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Effects of drugs on the phospholipid metabolism of the pineal body of rats

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MANY investigators have shown that cholinergic stimulation results in an increased incorporation of ^{32}P into phospholipids, particularly phosphatidyl inositol and phosphatidic acid, in a number of glandular and neural tissues.[1] In the salivary gland, which is controlled also by the sympathetic nervous system, epinephrine was reported to stimulate the incorporation of ^{32}P into phosphatidyl inositol.[2] The pineal body is also known to be innervated by the sympathetic nervous system,[3] and effects of norepinephrine and dibutyryl cyclic AMP on the metabolism of phospholipids have been reported.[4] The present paper describes the changes in the phospholipid metabolism of the pineal body of rats by several autonomic drugs *in vitro*.

Male Wistar rats were killed by decapitation between 1000 and 1300 hr. The pineal bodies were removed and immediately incubated under 95% O_2 + 5% CO_2 for 3 hr at 37° in 1 ml of sterilized Krebs–Ringer bicarbonate, pH 7·4, which contained 5·5 μmoles/ml of glucose and 10–100 μC/ml of ^{32}P-orthophosphate. All radioactivities were corrected to a specific activity of 10^5 cpm/μg of phosphorus in the medium. After the incubation, the tissues were weighed and the phospholipids were extracted with a mixture of 2:1 (v/v) chloroform–methanol. The phospholipids were separated two-dimensionally on a silicic acid-impregnated paper (Whatman SG 81) by the method of Wuthier[5] using solvents 1 and 3. The air-dried papers were stapled to Sakura medical X-ray film and the films were exposed for 3 days. The autoradiograms were superimposed over the chromatograms and the individual labeled phospholipids were circled. The individual spots were cut out and transferred to liquid scintillation counting vials. The samples were counted in a Nuclear Chicago 6725 liquid scintillation spectrometer. The identification of most of the phospholipids was accomplished by comparison with reference standards. Phosphatidyl choline was obtained from Tokyo Kasei Company and phosphatidyl inositol was bought from Applied Science Laboratories, Inc. The standard for phosphatidic acid was made from egg yolk lethicin by the chloroplast of carrots and was purified by silicic acid column chromatography.[6] The fraction eluted with 3% methanol in chloroform corresponded to the spot of phosphatidic acid on Wuthier's[5] chromatogram, although the identity of phosphatidic acid has been questioned.[7] Phosphatidyl glycerol was extracted from *Micrococcus lysodeikticus* and was purified by silicic acid column chromatography.[8] The fraction eluted by 15% methanol in chloroform was used as the standard. Phosphatidyl ethanolamine was located with ninhydrin and identified by

TABLE 1. EFFECTS OF DRUGS ON ^{32}P INCORPORATION INTO PHOSPHOLIPIDS OF PINEAL BODY

Drugs	Concn in medium (M)	No. of expts.	Phospholipids (cpm/mg wet wt.) Mean ± S.E.				
			Phosphatidyl choline	Phosphatidyl inositol	Phosphatidyl ethanolamine	Phosphatidic acid	Phosphatidyl glycerol
Control		6	127 ± 16	92 ± 12	69 ± 9	51 ± 2	15 ± 2
Norepinephrine	10^{-6}	6	167 ± 20	259 ± 43†	80 ± 7	69 ± 1	34 ± 6†
Norepinephrine	10^{-5}	6	213 ± 19†	652 ± 75‡	81 ± 3	58 ± 10	82 ± 9‡
Norepinephrine	10^{-4}	7	161 ± 10	787 ± 56‡	38 ± 6†	62 ± 9	119 ± 15‡
Tolazoline	10^{-4}	6	172 ± 18	176 ± 5‡	90 ± 5*	42 ± 4	20 ± 1
Tolazoline + norepinephrine	10^{-4} 10^{-4}	6	278 ± 27‡‖	1176 ± 122‡‖	94 ± 13‖	53 ± 12	90 ± 6‡
Dichloroisoproterenol	10^{-4}	6	167 ± 24	214 ± 17‡	62 ± 6	41 ± 3*	182 ± 18‡
Dichloroisoproterenol + norepinephrine	10^{-4} 10^{-4}	6	248 ± 26†‖	1180 ± 111‡	67 ± 6‖	99 ± 15†§	262 ± 27‡¶
Acetylcholine + physostigmine	10^{-4} 10^{-4}	8	282 ± 29†	243 ± 12‡	74 ± 5	63 ± 2†	16 ± 1
Serotonine	10^{-4}	8	179 ± 9	186 ± 6‡	98 ± 8*	53 ± 5	19 ± 1
Histamine	10^{-4}	8	153 ± 10	117 ± 5	67 ± 5	47 ± 3	14 ± 1

* $0.01 < P < 0.05$ vs. control.
† $0.001 < P < 0.01$ vs. control.
‡ $P < 0.001$ vs. control.
§ $0.01 < P < 0.05$ vs. 10^{-4} M norepinephrine.
‖ $0.001 < P < 0.01$ vs. 10^{-4} M norepinephrine.
¶ $P < 0.001$ vs. 10^{-4} M norepinephrine.

comparing the spot to the chromatogram reported by Wuthier.[5] The data were statistically analyzed by the Student *t*-test.

