epinephrine secretion by acetylcholine (15).

From the above observations, we may suggest that increases recorded in phosphatidylserine and phosphatidylinositol of the synaptosomal fraction in different brain areas in the present work especially after drug withdrawal represents a direct effect on the biosynthesis of phosphatidylserine and phosphatidylinositol in the synaptosomes of the brain areas which showed elevations in its concentration of these phosphatides.

Overall the results of the present study confirm and extend previous observations on the effects of morphine on brain phospholipid metabolism in guinea pig (24,25). Also, results suggest that the mechanisms of morphine action are closely associated with changes in the turnover of the brain phospholipids in rats (16,35).

Finally, the data presented here illustrate that narcotics like barbiturates and morphine are capable of having a marked effect on phospholipids (16, 28,29).

REFERENCES

1. Abdel-latif AA : Effect of neurotransmitters and neuropharmacological agents on phospholipid metabolism in rabbit iris muscle. In function and metabolism of phospholipids in the central and peripheral nervous systems. Ed by G Porcellati, L Amaducci and C Calli, Plenum publishing Co New York, pp 227-256, 1976.

2. Abood LG and Hoss W : Stereospecific morphine adsorption to phosphatidylserine and other membranous components of brain. Eur J Pharmacol, 32:66-75, 1975.

3. Ambrosini A and Meldolesi J : Muscarinic and quisqualate receptor-induced phosphoinositide hydrolysis in primary cultures of striatal and hippocampal neurons. Evidence for differential mechanisms of activation. J Neurochem, 53:825-833, 1989.

4. Brindley DN and Bowley M : Drugs affecting the synthesis of glycerides and phospholipids in rat liver. The effects of clofibrate, halofenate, fenfluramine, amphetamine, cinchocaine, chlorpronazine, mepyramine and some of their derivatives. Biochem J, 148:461-469, 1975.

5. Dixon W, Ting YW, Chang PL : The effects of morphine on norepinephrine-stimulated phosphoinositol response in rat cerebral cortex. Prog Clin Biol Res, 328:279-282, 1990.

6. Dum J, Meyer G, Hollt V, Herz A : In vivo opiate binding unchanged in tolerant/dependent mice. Eur J Pharmacol, 58:453-460, 1979.

7. Eddy NB : The phenomena of tolerance. In origins of resistance to toxic agents. Ed by MG Sevag, RD Reid, OE Reyndds, pp 223-243, Academic Press, New York, 1955.

8. Folch J, Lees M, Sloane-Stanley GH : A simple method for the isolation and purification of total lipids from animal tissue. J Biol Chem, 226:497-509, 1957.

9. Friebel H, Jacob R, Cros J : Gewöhnung von meerschweinchen an die hustengrace und schmerzstillende wirkung von morphine. Medna Pharmac Exp, 12:97-104, 1965.

10. Gaiti A, Brunetti M, Woelk H, Porcellati G : The relationships between the phospholipid pool and the base-exchange reaction in the Ca^{2+} -stimulated incorporation of ethanolamine into brain microsomal phospholipids. FEBS Lett, 49:361-364, 1975.

11. Hauser G and Eichberg J : Identification of cytidine diphosphate-diglyceride in pineal gland of the rat and its accumulation in the presence of Dl-propranolol. J Biol Chem, 250:105-112, 1975.

12. Helmy FM and Hack MH : Some contributions to the thin-layer chromatographic analysis of complex natural phospholipid and neutral lipid mixtures. J Chromatography, 374:61-72, 1986.

13. Hitzeman RJ and Loh HH : Influence of chronic phenobarbital or morphine treatment on the incorporation of $^{32}P_i$ and ^{3}H-choline into rat brain synaptic plasma membrane. Biochem Pharmacol, 26:1087-1089, 1977.

14. Hokin LE and Hokin MR : Phosphatidic acid metabolism and active transport of sodium. Fedn Proc Fedn Am Socs Exp Biol, 22:8-18, 1963.

15. Hokin MR, Benfey BG, Hokin LE : Phospholipids and adrenaline secretion in guinea pig adrenal medulla. J Biol Chem, 233:814-817, 1958.

16. Hula NM, Marhitych VM, Hovseieva NM, Klimashevskyi VM, Zhukov OD, Synytskyi VM : Brain lipid composition of the rat with experimental morphine dependence. Ukr Biokhim Zh, 70:98-105, 1998.

