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Effects of Adenine Nucleotides, Biogenic Amines and Oleic Acid on Synthesis of Choline and Ethanolamine Glycerophospholipids in Neuronal and Glial Cells from Adult Rabbit Brain

by

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Summary. Neuronal perikarya and astroglial cells were isolated from the gray matter of adult rabbit brain. Activities of CDPethanolamine; 1,2-diradyl-*sn*-glycerol phosphoethanolaminetransferase (EC 2.7.8.1) and CDP-choline; 1,2-diradyl-*sn*-glycerol phosphocholinetransferase (EC 2.7.8.2) were examined with and without potential regulators of the enzymes. The enzyme activities were increased several-fold by the addition of 1-alkyl-2-acyl-*sn*-glycerols which are intermediates in the pathway of plasmalogen biosynthesis. Both enzymes were more active in neural perikarya than in astroglia. The Km values for CDPethanolamine and for CDPcholine are similar in microsomes to those in isolated cells. Both transferases were more active with 10 mM-Mn^{2+} than with 10 mM-Mg^{2+} and were substantially inhibited by 10 mM-Ca^{2+}. Oleic acid at physiological concentration (50 μM) inhibited phosphocholinetransferase with added alkylacylglycerols in both cell types. With endogenous diradylglycerols, the glial phosphocholinetransferase activity was doubled by oleic acid. Phosphoethanolaminetransferase was not affected by 50 μM-oleic acid. Both 1 mM-ATP and 1 μM-cAMP inhibited neuronal transferases and did not affect glial transferase. Neuronal phosphocholinetransferase was inhibited by 1 mM 5-hydroxytryptamine. With endogenous diradylglycerols, both neuronal transferases were inhibited by 1 mM-acetylcholine. Phosphoethanolaminetransferases from neuronal perikarya and synaptosomes but not from astroglia are inhibited by ATP and cAMP. However, with endogenous diradylglycerols, the phosphocholinetransferases from neuronal perikarya and synaptosomes are affected neither by ATP, cAMP, 5-HT nor ACh.

In the present work we have studied the synthesis of the alkylacyl types of choline and ethanolamine glycerophospholipids with respect to possible control mechanisms.

Materials and methods

Sources of chemicals including CDP Me-^{14}C choline (diluted to 0.53 Ci/mol) were reported previously [22]. Preparations of CDP[1,2-^{14}C]ethanolamine (0.07 Ci/mol) and alkylacylglycerols were described previously [17]. Trace amounts of alkenylacylglycerols were removed from alkylacylglycerols by acidic hydrolysis before development of the preparative thin-layer chromatogram. Emulsions of alkylacylglycerols (20 mM) were prepared in 0.1 M-Tris-HCl, pH 7.4, that

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contained 0.1% Tween 20, by warming in a water bath, agitating with a Vortex mixer for 10 min, and sonicating (MSE Ultrasonic Disintegrator) for 1 min. CDP Me-^{14}C choline (diluted to 0.53 Ci/mol) was purchased from the Radiochemical Centre, Amersham, Bucks, England.

Fractions enriched in neuronal perikarya and in glial cells were prepared from rabbit brains (1.8–2.0 kg body wt) according to Blomstrand Hamberger [4] as described previously [21]. The neuronal fraction contained about 90% neuronal perikarya with capillaries and free nuclei as the major contaminant. Glial preparations appeared to be 80–90% astroglia with neuronal perikarya and endothelial cells as major contaminants.

Phosphocholinetransferase and phosphoethanolaminetransferase assay mixtures are described in legends of the tables and figures. Methods of processing and liquid scintillation counting were described previously [22].

Results

Characterization of transferase activities. The incorporation of radioactivity from CDP [^{14}C] choline and CDP [^{14}C] ethanolamine into glycerophospholipids was proportional to the amounts of neuronal or glial protein over the range of 0.25 to 1.2 mg/ml. In subsequent experiments the protein concentration was 0.5 mg/ml. Incorporations were linear with time to 20 min, so further incubation times were 15 min. Values of Km for CDP-choline were 2.2×10^{-4} and 1.5×10^{-4} M, for CDPethanolamine they were 1.8×10^{-4} M and 1.2×10^{-4} M for neuronal perikarya and glial cells, respectively. Apparent Km values for alkylacylglycerols were from 1.5 to 2.0 mM. In the present experiments we used lower protein concentrations and shorter times than we applied previously [21]. The present Km values are similar to our previous finding for microsomes from that brain [17] and to values for CDPcholine in isolated cells [3] but are higher than the Km value for CDPethanolamine in isolated cells reported by Roberti *et al.* [18].

