Glyceride Lipases in Nerve Endings of Guinea-Pig Brain and their Stimulation by Noradrenaline, 5-Hydroxytryptamine and Adrenaline

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1. Combined guinea-pig cortex and cerebellum was shown to contain triglyceride lipase, diglyceride lipase and monoglyceride lipase, which were assayed by the release of [$1\text{-}^{14}\text{C}$]palmitate from [$1\text{-}^{14}\text{C}$]palmitoylglycerol esters. Triglyceride lipase and diglyceride lipase were found in all particulate fractions. 2. With osmotically ruptured synaptosomes the rates of release of palmitate from glyceryl tripalmitate and glyceryl dipalmitate were $7\text{--}25\text{ }\mu\text{mol/h}$ per g of protein and $0.18\text{--}0.69\text{ mmol/h}$ per g of protein respectively. The logarithm of the rate of hydrolysis of glyceryl monopalmitate increased linearly with the logarithm of protein concentration. The pH optima of triglyceride lipase and diglyceride lipase were between 7 and 8. The pH optimum for monoglyceride lipase was approx. 8. 3. Triglyceride lipase and diglyceride lipase of osmotically ruptured synaptosomes were stimulated by noradrenaline, 5-hydroxytryptamine and adrenaline. Triglyceride lipase of isolated synaptic membranes was stimulated by $0.01\text{--}1\text{ mM}$ -noradrenaline. Aging of membranes at 0°C decreased activity, which could still be stimulated by noradrenaline. Diglyceride lipase of isolated membranes was stimulated by $1\text{ }\mu\text{M}\text{--}1\text{ mM}$ -noradrenaline. The activity of triglyceride lipase in isolated synaptic vesicles was diminished by 1 mM -5-hydroxytryptamine.

Glyceride lipases have been described in adipose tissue (Gorin & Shafir, 1964; Strand *et al.*, 1964; Vaughan *et al.*, 1964), rat liver (Irwin *et al.*, 1966; Carter, 1967; Waite & van Deenan, 1967; Biale *et al.*, 1968; Guder *et al.*, 1969), kidney, heart, diaphragm, lung and spleen of rat (Biale *et al.*, 1968), skeletal muscle (Wallach, 1968), and polymorphonuclear leucocytes (Elsbach & Kayden, 1965). Little work seems to have been done on the hydrolysis of long-chain esters of glycerol in brain. Rat brain contains small quantities of triglyceride (Davison & Wajda, 1962; Rowe, 1969; Sun & Horrocks, 1969). Acetate is incorporated rapidly into triglyceride of rat brain *in vitro* (Rowe, 1969) and is incorporated into triglyceride of developing mouse brain *in vivo* (Kanazawa *et al.*, 1972). Palmitate is incorporated into triglyceride of mouse brain *in vivo* (Sun & Horrocks, 1969), and palmitate and stearate are incorporated readily into triglyceride of brain explants (Menkes, 1971). These observations indicate that triglyceride in brain is metabolically active. The concentration of triglyceride in cortical slices of rat brain diminished when the slices were incubated in buffer containing EDTA (Rowe, 1969), suggesting the presence of lipases acting on endogenous substrates.

Noradrenaline and 5-hydroxytryptamine have been shown to be present in certain nerve endings of rat brain (Michaelson & Whittaker, 1963; Michaelson *et al.*, 1963; Zieher & De Robertis, 1963; Levi & Maynert, 1964). They are closely associated with

vesicles (Michaelson *et al.*, 1963; Maynert *et al.*, 1964) and there is evidence that they are putative transmitter substances in the central nervous system of mammals (see Curtis, 1969). As transmitter substances they would be present in unbound forms in contact with synaptic membranes during synaptic transmission. Noradrenaline and 5-hydroxytryptamine increase the concentration of unesterified fatty acids *in vitro* in nerve endings and in preparations of synaptic membranes (Price & Rowe, 1971, 1972), and the question arises whether breakdown of endogenous glycerides contributes to this increase. The present paper reports the presence of triglyceride lipase, diglyceride lipase and monoglyceride lipase in combined guinea-pig cerebral cortex and cerebellum. It reports the stimulation of triglyceride lipase and diglyceride lipase of nerve endings by noradrenaline, 5-hydroxytryptamine and adrenaline, and the stimulation of the triglyceride lipase of isolated synaptic membranes by noradrenaline and 5-hydroxytryptamine. A preliminary communication has been given (Vyvoda & Rowe, 1972).

