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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*

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Abstract—The effect of acute and chronic morphine treatment on the synthesis and turnover of ^{3}H-leucine-labeled protein and ^{14}C-choline-labeled phosphatidylcholine was measured in discrete regions of the rat brain. Chronic morphine treatment had the following effects on turnover. In the crude mitochondrial fraction of all brain regions studied, the turnover of ^{3}H-protein was decreased. Microsomal ^{3}H-protein turnover was increased in the cerebellum and hypothalamus and decreased in the cortex. The turnover of ^{14}C-phosphatidylcholine was increased in the crude mitochondrial fraction of the brain stem, hypothalamus and diencephalon, but decreased in the cortex. In the microsomal fraction, the turnover of ^{14}C-phosphatidylcholine was decreased in the cortex, brain stem and caudate nucleus, but increased in the diencephalon. Acute morphine treatment decreased ^{14}C-phosphatidylcholine synthesis in the cortex (58 per cent) and cerebellum (49 per cent), but increased synthesis in the hypothalamus (95 per cent) and diencephalon (285 per cent). Acute morphine treatment decreased ^{3}H-protein synthesis in the cortex (77 per cent) and diencephalon (73 per cent), but increased ^{3}H-protein synthesis in the hypothalamus (55 per cent) and caudate nucleus (146 per cent). The relevance of these findings to current theories of narcotic tolerance and physical dependence development is discussed.

IN LINE WITH various theories of the mechanisms responsible for the development of morphine tolerance and physical dependence,[1–3] protein synthesis inhibitors have been found to block tolerance and dependence development.[4–6] Way *et al*,[7] Shen *et al*,[8] as well as Knapp and Mandell,[9] have suggested that the proteins affected by the repeated administration of morphine may be enzymes associated with the putative neurotransmitters, especially serotonin. However, at present, the role of serotonin is still controversial.[10] More generally, some workers have attempted to isolate the protein or proteins principally affected by morphine treatment.[11,12] The results of these experiments have been negative in that chronic morphine treatment was not found to alter the incorporation of labeled amino acids into brain proteins isolated by disc gel electrophoresis.

The crucial argument in the macromolecular theories of morphine tolerance and physical dependence development is the effect of protein synthesis inhibitors. However, it is known that these compounds have many side effects.[13] One important side effect of one inhibitor which blocks tolerance and dependence development, namely

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actinomycin D, is that this compound inhibits not only protein but also phospholipid synthesis.[14] Although it is not clear if other protein synthesis inhibitors, e.g. cycloheximide and puromycin, also block phospholipid synthesis, some effects from these compounds would not be unexpected. A role for brain phospholipids in the mechanisms of morphine action has been suggested by Mulé.[15–18] The author has presented evidence indicating that analgesia may be related to a competition between morphine and divalent cations, especially Ca^{2+}, for phospholipids, resulting in decreased ion transport. Furthermore, morphine was found to alter phospholipid synthesis, which could result in changes in active transport,[19] protein synthesis,[20,21] mitochondrial function[22] or the activity of some membrane-bound enzymes.[23–24]

The exact role brain proteins and phospholipids have in morphine tolerance and physical dependence development is not known. However, as a general working hypothesis, we suggest that in order to complement present theories of tolerance and physical dependence development, the synthesis of brain proteins or phospholipids (or both) should increase during chronic morphine treatment and that these changes must at least occur in those subcortical brain regions where the principal sites of morphine action reside.[25,26] In order to test this hypothesis, we have examined in the present study the effect of acute and chronic morphine treatment on the synthesis and turnover of ^{14}C-choline-labeled phosphatidylcholine and ^{3}H-leucine-labeled protein in discrete regions of the rat brain. We have chosen to examine morphine effects on phosphatidylcholine, since this phospholipid is a major component of nerve-ending vesicular and plasma membranes[27] and since some evidence suggests that phosphatidylcholine may play an important role in neurotransmitter release through the formation of lysophosphatidylcholine.[28]

MATERIALS AND METHODS

Materials. Methyl-^{14}C-choline (sp. act., 54 mCi/m-mole) and 4,5-^{3}H-leucine (sp. act., 25 Ci/m-mole) were obtained from Amersham-Searle Corp. Phosphatidylcholine, lysophosphatidylcholine and sphingomyelin standards were purchased from Supelco Inc. All other reagents and supplies were obtained locally.

