the isolated seminal vesicle of the guinea pig.[2] Still, in view of the present results, the possibility that the intrinsic vasodepressor activity of prostaglandin is related to an effect on endogenous catecholamine action deserves further exploration.

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Effects of morphine and Tofranil on the incorporation of phosphate (^{32}P) into phospolipids of rat brain slices

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RESULTS of previous work from this laboratory[1, 2] have shown that the addition of a high concentration of potassium ions (105 mEq/L) to, or the omission of calcium ions from, the incubation medium markedly stimulates the incoporation of labeled phosphate (^{32}P) into the phospholipids of slices of cortex from rat brain. In these experiments, the factors influencing the cationic stimulation of ^{32}P incorporation have been found to be similar to those affecting the acetylcholine stimulation of phospholipid labeling. We now find, in confirmation of some earlier results obtained with morphine,[3] that the labeling of phospholipids from inorganic ^{32}P in brain cortex slices is strongly enhanced by the addition of morphine (>2 mM) or by the antidepressant drug, Tofranil (N-(3-dimethylaminopropyl)-iminodibenzyl hydrochloride).

The results presented in Table 1 show that, although morphine at the concentrations tested has no effect within experimental error on the rate of oxygen uptake of brain cortex slices of rat, an increase in the labeling of phospholipids takes place with concentrations of 5 and of 10 mM. Similarly, addition of Tofranil, at concentrations of 0·05 and 0·1 mM, which does not affect the respiration significantly, brings about increases of 39 and 70 per cent, respectively, in the incorporation of ^{32}P into phospholipids. At a higher concentration of Tofranil (0·5 mM) the respiration and the labeling of phospholipids are markedly inhibited.

TABLE 1. EFFECTS OF MORPHINE AND TOFRANIL ON INCORPORATION OF PHOSPHATE (^{32}P) INTO PHOSPHOLIPIDS OF RAT BRAIN CORTEX SLICES

	Oxygen uptake (μl/mg dry wt/2 hr)	Incorporation of ^{32}P into phospholipids (counts per min/100 mg wet wt tissue)	Change (%)
Morphine 0	19·5	44,800	
Morphine 2 mM	19·8	46,000	+ 4
Morphine 5 mM	19·4	58,500	+30
Morphine 10 mM	18·0	66,700	+48
Tofranil 0	19·8	41,700	
Tofranil 0·05 mM	19·1	58,000	+39
Tofranil 0·1 mM	18·3	71,400	+70
Tofranil 0·5 mM	11·4	16,500	−63

Rat brain cortex slices of 50 to 70 mg wet weight were incubated at 37° in Krebs-Ringer medium with 0·01 M glucose and 20 μc of ^{32}P (20 × 10^6 counts per min). After 2-hr incubation, the slices were homogenized in cold trichloroacetic acid (10%), and the lipids were extracted in 1:1 chloroform:ethanol.[4]
The mean deviation in the experimental results does not exceed ±5%.

The brain phospholipids were fractionated by chromatography on silicic acid-impregnated paper with diisobutyl ketone:acetic acid:water (40:25:5) according to the method of Marinetti *et al.*,[5] and the radioactive spots were developed by radioautography and various color reagents. It can be seen (Table 2) that the stimulation of ^{32}P incorporation into the total lipid fraction is mainly due to increased ^{32}P incorporation into phosphatidic acid, phosphoinositide, and to a lesser extent into phosphatidyl serine. An appreciable stimulation is also observed in the unidentified spot 1. The Rf of the unidentified phospholipid spot is approximately that reported for diphosphoinositide[6] under similar experimental conditions. Morphine and Tofranil, however, have no effect at the concentrations tested on the labeling of phosphatidyl choline and phosphatidyl ethanolamine.

TABLE 2. EFFECT OF 10^{-2}M MORPHINE AND 10^{-4}M TOFRANIL ON INCORPORATION OF PHOSPATE (^{32}P) INTO INDIVIDUAL PHOSPHOLOPIDS OF RAT BRAIN CORTEX SLICES

Phospholipid	Total radioactivity (counts per min/100 mg wet wt tissue) Control	Morphine	Change (%)	Tofranil	Change (%)
Unidentified spot 1	1,590	2,405	+ 51	2,760	+ 73
Phosphoinositide	1,870	3,925	+110	4,265	+128
Phosphatidyl choline	2,695	2,142	− 21	2,675	− 1
Phosphatidyl serine	530	650	+ 22	810	+ 53
Phosphatidyl ethanolamine	861	842	− 2	1,037	+ 20
Phosphatidic acid	1,995	5,424	+172	6,100	+205
Total $CHCl_3$ extract	34,400	50,700	+ 47	60,262	+ 75

The conditions were as given in Table 1. Brain cortex slices were incubated with 50 to 100 μc of ^{32}P. The counts per minute are corrected to a specific activity of 10^6 counts per min/μmole of inorganic phosphate added to the medium. The values are the averages of the results of four experiments with a mean deviation not exceeding ±10%.

It is evident that both morphine and Tofranil stimulate the rate of incorporation of phosphate into phosphatidic acid and phosphoinositide without affecting the oxidative metabolism of the brain slices. In this respect, these two drugs have effects similar to those produced by acetylcholine[7] and chlorpromazine,[8] both of which enhance the incorporation of ^{32}P into phospholipids without any significant change in the cell ATP.

It is possible that Tofranil by its suppression of at least one ATP-utilizing system—e.g. the ATP-phosphate exchange reaction[9]—will cause more ATP to be involved in the labeling of phospholipids. A somewhat similar explanation[10] has been advanced for the observation that ouabain[11] stimulates the incorporation of phosphate-^{32}P into phospholipids. Morphine may behave in some ways like acetylcholine,[12] for it is already known that morphine and acetylcholine may become attached to similar receptor groups—e.g. in choline esterase[13, 14] or in leech muscle.[15]

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