TABLE I

Effect of divalent cations on transferase activities

Additions	Phosphocholinetransferase			Phosphoethanolaminetransferase		
	Neurons	Glia	Ratio	Neurons	Glia	Ratio
Mg^{2+}	8.9	3.0	3.0	8.1	1.8	4.5
Mn^{2+}	16.4	9.1	1.8	14.0	7.8	1.8
Mg^{2+}, Ca^{2+}	3.2	1.8	1.7	2.0	1.1	1.8
Mn^{2+}, Ca^{2+}	4,8	3.6	2.1	7.4	4.6	1.8
AAG, Mg^{2+}	89.8	12.6	7.1	19.9	5.1	3.9
AAG, Mn^{2+}	93.2	19.6	4.9	34.5	21.3	1.6
AAG, Mg^{2+}, Ca^{2+}	25.6	5.1	5.0	4.8	2.3	2.1
AAG, Mn^{2+}, Ca^{2+}	27.4	7.4	3.7	16.1	9.8	1.6

Activities of radioactivity incorporation into choline or ethanolamine glycerophospholipids are expressed as nmol/mg protein/h or as the neuronal/glial ratio. Values are means from 2 or 3 experiments. Neuronal perikarya or glial cell preparations (about 100 μg protein) were incubated in a medium containing 50 mM Tris-HCl at pH 8.0, 10 mM $MnCl_2$, 0.01% Tween 20, 4 mM alkylacylglycerols when added, 0.51 mM CDP[^{14}C]choline (1.2×10^5 disint/min) or 0.56 mM CDP[^{14}C]ethanolamine (1.8×10^4 disint/min), and 10 mM divalent cations as stated under additions in a volume of 0.20 ml at 37° for 15 min. The washed lipid extract was assayed for radioactivity.

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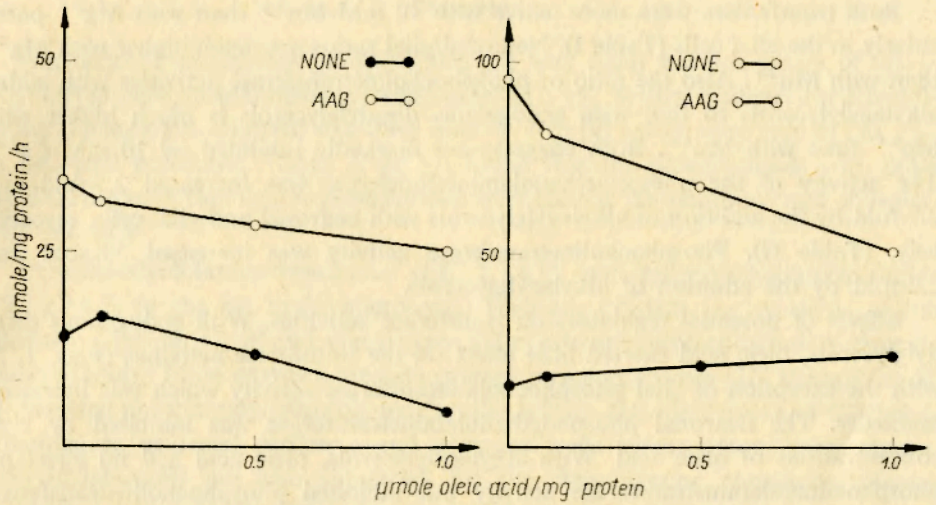