Turnover of ^{14}C-phosphatidylcholine and ^{3}H-protein. Under light ether anesthesia, male Sprague-Dawley rats weighing 150 g (age 60–70 days) were given 5 μCi ^{14}C-choline and 20 μCi ^{3}H-leucine intracisternally (i.c.).[29] Five days later, twice daily injections (s.c.) of 10 mg/kg of morphine sulfate were begun. The dose of morphine was doubled every 5 days with the experiment terminating 15 days after the first morphine injection. Animals were sacrificed 12 hr after the last morphine injection on days 10, 15 and 20. Controls sacrificed on day 5 received no drug treatment. 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. 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 a crude mitochondrial pellet containing nerve endings, myelin fragments and mitochondria.[27] The post-mitochondrial supernatant was centrifuged for 1 hr at 105,000 *g* to yield the microsomal pellet and the soluble fraction. Protein and phospholipid were precipitated from the various fractions by adding sufficient trichloroacetic acid (TCA) to bring the final concentration to 10 per cent (w/v) and centrifuging. The pellet

was then washed twice with 5 per cent TCA containing 1 per cent leucine and 1 per cent choline.

^{14}C-phosphatidylcholine and ^{3}H-protein synthesis. Animals were given 40 mg/kg of morphine sulfate s.c. 0·5 hr prior to the injection i.c. of 5 μCi ^{14}C-choline and 20μCi ^{3}H-leucine; 0·5 hr later, the animals were sacrificed, brains removed and dissected. The brain parts were immediately homogenized in 10 per cent cold TCA and centrifuged. The TCA supernatant was saved, the pellet was washed twice with 5 per cent TCA, and the supernatant and washes were saved for ^{14}C-choline analysis. The pellet was then washed twice with 5 per cent TCA containing 1 per cent leucine and 1 per cent choline.

Phospholipid analysis. The TCA pellet was vigorously suspended in 1–4 ml of 5% TCA and divided into two portions. One portion was extracted twice with 19 vol. of chloroform–methanol (2 : 1, v/v).[30] The lipid extract was partitioned with 0·1 M KCl and then washed several times 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 of chloroform–methanol (2 : 1). Twenty-five μl was spotted on Silica gel G thin-layer chromatography plates (Merck). The spots were overspotted with a stock solution of phosphatidylcholine, lysophosphatidylcholine and sphingomyelin. Plates were developed in a solvent system of chloroform–methanol–acetic acid–water (50:25:9:4, v/v).[31] Spots were identified by spraying with a dilute solution of iodine, scraped into scintillation vials containing 1 ml methanol and extracted overnight. Ten ml of scintillation mixture was added and the samples were counted in a Beckman LS-100 liquid scintillation counter using the narrow ^{14}C-window setting. The scintillation solution contained one part Triton X-100 (Rohm & Haas. Philadelphia, Pa.) and two parts toluene plus 6 g/l. of 2,5-diphenyloxazole). ^{14}C counting efficiency as judged by an external standard was in the range of 48–52 per cent. A portion of the chloroform-methanol extract was assayed for lipid phosphorus in phosphoglycerides containing two acid-hydrolyzable groups.[32] Data are given as dis./min/μmole of lipid phosphorus.

^{14}C-choline determination. In some experiments, the TCA supernatant was analyzed for ^{14}C-choline by a modification of the procedure described by Gomez *et al.*[33] TCA was removed by repeated extractions with diethyl ether, resuspended in 100 μl methanol-HCl (21 : 1, v/v) and 50 μl was spotted on microcrystalline cellulose thin-layer chromatography plates. Plates were developed on a solvent system of *n*-butanol–ethanol–acetic acid–water (8 : 2 : 1 : 3). The choline spot was visualized with a dilute iodine spray, scraped out and counted as previously described. This procedure effectively separates choline from the major choline metabolites, namely, phosphorylcholine and cytidine diphosphorylcholine.