Experimental

Materials

Female guinea-pigs of Dunkin-Hartley strain of age greater than 2 months were used throughout. Glyceryl tri[$1\text{-}^{14}\text{C}$]palmitate was supplied by The Radiochemical Centre, Amersham, Bucks., U.K.

Glyceryl dipalmitate, glyceryl 1-monopalmitate, L-noradrenaline hydrochloride, 5-hydroxytryptamine creatine sulphate, tranlycypromine (*trans*-2-phenylcyclopropylamine), iproniazid (*N*-isonicotinoyl-*N'*-isopropylhydrazine) phosphate and pancreatic lipase (steapsin) were supplied by Sigma (London) Chemical Co. Ltd., London S.W.6, U.K. Glyceryl dipalmitate used in preparation of substrates was a mixture of the 1,2- and 1,3-diester. Glyceryl 1,2-dipalmitate and glyceryl 1,3-dipalmitate were supplied by Koch-Light Laboratories Ltd., Colnbrook, Bucks., U.K. Glyceryl 2-monopalmitate was supplied by Serdary Research Laboratories, Ontario, Canada. Bovine serum albumin was supplied by Armour Pharmaceutical Co. Ltd., Eastbourne, Sussex, U.K. Triton X-100 was supplied by Lennig Chemicals, Croydon, U.K. *N*-Benzyl-*N*-methylprop-2-nylamine (pargyline) was a gift from Abbott Laboratories Ltd., Queenborough, Kent, U.K. Brij 35 (polyoxyethylene lauryl alcohol), glyceryl tripalmitate and methylamine hydrochloride were supplied by BDH Chemicals Ltd., Poole, Dorset, U.K. Silicic acid (100 mesh) used for chromatography was manufactured by Mallinckrodt Chemical Works, St. Louis, Mo., U.S.A., and supplied by Camlab, Cambridge, U.K. Phosphor used for the determination of radioactivity by liquid-scintillation counting consisted of 0.6% (w/v) 2,5-diphenyloxazole and 0.15% (w/v) 1,4-bis-(5-phenyloxazol-2-yl)benzene dissolved in xylene. All solvents were redistilled before use.

Methods

Preparation of synaptosomes and synaptic fractions. Synaptosomes were prepared from combined cerebral cortex and cerebellum of guinea-pig brain by the method of Eichberg *et al.* (1964). They were osmotically ruptured by suspension in water (2ml/g of original tissue) at 0°C for 30min (Whittaker *et al.*, 1964). Synaptic membranes were prepared by the procedure of Rodriguez de Lores Arnaiz *et al.* (1967). Suspensions of fractions were diluted to 0.45M-sucrose with water. Synaptic vesicles were isolated as described by Lapetina *et al.* (1967). Vesicles at the top of the 0.5M-sucrose layer (M2A fraction) were collected and diluted to 0.32M-sucrose with water. The dilution of each fraction was based on the molarity of the lower layer of sucrose at the interface from which the fraction had been collected. Suspensions of fractions in diluted sucrose were stored at 0°C for 17h. Immediately before use they were centrifuged at 100000g and the sedimented pellets were resuspended in water.

Enzyme assays. Succinate dehydrogenase [succinate-(acceptor) oxidoreductase; EC 1.3.99.1] was determined by the method of Pennington (1961). Lactate dehydrogenase (L-lactate-NAD oxido-

reductase; EC 1.1.1.27) was determined by the method of Wroblewski & La Due (1955). Acetylcholinesterase (acetylcholine acetylhydrolase, EC 3.1.1.7) was determined by the method of Ellman *et al.* (1961).