Radioactivity was usually found in seven spots on the chromatogram, two of which were not identified (Fig. 1). In the control pineal body, the rate of ^{32}P incorporation into phospholipids was highest in phosphatidyl choline, followed by phosphatidyl inositol, phosphatidyl ethanolamine, phosphatidic acid and phosphatidyl glycerol (Table 1). Norepinephrine inceased the ^{32}P incorporation into phosphatidyl inositol and phosphatidyl glycerol, although this effect was not specific with norepinephrine. These results are in accord with those of Berg *et al.*,[4] who reported that norepinephrine stimulated the ^{32}P incorporation into phosphatidyl inositol + phosphatidyl serine fraction in the

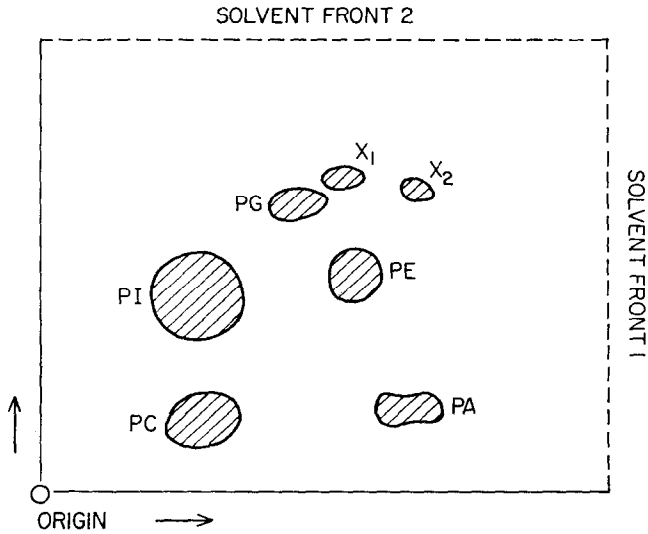

FIG. 1. Two-dimensional paper chromatogram of phospholipids of the pineal body. Abbreviations: PC, phosphatidyl choline; PI, phosphatidyl inositol; PE, phosphatidyl ethanolamine, PA, phosphatidic acid; PG, phosphatidyl glycerol; X_1 and X_2, unidentified spots.

pineal body. The incorporation of the radioactivity into phosphatidyl serine was too little to be determined in the present experiment. The increased phosphate incorporation into phosphatidyl glycerol and phosphatidyl inositol was dependent on the concentration of norepinephrine in the medium. Norepinephrine did not show the concentration-dependent effect on phosphate incorporation into the other phospholipids, which have different metabolic pathways from phosphatidyl inositol and phosphatidyl glycerol.[9] This might suggest that norepinephrine increases ^{32}P incorporation into the two phospholipids by raising the production of CDP-diglyceride, a mutual precursor. Tolazoline, an alpha blocking agent, also elevated the ^{32}P incorporation into phosphatidyl inositol and phosphatidyl ethanolamine. When tolazoline and norepinephrine were added together into the medium, a potentiation was seen in increasing the ^{32}P incorporation into phosphatidyl choline and phosphatidyl inositol. Dichloroisoproterenol, a beta blocking agent, increased the ^{32}P incorporation into phosphatidyl inositol and phosphatidyl glycerol, but decreased the ^{32}P incorporation into phosphatidic acid. Dichloroisoproterenol and norepinephrine together also revealed a potentiative effect on the ^{32}P incorporation into all the phospholipids, except phosphatidyl ethanolamine, which showed no difference from control. The addition of acetylcholine and physostigmine to the medium increased the incorporation of radioactivity into phosphatidyl choline, phosphatidic acid and phosphatidyl inositol. Serotonin caused a slight increase in the ^{32}P incorporation into phosphatidyl inositol and phosphatidyl ethanolamine. Histamine at 10^{-4} M did not affect the ^{32}P incorporation into any of the phospholipids. In conclusion, norepinephrine was the most potent drug to increase the ^{32}P incorporation into phosphatidyl inositol in the rat pineal body; however, this effect was not reversed by either alpha or beta blocking agents.

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Effect of cycloheximide and emitine on ^{14}C-amino acids incorporation by different subcellular fractions from rat liver

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EMETINE, an alkaloid having some antitumor and antiviral activities has been reported to inhibit protein synthesis by the HeLa cells.[1,2] Cycloheximide, a glutarimide antibiotic and a known inhibitor of protein synthesis, has no effect on mitochondrial protein synthesis.[3] It has been reported that emetine, in its mode of action, resembles cycloheximide and related glutarimide antibiotics.[4] This communication is mainly concerned with the effects of emetine and cycloheximide on the incorporation of ^{14}C-amino acids by different subcellular fractions when cell-free extracts of rat liver were incubated with ^{14}C-amino acids.

Rat liver was homogenized with Potter–Elvehjem homogenizer in 8 vol. of ice-cold medium (0·25 M, sucrose; 0·05 M, Tris–HCl buffer, pH 7·4; 0·025 M, potassium phosphate buffer, pH 7·4 and 0·025 M, KCl). Homogenate thus obtained was centrifuged to a cell-free extract at 1000 *g* for 10 min at 0°.

Complete incubation system contained 50 μmoles ATP; 25 μmoles Mg^{2+}; 625 μmoles Tris–HCl buffer (pH 7·4); 625 μmoles sucrose; ^{14}C-algal protein hydrolysate (having total count/min: $1{\cdot}25 \times 10^6$); 30–32 mg protein of cell-free extract. Total volume of the incubation mixture was 5 ml. The incubation was carried out for 120 min at 37°, with constant shaking and was stopped by the addition of 0·3 ml of casein hydrolysate (5 mg/ml). The incubation mixture was then fractionated into different subcellular constituents and processed according to Schneider and Hogeboom.[5] The radioactivity in different subcellular fractions was determined by processing the protein according to the method of Stachiewicz and Quastel,[6] as described by Banerjee *et al.*[7]. Radioactive counts were taken in a gas-flow counter (Nuclear-Chicago). Protein content of the tissue extract was determined by the biuret method.[8]

The results given in Table 1 indicate that different subcellular fractions *viz.* mitochondria, microsomes and soluble supernatants from rat liver can incorporate amino acids when cell-free extracts