Protein analysis. A portion of the TCA pellet suspension was analyzed for ^{3}H-protein essentially as described by Jones and McIlwain.[34] The tissue suspension was heated at 80° for 15 min. After centrifugation, the pellet was serially extracted with 5 ml ethanol–ether (1 : 1, v/v), 5 ml chloroform–methanol (1 : 1, v/v) and 3 ml ether. The final tissue residue was gently shaken to form a thin film on the walls of the centrifuge tube and air dried. The residue was dissolved in 1–3 ml of 97 per cent formic acid. Formic acid extract (100 μl) was added to a counting vial containing 0·9 ml distilled water. After gentle mixing, 20 ml of the Triton X-100 toluene scintillation mixture was added. ^{3}H–efficiency as judged by an external standard was in the range of 10–12

per cent. ^{14}C contamination was found to be less than 1 per cent of the total ^{3}H cpm. The formic acid extract was neutralized with 5 M NaOH prior to protein determination by the method of Lowry *et al.*[35] as modified by Miller.[36]

Statistical analysis. 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

Effect of acute morphine treatment on the synthesis of ^{3}H-protein and ^{14}C-phosphatidylcholine. Animals were given 40 mg/kg of morphine sulfate s.c. 0·5 hr prior to the injection of 5μCi ^{14}C-choline and 20 μCi ^{3}H-leucine intracisternally (i.c.). Thirty min later, the animals were sacrificed and the levels of ^{14}C-choline, ^{14}C-phosphatidylcholine, unincorporated soluble ^{3}H, and ^{3}H-protein were determined (Table 1). Morphine treatment decreased ^{14}C-choline levels in the brain stem 55 per cent, decreased ^{14}C-phosphatidylcholine synthesis in the cortex 58 per cent and cerebellum 49 per cent, but increased synthesis in the hypothalamus 95 per cent and diencephalon 285 per cent. Morphine increased the levels of unincorporated soluble ^{3}H in the cortex 49 per cent, cerebellum 83 per cent, caudate nucleus 43 per cent and diencephalon 55 per cent. The synthesis of ^{3}H-protein was decreased in the cortex 67 per cent, cerebellum 86 per cent, brain stem 77 per cent and diencephalon 73 per cent, but increased in the hypothalamus 55 per cent and caudate nucleus 146 per cent.

Turnover of ^{3}H-protein and ^{14}C-*phosphatidylcholine in discrete regions of normal rats.* Rats were given 5 μCi ^{14}C-choline and 20 μCi ^{3}H-leucine i.c. After waiting 5 days to allow for the decay of proteins that were turning over rapidly, animals were sacrificed and the specific activity of ^{3}H-protein and ^{14}C-phosphatidylcholine was determined in the microsomal, soluble and crude mitochondrial fractions from discrete brain regions. Subsequently, brains were analyzed on days 10, 15 and 20 after the injection of labeled material. Although brains were analyzed for the levels of ^{14}C-phosphatidyl choline and ^{14}C-sphingomyelin, the levels of these two compounds were less than 40 cpm above background and an accurate determination of the turnover rate could not be made. Additionally, the levels of ^{14}C-phosphatidylcholine in the soluble fraction of all brain regions and the levels of ^{3}H-protein in the soluble fraction of the caudate nucleus and hypothalamus were not sufficiently greater than background activity to warrant presentation.

For reasons discussed in detail by Lajtha and Marks,[37] the half-life ($T_{1/2}$) values calculated for ^{3}H-protein from the data in Figs. 1–5 can only, at best, be considered approximations. Realizing this limitation, it is interesting to note that the $T_{1/2}$ values for the mitochondrial, microsomal and soluble fractions are both similar and uniform for all brain regions except the brain stem. In this brain region, the $T_{1/2}$ values for the microsomal and soluble fractions are larger than in other areas, indicating a slower turnover. The possibility that the i.c. injection affected turnover in this region must be considered. The turnover of soluble protein in the cerebellum ($T_{1/2}$ = 22 days) was also found to be slower than the rate generally observed ($T_{1/2}$ = 9–12 days). Regional changes in phospholipid turnover have been described by this laboratory elsewhere.[38] In general, ^{14}C-phosphatidylcholine turnover shows less uniformity than the turnover of ^{3}H-protein. In all brain regions, the turnover of ^{14}C-phosphatidylcholine in the mitochondrial fraction was slower than that observed in