Preparation of glyceryl di[1-¹⁴C]palmitate and glyceryl mono[1-¹⁴C]palmitate. Glyceryl di[1-¹⁴C]palmitate and glyceryl mono[1-¹⁴C]palmitate were prepared from the hydrolysis of glyceryl tri[1-¹⁴C]palmitate with pancreatic lipase. The following procedure, which was based on that described by Bockerhoff (1965), was used. Glyceryl tri[1-¹⁴C]palmitate (0.1μCi, specific radioactivity 86Ci/mol), mixed with glyceryl tripalmitate (11mg), was dissolved in hexane (0.50ml). The solution was mixed with Tris-HCl buffer (0.30ml, 1.0M, pH 7.4), Bromophenol Blue [0.10ml; 0.1% (w/v) aqueous solution] and CaCl₂ [0.50ml; 4% (w/v) aqueous solution of CaCl₂·6H₂O], and incubated at 37°C with shaking for 10min. Pancreatic lipase (50mg) suspended in 1.0M-Tris-HCl buffer (1.0ml, pH 7.4) was added and the incubation was continued for 30min. Hydrolysis was terminated by the addition of ethanol (1.0ml). The mixture was cooled to 0°C, made just acid to Bromophenol Blue by the addition of 1M-HCl, and immediately extracted with 3×5.0ml of light petroleum (b.p. 40–60°C). The combined extracts were dried over anhydrous Na₂SO₄.

The extract in light petroleum was evaporated to dryness in a rotary evaporator and the residue was redissolved in light petroleum (b.p. 40–60°C, 5.0ml). It was then chromatographed on a column (2.5cm×1cm diam.) of activated silica gel (2.0g). Glyceryl tri[1-¹⁴C]palmitate, glyceryl di[1-¹⁴C]palmitate and glyceryl mono[1-¹⁴C]palmitate were successively eluted with 100ml volumes of diethyl ether–light petroleum (b.p. 40–60°C) (2:23, v/v), diethyl ether–light petroleum (b.p. 40–60°C) (1:3, v/v), and diethyl ether, respectively. The purities of the products were checked every 14 days by t.l.c. on Kieselgel G (0.25mm thick) with the solvent mixture light petroleum (b.p. 40–60°C)–diethyl ether–acetic acid (90:53:7, by vol.). Areas of Kieselgel G in the developed chromatogram corresponding to the three glycerides and to palmitic acid were transferred to vials for the determination of radioactivity. Ethanol–water (1:1, v/v; 1.0ml) and phosphor–Triton X-100 (2:1, v/v; 9.0ml) were added and stirred. Radioactivity was determined by liquid-scintillation counting in a Nuclear–Chicago 720 or Luxor spectrometer. Counts were corrected to 100% efficiency by the two-channels-ratio method (Bruno & Christian, 1961). From the determination of radioactivity all components were found to have a purity greater than 99.5%.

Preparation of emulsions of glycerides. Emulsified radioactive tripalmitin was prepared by a modification of the procedure of Carter (1967) as follows. A

mixture of glyceryl tri[1-¹⁴C]palmitate (2–3 μ Ci) and non-radioactive glyceryl tripalmitate (20.2mg) dissolved in ethanol (5.0ml) at 65°C was injected rapidly through a fine-bore needle (16mm long $\times$ 0.5mm diam.), with a glass syringe of 1ml capacity (5mm bore), into water (5.0ml) at 65°C. Ethanol was subsequently removed by evaporation on a boiling-water bath. The emulsion was cooled to 0°C, immersed in crushed ice and sonicated at 18000–20000Hz for 5min. The final emulsion contained 5 μ mol of glyceryl tripalmitate/ml. Emulsions of glyceryl di[1-¹⁴C]palmitate were prepared in the same way, except that 1.0ml of a ethanolic solution of dipalmitin was injected into 3.0ml water and sonication was omitted. A non-radioactive mixture of glycerol 1,2-dipalmitate and glycerol 1,3-dipalmitate was used as a carrier. Emulsions of radioactive monopalmitin were prepared by injection of a mixture of glyceryl mono[1-¹⁴C]palmitate and non-radioactive glyceryl 1-monopalmitate, dissolved in 1.0ml of ethanol, into 5.0ml of water. Sonication was omitted. Emulsions of glyceryl tri[1-¹⁴C]palmitate were stable for at least 24h. Emulsions of glyceryl di[1-¹⁴C]palmitate and glyceryl mono[1-¹⁴C]palmitate were stable for 4h.

Determination of protein. Protein was determined by the method of Lowry *et al.* (1951) with bovine serum albumin as standard.