17. Kennedy EP : Biosynthesis of complex lipids. Fedn Proc Fedn Am Socs Exp Biol, 20:943, 1961.

18. Loh HH and Hitzemann RJ : Effect of morphine on the turnover and synthesis of (leu-^{3}H)-protein and (ch-^{14}C)-phosphatidylcholine in discrete regions of the rat brain. Biochem Pharmacol, 23:1753-1765, 1974.

19. Mansour A, Khuchaturian H, Lewis ME, Akil H, Watson SJ : Anatomy of CNS opioid receptors. Trends Neurosci, 11:308-314, 1988.

20. Mansour A, Khuchaturian H, Lewis ME, Akil H, Watson SJ : Autoradiographic differentiation of mu, delta and kappa opioid receptors in the rat forebrain and midbrain. J Neurosci, 7:2445-2464, 1987.

21. Manzoni OJJ, Finiels-Marlier F, Sassetti I, Blockaert J, Le Peuch C, Sladeczek FAJ : The glutamate receptor of the Q_p-type activities protein kinase C and is regulated by protein kinase C. Neurosci Lett, 109:146-151, 1990.

22. Michell RH : Inositol phospholipids and cell surface receptor function. Biochim Biophys Acta, 415:81-147, 1975.

23. Mulé SJ : Effect of morphine and nalorphine on the metabolism of phospholipids in guinea pig cerebral cortex slices. J Pharmacol Exp Ther, 154:370-383, 1966.

24. Mulé SJ : Morphine and the incorporation of $^{32}P_i$ into brain phospholipids of non-tolerant, tolerant and abstinent guinea pigs. J Pharmacol Exp Ther, 156:92-100, 1967.

25. Mulé SJ : Morphine and incorporation of $^{32}P_i$ orthophosphate in vivo into phospholipids of the guinea pig cerebral cortex, liver and subcellular fraction. Biochem Pharmacol, 19:581-593, 1970.

26. Mulé SJ and Woods LA : Distribution of N-C^{14} - methyl labeled morphine I- In central nervous system of non-tolerant and tolerant dogs. J Pharmacol Exp Ther, 136:232-241, 1962.

27. Natsuki R : Relationship between incorporation of choline into phosphatidylcholine and morphine binding sites. Jpn J Alcohol and Drug dependence, 27:57-70, 1992.

28. Natsuki R, Hitzemann RJ, Hitzemann BA, Loh HH : Effect of phenobarbital on regional brain phospholipid synthesis. Biochemical Pharmacology, 26:2095-2105, 1977.

29. Natsuki R, Hitzemann RJ, Loh HH : Influence of morphine, B-endorphine and naloxone on the synthesis of phosphoinositides in the rat mid brain. Res Commun Chem Pathol Pharmacol, 24:233-250, 1979.

30. Periyasamy S and Hoss W : Kappa opioid receptors stimulate phosphoinositide turnover in rat brain. Life Sci, 47:219-225, 1990.

31. Plager MD and Vogt BA : Mu- and delta-opioid receptor binding peaks and kappa-homogeneity in the molecular layers of rat hippocampal formation. Brain Res, 460:150-154, 1988.

32. Raffa RB and Martinez RP : Morphine antinociception is mediated through a LiCl-sensitive, Ip_3 -restorable pathway. Eur J Pharmacol, 215:357-358, 1992.

33. Schoepp DD and Conn PJ : Metabotropic glutamate receptors in brain function and pathology. Trends Pharmacol Sci, 14:13-25, 1993.

34. Seevers MH and Woods LA : The phenomena of tolerance. Am J Med, 14:546-557, 1953.

35. Selevich MI and Lelevich VV : The effect of chronic morphine administration on the concentrations of individual lipids in various rat brain structures. Ukr Biokhim Zh, 69:107-110, 1997.

36. Sivan SP, Nabeshima T, Ho IK : Alterations of synaptic high and low affinity opiate binding site after acute and chronic morphine administration in mice. Prog Neuro-Psycholpharmacol Biol Psychiat, 6:119-127, 1982.

37. Sun GY, Navidi N, Yoa F, Wood WG, Sun AY : Effects of chronic ethanol administration on poly-phosphoinositide metabolism in mouse brain: variance with age. Neurochem Int, 22:11-17, 1993.

38. Way EL and Adler TK : The pharmacologic implications of the fate of morphine and its surrogates. Pharmac Rev, 12:383-446, 1960.

39. Winder CV : Quantitative evaluation of analgesic action in guinea pigs, morphine, ethyl-1-methyl-4-phenylpiperidine-4-carboxylate (Demoerol), and acetylsalicylic acid. Archs Int Pharmacodyn Ther, 74:219-232, 1947.

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