TABLE 1. EFFECT OF MORPHINE ON THE SYNTHESIS OF ^{3}H-PROTEIN AND ^{14}C-PHOSPHATIDYLCHOLINE*

Brain region	Treatment†	^{14}C-choline (dis./min/g ± S.E. × 10^{-5})	^{14}C-phosphatidylcholine (cpm/μmole lipid P ± S.E.)	Soluble ^{3}H (cpm/g ± S.E. × 10^{-4})	^{3}H-protein (cpm/mg protein ± S.E. × 10^{-1})
Cortex	C	12 ± 4	26 ± 4	17 ± 3	11 ± 3
	M	15 ± 3	11 ± 2‡	25 ± 2‡	3 ± 1‡
Cerebellum	C	104 ± 20	187 ± 28	89 ± 6	109 ± 18
	M	89 ± 13	99 ± 28‡	163 ± 18‡	15 ± 4‡
Hypothalamus	C	8 ± 2	39 ± 4	6 ± 1	48 ± 11
	M	6 ± 2	75 ± 17‡	6 ± 1	74 ± 4‡
Caudate nucleus	C	7 ± 1	10 ± 3	14 ± 1	11 ± 1
	M	8 ± 2	11 ± 4	19 ± 1‡	29 ± 2‡
Brain stem	C	114 ± 16	35 ± 4	103 ± 28	298 ± 51
	M	51 ± 7‡	76 ± 10‡	73 ± 11	69 ± 7‡
Diencephalon	C	36 ± 5	6 ± 2	37 ± 3	73 ± 15
	M	36 ± 7	24 ± 7‡	55 ± 5‡	19 ± 1‡

* Animals were given 40 mg/kg of morphine sulfate s.c. 0·5 hr prior to the injection of 5 μCi ^{14}C-choline and 20 μCi ^{3}H-leucine i.c.; 0·5 hr later, the animals were sacrificed and the levels of ^{14}C-choline, ^{14}C-phosphatidylcholine, unincorporated soluble ^{3}H and ^{3}H-protein were determined in discrete brain regions. N equals six animals/determination.

† C = control; M = morphine.

‡ Significantly different than control ($P < 0.05$).

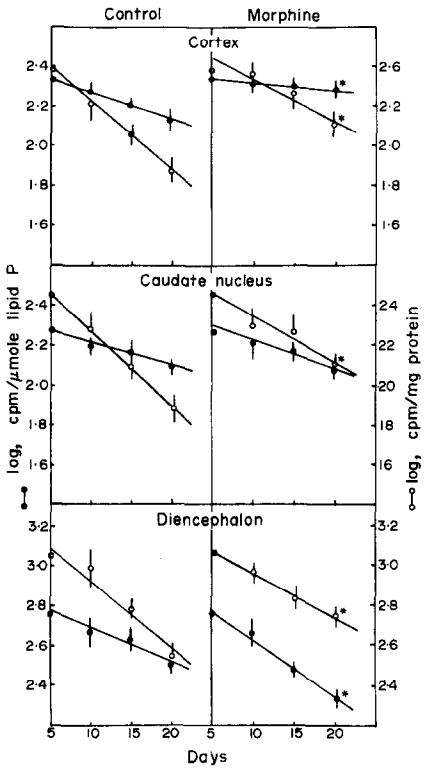

FIG. 1. Turnover of ^{3}H-protein and ^{14}C-phosphatidylcholine in the P_2 fraction of the cortex, caudate nucleus and diencephalon from normal and chronic morphine-treated animals. Animals were given 5 μCi ^{14}C-choline (sp. act., 54 mCi/m-mole) and 20 μCi ^{3}H-leucine (sp. act., 25 Ci/m-mole) i.c. Beginning 5 days after administration of the labeled compound, animals were given 10 mg/kg of morphine sulfate s.c. twice daily. The dose of morphine was doubled 5 days later and doubled once again 10 days later. Control groups were sacrificed on days 5, 10, 15 and 20. Morphine-treated groups were sacrificed 12 hr after their last injection on days 10, 15 and 20. Data in Fig. 1 are for the P_2 fraction of the three brain regions and are plotted as log dis./min μmole of lipid P ± S.E. vs time and as log dis./min/mg of protein ± S.E. vs time. Each point represents the mean of six determinations. The asterisk indicates values that are significantly different from control on day 20 ($P < 0.05$).