Fig. 1

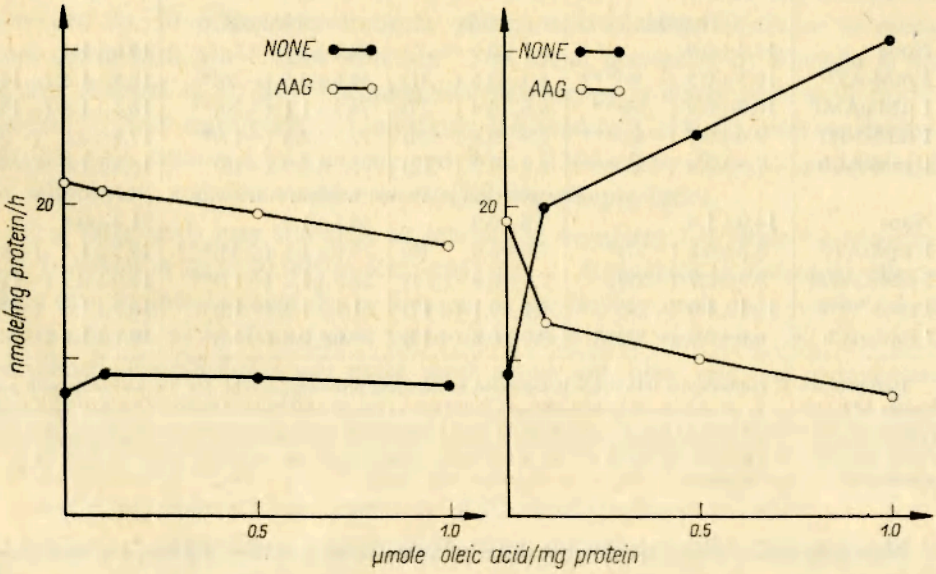

Fig. 2

Figs. 1–2 Effect of increasing concentrations of oleic acid and ethanolamine phosphotransferase (left) and choline phosphotransferase (right) activities in neuronal perikarya (Fig. 1) and glial cells (Fig 2)

Incubations were carried out as described in Table II. Oleic acid concentrations were 0, 50, 250 and 500 μM

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Both transferases were more active with 10 mM-Mn^{2+} than with Mg^{2+} particularly in the glial cells (Table I). Neuronal/glial ratios are much higher with Mg^{2+} than with Mn^{2+}. Also the ratio of phosphocholinetransferase activities with added alkylacylglycerols to that with endogenous diradylglycerols is much higher with Mg^{2+} than with Mn^{2+}. Both enzymes are markedly inhibited by 10 mM-Ca^{2+}. The activity of the phosphoethanolaminetransferase was increased 2.5-fold and 2.7-fold by the addition of alkylacylglycerols with neuronal and glial cells, respectively, (Table II). Phosphocholinetransferase activity was increased 5.8-fold and 2.2-fold by the addition of alkylacylglycerols.

Effects of potential regulators on transferase activities. With endogenous diradylglycerols, oleic acid exerted little effect on the transferase activities (Figs. 1, 2) with the exception of glial phosphocholinetransferase activity which was increased markedly. The neuronal phosphoethanolaminetransferase was inhibited by high concentrations of oleic acid. With alkylacylglycerols, oleic acid had no effect on phosphoethanolaminetransferase activity but inhibited phosphocholinetransferase activities, especially with the glial enzyme.

TABLE II

Effects of ATP, 5-HT, and ACh on transferase activities

Addition	Phosphocholinetransferase activity			
	Endogenous diradylglycerols		Alkylacylglycerols	
	Neurons	Glia	Neurons	Glia
None	16.4±0.9	9.1±0.9	93.2±2.5	19.6±1.4
1 mM-ATP	10.5±0.5 (−36)***	6.3±0.6 (−31)	68.6±5.5 (−26)*	16.8±1.6 (−14)
1 μM-cAMP	10.0±0.9 (−39)**	6.6±0.4 (−27)	74.3±1.1 (−20)**	16.6±1.3 (−15)
1 mM-5-HT	9.0±0.7 (−45)***	6.9±0.6 (−24)	77.3±4.4 (−17)*	17.8±0.6 (−9)
1 mM-ACh	7.3±0.4 (−55)****	8.0±0.3 (−12)	83.9±5.9 (−10)	18.9±1.6 (−4)
	Phosphoethanolaminetransferase activity			
None	14.0±1.4	7.8±0.9	34.5±1.5	21.3±0.4
1 mM-ATP	8.8±0.8 (−37)*	7.8±0.8 (0)	23.0±1.3 (−33)***	19.3±1.5 (−9)
1 μM-CAMP	9.9±0.7 (−29)	7.9±0.8 (+1)	28.5±1.2 (−17)**	18.9±0.9 (−11)
1 mM-5-HT	10.1±1.0 (−28)	9.1±0.1 (+17)	31.1±1.3 (−10)	19.3±1.8 (−9)
1 mM-ACh	6.6±0.3 (−53)**	9.0±0.8 (+15)	29.6±0.8 (−14)	19.1±1.8 (−10)

Incorporation of radioactivity into lipids is expressed as nmol/mg protein/h±S.E.M. for 3 experiments with percentages of inhibition in parentheses. Incubation were carried out as described in Table I with or without 4 mM alkylacylglycerols. Incubations with cAMP or 5-HT included 1 mM theophylline and incubations with ACh included 1 mM eserine. Values that are significantly different from controls by Student's *t* test are marked*, $P<0.05$**, $P<0.01$; ***; $P<0.005$; and ****, $P<0.001$.