the microsomal fraction. Furthermore, marked regional differences in turnover were observed. For example, microsomal ^{14}C-phosphatidylcholine turnover in the cerebellum ($T_{1/2}$ = 7 days) occurred at twice the rate observed in the diencephalon ($T_{1/2}$ = 14 days). A comparison of the turnover rates for ^{3}H-protein and ^{14}C-phosphatidylcholine shows that, in general, the turnover of ^{14}C-phosphatidylcholine was slower than that of ^{3}H-protein in the mitochondrial fraction but faster than that of ^{3}H-protein in the microsomal fraction.

Effect of chronic morphine treatment on the turnover of ^{3}H-protein and ^{14}C-phosphatidylcholine. Beginning on day 5 after the injection of labeled material, animals were given 10 mg/kg of morphine sulfate twice daily. The dose of morphine was doubled every 5 days so that, during days 15-20, the animals received two daily injections of 40 mg/kg, s.c. Significant changes in turnover were judged to have occurred when the specific activity of ^{3}H-protein or ^{14}C-phosphatidylcholine at day 20 in the morphine group was significantly different from that of control (Tables 2 and 3). In the

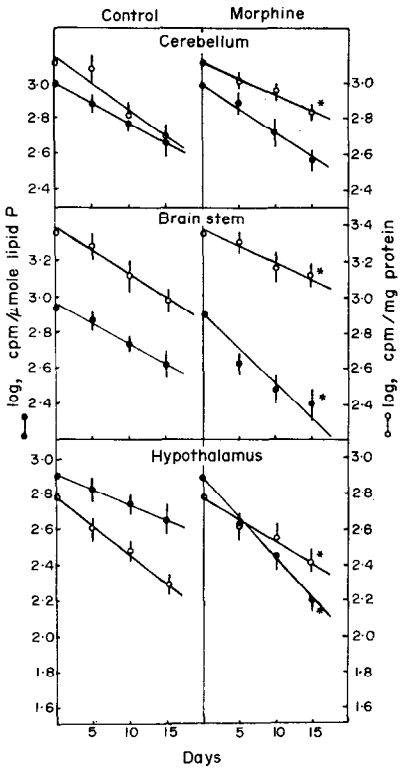

FIG. 2. Turnover of ^{3}H-protein and ^{14}C-phosphatidylcholine in the mitochondrial fraction of the cerebellum, brain stem and hypothalamus from normal and morphine-treated animals.

mitochondrial fraction, chronic morphine treatment decreased ^{3}H-protein turnover in all brain regions. Microsomal ^{3}H-protein turnover was decreased only in the cortex and significant increases in turnover were observed in the cerebellum and hypothalamus. The turnover of soluble ^{3}H-protein was decreased in the cortex and diencephalon. As with ^{3}H-protein, chronic morphine treatment decreased 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 fraction of the brain stem, diencephalon and hypothalamus, decreased in the microsomal fraction of the caudate nucleus and brain stem, and increased in the microsomal fraction of the diencephalon.

DISCUSSION

Although there is strong pharmacological evidence[4–9] to support the hypothesis that brain proteins (and perhaps phospholipids) play an important role in the development of morphine tolerance and physical dependence, supporting chemical evidence has not been forthcoming. In the present study, we have attempted to consider the possibility that this lack of evidence may be the result of not examining the effects of morphine in descrete regions of the brain. By analogy with the effects of psychoactive drugs on the putative neurotransmitters where regional changes are known to occur, it must be considered, and perhaps expected, that a psychoactive agent such

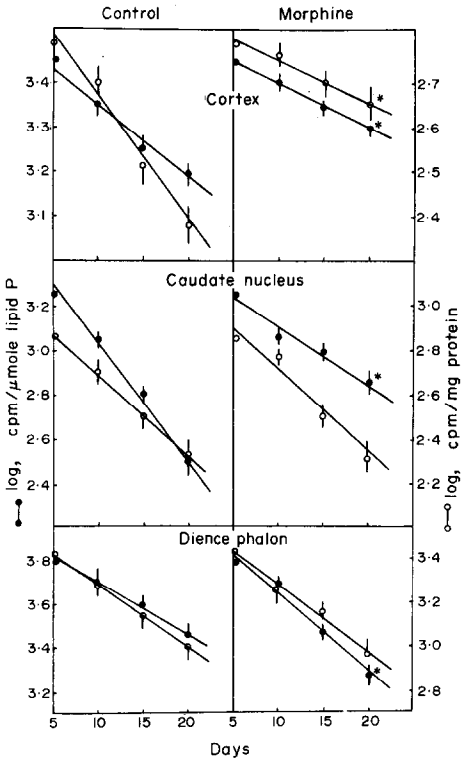

FIG. 3. Turnover of ^{3}H-protein and ^{14}C-phosphatidylcholine in the microsomal fraction of the cortex, caudate nucleus and diencephalon from normal and morphine-treated animals.

as morphine will show regional effects on brain protein and phospholipid synthesis.

Previously, it has been demonstrated that a single injection of morphine increases the amount of unincorporated radioactivity after the injection of a labeled amino acid, but decreases the incorporation of the radioactivity into protein.[39,40] The present experiments (Table 1) confirmed these findings of Clouet *et al.*, [39,40] but also demonstrated that in at least two brain regions, namely the caudate nucleus and hypothalamus, acute morphine treatment increased ^{3}H-protein synthesis. An increase in protein synthesis caused by morphine has been reported by Stolman and Loh,[41] using a system composed of brain polysomes and soluble enzymes *in vitro*. These authors concluded that one mechanism by which morphine can increase protein synthesis is by preventing the breakdown of brain polysomes into ribosomes.

The effect of acute morphine treatment on ^{14}C-phosphatidylcholine synthesis was found to be more heterogeneous than that observed for ^{3}H-protein synthesis. 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. Although acute morphine treatment increased ^{3}H-protein synthesis in the caudate nucleus, no effect on ^{14}C-phosphatidylcholine synthesis was observed. Preliminary experiments now in progress suggest that the mechanism by which morphine increases phosphatidylcholine synthesis in the diencephalon involves the final enzyme in the biosynthetic sequence, cytidine phosphorylcholine diglyceride transferase.

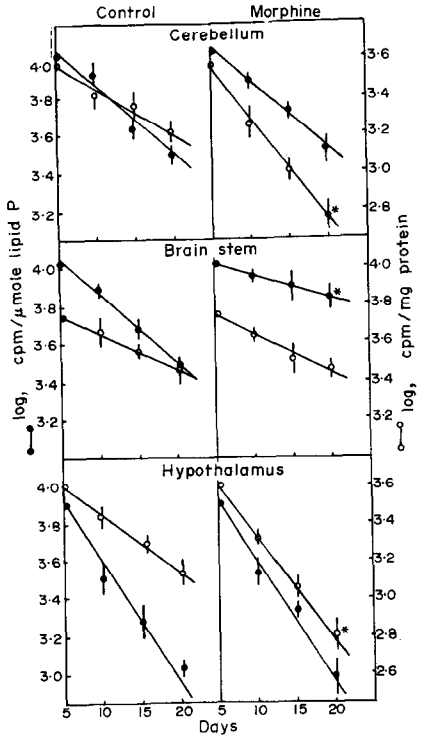

FIG. 4. Turnover of ^{3}H-protein and ^{14}C-phosphatidylcholine in the microsomal fraction of the cerebellum, brain stem and hypothalamus from normal and morphine-treated animals.