Neuronal transferases were substantially inhibited by 1 mM-ATP and 1 μM-cAMP (Table II). The nucleotides exerted little effect on glial transferases with the exception of an inhibition of glial phosphocholinetransferase in the presence of endogenous diradylglycerols. Acetylcholine and 5-hydroxytryptamine substantially inhibited both neuronal transferases in the presence of endogenous diradylglycerols. These inhibitions were much smaller with added alkylacylglycerols.

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Discussion

Alkylacyl or alkylglycerophospholipids are very likely the precursors of alk-1--enylacyl glycerophospholipids (plasmalogens) [11, 19]. Recent evidence for ethanolamine plasmalogen synthesis has come from in vivo [9] and in vitro studies [20, 15]. In neuronal perikarya, Freysz *et al.* [7] found that the phospholipids with most rapid turnover of radioactive phosphorous were choline plasmalogens and phosphatidyl inositols.

Phosphoethanolaminetransferase (EC 2.7.8.1) and phosphocholinetransferase (EC 2.7.8.2) in the rat brain microsomal fractions catalyze the synthesis of the alkylacyl types [3, 17, 8] and diacyl types [2] of ethanolamine and choline glycerophospholipids. These enzymes are also present in synaptosomes [22] Binaglia *et al.* [3] detected phosphatidyl ethanolamine synthesis from diacylglycerols and CDPethanolamine in neuronal perikarya and astroglial cells isolated from rabbit brain. Alkylacylglycerols are also substrates for the ethanolamine phosphotransferase in isolated cells [18]. We have also compared choline and ethanolamine phosphotransferase activities with both diacylglycerols and alkylacylglycerols in neuronal perikarya and astroglial cells isolated from rabbit brain [21] Specific activities of both enzymes are 2 to 5 fold higher in neuronal perikarya than in glial cells [3, 5, 18, 21].

We have examined the effects of some potential regulators of phosphocholinetransferase and phosphoethanolaminetransferase activities in neuronal perikarya and astroglia isolated from gray matter. Divalent cations influence the transferase activities. At 10 mM concentrations, phosphoethanolaminetransferase is much more active with Mn^{2+} than with Mg^{2+}, as found previously by Binaglia *et al.* [3] and Roberti *et al.* [18]. Phosphocholinetransferase is active with both Mn^{2+} and Mg^{2+}. Both transferases are markedly inhibited by Ca^{2+}. Calcium ion inhibition has been described by Kennedy and Weiss [13] for liver, Coleman and Bell [6] for adipocytes, and Strosznajder *et al.* [22] for synaptosomes.

Free fatty acids may stimulate or inhibit the transferases in brain microsomes [17]. Increases of 10 or 20 fold in oleic acid (Figs. 1, 2) generally increased the effects noted with 50 μM-oleic acid. In the presence of alkylacylglycerols oleic acid inhibited the transferases as we found with microsomes from brain and liver [16]. The glial phosphocholinetransferase was much more active with oleic acid and endogenous diradylglycerols whereas the same enzyme in microsomes from brain and liver was somewhat inhibited with a higher concentration of oleic acid [16]. Concentrations used in this study may be more physiological since Cenedella *et al.* [5] have reported a free fatty acid concentration of 0.3 μmol/g of normal brain.

Inhibitions of transferase reactions by ATP are greater with alkylacylglycerols than with diacylglycerols [12, 22] and inhibitions by ATP with diacyglycerols are greater in the presence of CoA and related compounds [14]. Synaptosomal transferases are affected similarly by 1 mM-ATP and μM-cAMP [22]. The effects of these nucleotides on transferases in neuronal perikarya are similar to those exerted on transferases in synaptosomes with the exception of the phosphocholinetransferase

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with endogenous diradylglycerols. In this case, 1 μM-cAMP inhibited the activity 39% in perikarya and stimulated 96% in synaptosomes. The effects of ATP were also opposite in perikarya and synaptosomes for this activity. Nucleotides inhibited the activity of phosphocholinetransferase in both neuronal perikarya and glial cells. The activity of phosphoethanolaminetransferase in neuronal perikarya was inhibited significantly by the nucleotides and their glial activity was not affected.