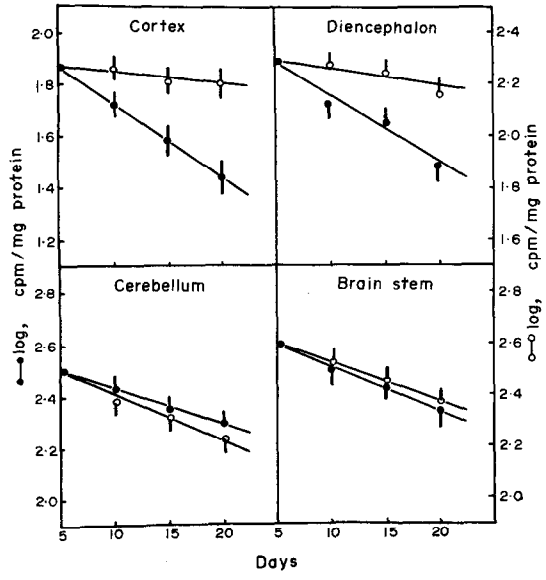

FIG. 5. Turnover of ^{3}H-protein in the soluble fraction of the cortex, cerebellum, brain stem and diencephalon from normal (○) and morphine-treated (●) animals.

TABLE 2. EFFECT OF CHRONIC MORPHINE TREATMENT ON THE THE TURNOVER OF ^{3}H-PROTEIN*

	$T_{1/2}$ (days)					
	Mitochondrial		Microsomes		Soluble	
Brain region	Control	Morphine	Control	Morphine	Control	Morphine
Cortex	9	14†	11	30†	11	48†
Cerebellum	11	18†	12	5†	22	17
Brain stem	10	15†	18	19	17	19
Diencephalon	10	14†	12	11	11	34†
Caudate nucleus	9	13†	9	7	ND‡	
Hypothalamus	9	12†	12	7†	ND	

* Animals were given 20 μCi ^{3}H-leucine i.c. Twice daily injections of morphine sulfate were begun 5 days later and continued for 15 days. Injections were begun at 10 mg/kg s.c. and doubled every 5 days. Animals were sacrificed at various times after beginning morphine injection and the specific activity of ^{3}H-protein was determined in the crude mitochondrial, microsomal and soluble fractions prepared from various brain regions. Half-life values ($T_{1/2}$) were determined by linear regression. Additional experimental details are given in the legend to Fig. 1.

† Significantly different from control, $P < 0.05$. (See text for test of significance.)

‡ ND = not determined.

The pulse-labeling technique was employed to estimate the turnover rates of proteins and phosphatidylcholine in different subcellular fractions of the discrete brain regions. Assuming that in the adult rat the rate of protein or phospholipid breakdown equals the rate of synthesis, i.e. a steady state exists, and where there is little reutilization of the label, the disappearance or the turnover rate of the labeled material may be used as an index of the rate of synthesis (or breakdown). Non-drug-related studies using ^{3}H-leucine and ^{14}C-choline to estimate the turnover of brain protein and phosphatidylcholine have been presented by others.[42,43] Turnover rates, as calculated in the present experiments, pertain only to those proteins or stores of phosphatidylcholine that turn over at a moderate rate ($T_{1/2}$ = 5–30 days). Rates of less than 5 days or more than 30 days would not be accurately measured. For example,

TABLE 3. EFFECT OF CHRONIC MORPHINE TREATMENT ON THE TURNOVER OF ^{14}C-PHOSPHATIDYLCHOLINE*

	$T_{1/2}$ (days)			
	Mitochondrial		Microsomes	
Brain Region	Control	Morphine	Control	Morphine
Cortex	22	90†	17	33†
Cerebellum	14	11	7	8
Brain stem	13	8†	8	22†
Diencephalon	17	11†	14	8†
Caudate nucleus	26	20	6	10†
Hypothalamus	18	7†	5	5

* Animals were given 5 μCi ^{14}C-choline i.c. Twice daily injections of morphine sulfate were begun 5 days later and continued for 15 days. Injections were begun at 10 mg/kg s.c. and doubled every 5 days. Animals were sacrificed at various times after beginning morphine injection and the specific activity of ^{14}C-phosphatidylcholine was determined in the crude mitochondrial and microsomal fractions prepared from the various brain regions. Half-life values ($T_{1/2}$) were determined by linear regression. Additional experimental details are given in the legend to Fig. 1.