Putative neurotransmitters may inhibit transferase activities in nerve endings [22]. With endogenous diradylglycerols, acetylcholine markedly inhibits the transferases in neuronal perikarya but had very little effect on transferases in glial cells or on synaptosomal phosphocholinetransferase. With alkylacylglycerols, acetylcholine had little effect on the transferases. Also 5-hydroxytryptamine affects slightly transferases with alkylacyglycerols. With endogenous diradylglyceols, 5-hydroxytryptamine inhibited neuronal and glial but not synaptosomal phosphocholinetransferase and it inhibited neuronal and synaptosomal but not glial phosphoethanolaminetransferase. Acetyl-choline and 5-hydroxytryptamine could influence transferase activities by stimulating the formation of cAMP. The effects of 1 μM-cAMP were quite similar to those of 1 mM-ACh and 1 mM 5-HT in direction and magnitude in neuronal perikarya and in glial cells.

Hokin [10] studied effects of ACh and 5-HT on ^{32}P phosphate incorporation into glycerophospholipids in slices from guinea pig brain. Its incorporation into phosphatidic acid and in some cases into phosphatidyl choline and phosphatidyl ethanolamine was decreased. The stimulation of labelling of phosphatidic acid and phosphatidyl inositol by neurotransmitters has also been reported by Yagihara and Hawthorne [23], Yagihara *et al.* [24] and Althaus *et al.* [2]. Abdel-Latif *et al.* [1] incubated slices of rat brain cortex with ^{32}P phosphate and ^{14}C choline. Acetylcholine increased the incorporation of ^{32}P and ^{14}C into the phospholipids of neuronal and glial fractions isolated from the slices. The effect was greater with neuronal perikarya and synaptosomes than with glial cells. The mechanism of these neurotransmitter effects on phospholipid metabolism is not well understood. In our experiments the synthesis of choline and ethanolamine glycerophospholipids was inhibited by neurotransmitters. They may act directly or through cAMP.

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Й. Строшнайдер, А. Радоминска-Пырек, Е. Лязаревич, Л. А. Хоррокс, **Влияние нуклеотидов аденины, биогенных аминов и олеиновой кислоты на синтез холины и глицерофосфонидов этаноламина в перикарных первных и глиальных клетках из мозга взрослых кроликов.**

Содержание. Перикарий нервных клеток и глиальные клетки выделяли из серого вещества мозга взрослых кроликов. Активность ЦДФэтаноламин: 1,2-дирадил-сн-глицерол фосфоэтаноламинтрансфераз и ЦДР: 1,2-диарил-*сн*-глицерол фосфохолинтрансферазы (ЕС 2.7.8.2) исследовали в присутствии или без потенциальных ферментных регуляторов. Активность ферментов увеличивалась в несколько раз при добавлении 1-алкил-2-ацил-*сн*-глицеролов, являющихся промежуточными метаболитами на пути биосинтеза плазмалогенов. Оба фермента были более активны в перикарпиях нейронов, чем в астроглии. Значения K_M для ЦДР этаноламины и ЦДР халины были такими же в микросомах и изолированных клетках. Обе трансферазы были более активны при 10 *мМ* Mn^{2+} чем при 10 *мМ* Mg^{2+} и значительно ингибировались 10 *мМ* Ca^{2+}. Олеиновая кислота в физиологических концентрациях (50 *М*) ингибировала фосфохолинтрансферазу при добавлении алкилацилглицеролов в обоих типах клеток. В присутствии эндогенных дирадилглицеролов олеиновая кислота удваивала

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активность глиальнойфосфохолинтрансферазы. Олеиновая кислота не влияла на фосфоэтаноламинтрансферазу. Как 1 *мМ* АТР так и 1 *М* cAMP ингибировали трансферазу нейронов и не влияли на глиальную трансферазу. Фосфохолинтрансфераза нейронов ингибировалась 1 *мМ* 5-гидрокситриптамином. В присутствии эндогенных дирадилглицеролов нейрональные трансферазы ингибировались 1 мМ ацетилхоламина. Ингибировались фосфоэтаноламинтрансферазы из перикарпиев нейронов и синаптом, однако не из астроглии. В присутствии эндогенных дирадилглицеролов, АТР, cAMP, 5-HT и АХ они оказывают неодинаковое влияние на фосфохолинтрансферазы перикарпия и синаптосом.

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