† Significantly different from control. $P < 0.05$. (See text for test of significance.)

sphingomyelin is known to turn over slowly in the adult rat[44] and, as a consequence, should incorporate only a small amount of radioactivity from the pulse of (Me-^{14}C)-choline. This was found to be true in the present study and confirms the earlier results of Abdel-Latif and Smith.[43] It should be noted that some of the turnover rates presented in Tables 2 and 3 exceed those values which can be accurately measured and, thus, must be considered only a rough approximation of the true turnover rate.

In a previous study, it was found that chronic *d*-amphetamine treatment inhibited the turnover of ^{14}C-phosphatidylcholine in all brain regions.[38] It was concluded that this effect was due either to a direct influence on phosphatidylcholine biosynthesis or to a secondary effect resulting from the inhibition of brain protein synthesis. Similar considerations can be made for interpreting the results of the present study. For example, the inhibition of phosphatidylcholine turnover in the cortex may result from an accumulative inhibitory effect on the synthesis of this compound and also from the marked inhibition of protein synthesis observed in this brain region. In other brain regions, more complex interactions between phospholipid and protein synthesis and metabolism may exist and should be particularly considered when interpreting the results of long-term turnover experiments. However, at this point in time when our knowledge of such interactions is still incomplete, it would seem more useful to examine the data on protein and phosphatidylcholine turnover on an individual basis.

Chronic morphine treatment inhibited the turnover of cortical ^{3}H-protein in all subcellular fractions studied. These data would suggest a general inhibitory action of morphine on protein synthesis in this brain region. Since acute morphine treatment also inhibited cortical protein synthesis, we may conclude that the chronic drug effect results from an accumulation of acute drug effects to which tolerance apparently does not develop. Similarly, Clouet[45] has shown that five daily injections of morphine do not prevent the acute inhibition of whole brain protein synthesis. In other brain regions, the effects of chronic morphine treatment on ^{3}H-protein turnover appear more complicated than those observed in the cortex. Although the turnover of ^{3}H-protein in the mitochondrial fraction is inhibited in all brain regions, a complementary effect was not always observed in the microsomal or soluble fractions. Presently, the mechanism(s) underlying the disassociation of drug effects between the various subcellular fractions is not clear. Almost any interpretation of the data would imply that morphine has a specific effect on the synthesis or metabolism of protein in the mitochondrial fraction and a different distinct effect on the synthesis or metabolism of microsomal protein.

We realize that the acute and chronic effects of morphine on brain protein synthesis and turnover can be interpreted only in general terms. However, in the present study, we were attempting to evaluate the hypothesis that morphine regionally alters protein synthesis and that in those brain regions associated with the site(s) of morphine action, protein synthesis or turnover increases. Various investigators have suggested that the principal neuronanatomical sites associated with acute morphine action and physical dependence are located in the diencephalon,[25,26] although other brain regions may also be involved.[46–48] Acute and chronic morphine treatment was found to inhibit ^{3}H-protein synthesis and turnover in the diencephalon. Only in the hypothalamus were we able to observe a consistent stimulation of these parameters.

Differently than with ^{3}H-protein, in the diencephalon acute morphine treatment increased ^{14}C-phosphatidylcholine synthesis and chronic morphine treatment increased ^{14}C-phosphatidylcholine turnover. This increase in turnover, however, was not entirely specific to the diencephalon but was also observed in the mitochondrial fraction of the brain stem and hypothalamus. The functional significance of an increase in the turnover of the choline moiety of phosphatidylcholine is presently unclear. Choline is strongly basic in character, thus making it less likely that phosphatidylcholine will interact with divalent or monovalent cations in preference to the more acidic phospholipids. Possible functional roles of phosphatidylcholine are discussed in the introductory section, but the exact role the choline moiety plays in these processes is not known. The question also arises as to whether or not the increase in turnover is specific for choline. Other components of phospholipid molecules may also exhibit enhanced turnover, which would indicate a more general rather than a specific drug effect. Although there are many questions yet to be answered, the present experiments demonstrate that enhanced synthesis and turnover of at least one phospholipid component occurs in a brain region associated with tolerance and dependence development.

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