Determination of lipolytic activity. To 0.25ml of 0.2M-Tris-HCl buffer, pH7.4, tissue (0.03–4.60mg of protein) suspended in water was added. Emulsion of glyceride was added to give a final volume of 1.0ml. Unless otherwise stated, when glyceryl tri[1-¹⁴C]palmitate was the substrate 0.20ml of emulsion containing 1 μ mol was used. With glyceryl di[1-¹⁴C]palmitate and glyceryl mono[1-¹⁴C]palmitate 0.40ml of emulsion containing 2 μ mol of glyceride was used. The mixture was incubated with shaking (40 shakes/min, path-length 3.8cm) at 37°C for 60min unless stated otherwise. Lipolysis was terminated by the addition of 5.0ml of a mixture of propan-2-ol-iso-octane–0.25M-H₂SO₄ (40:10:1, by vol.) containing 1.5% (w/v) Brij 35, palmitic acid (5mg) and non-radioactive glyceride (5mg). The glyceride added was that used as non-radioactive carrier in the preparation of the substrate, and together with the palmitate acted as carrier for the extraction.

The mixture was separated into two phases by the addition of 2.0ml of 0.25M-H₂SO₄ and 4.0ml of iso-octane. The upper layer was removed and the lower aqueous layer was extracted twice with 5.0ml of iso-octane. The upper layer, combined with the extracts of the lower layer, was concentrated to dryness. The residue was dissolved in 5.0ml of ethanol-water (1:1, v/v). [1-¹⁴C]Palmitate was isolated as described by Rowe (1964), by using phenolphthalein and Methyl Red as indicators. The [1-¹⁴C]palmitic acid was determined by measurement of radioactivity

by scintillation counting in 5.0ml of the phosphor. In all assays blanks were determined by using tissue that had been immersed in boiling water for 60min. The values obtained from the blanks were subtracted from the experimental values.

Preparation of tissue with monoamine oxidase inhibitors and amines. In experiments with monoamine oxidase inhibitors (iproniazid phosphate, tranlylcypromine or pargyline), tissue suspended in water was mixed with 0.2M-Tris-HCl buffer, pH7.4. The mixture was preincubated for 10min with inhibitor and then for a further 10min with amine inhibitor plus amine before the addition of substrate. The following procedures were used. (1) In the determination of the hydrolysis of glyceryl tripalmitate by synaptosomes, tissue suspended in 0.55ml of water was mixed with 0.05ml of buffer. Inhibitor dissolved in 0.10ml of buffer was added and the mixture incubated at 37°C, with shaking for 10min. Amine dissolved in 0.10ml of buffer was added and mixed and the incubation was continued for 10min. Emulsion of glyceryl tripalmitate (0.20ml; 1 μ mol) was then added and mixed and the incubation continued for a further 60min. (2) With synaptic membranes and vesicles, tissue was suspended in 0.35ml of water and then mixed with 0.25ml of buffer. Inhibitor and amine, both dissolved in water, and substrate were added as described in (1) above. (3) In the determination of the hydrolysis of glyceryl dipalmitate and glyceryl monopalmitate by synaptosomes, and the hydrolysis of glyceryl dipalmitate by synaptic membranes, the procedure was as described in (2) above except that tissue was suspended in 0.15ml of water and 0.40ml of glyceryl dipalmitate (2 μ mol) or glyceryl monopalmitate (2 μ mol) was added instead of glyceryl tripalmitate.

Statistical treatment of results

Values given are means from independent incubations $\pm$ s.d., with the numbers of incubations given in parentheses. The significance of stimulation by amines was calculated from Student's *t* test. The upper values of *P* are quoted. Values of *P* > 0.05 were considered to be not significant. With duplicate determinations the means $\pm$ the average error are given.

Results and Discussion

Determination of hydrolysis

Hydrolysis of glycerides was measured by the determination of [1-¹⁴C]palmitate released from emulsions of [1-¹⁴C]palmitoyl esters of glycerol. Emulsifying agents were not used in the preparation of emulsions because of their likely effects on both membrane structure and the activities of membrane-bound enzymes. Glyceryl di[1-¹⁴C]palmitate was

prepared from the hydrolysis of glyceryl tri[1-¹⁴C]-palmitate by pancreatic lipase. This would be expected to give predominantly glyceryl 1,2-di[1-¹⁴C]-palmitate (Borgström, 1954; Mattson & Beck, 1956; Savary & Desnuelle, 1956; Entressangles *et al.*, 1966) with some isomerization to glyceryl 1,3-di[1-¹⁴C]palmitate (cf. Entressangles *et al.*, 1966). Glyceryl 1,2-dipalmitate alone gave poor emulsions under the conditions used. For the assay of lipase activity glyceryl di[1-¹⁴C]palmitate was diluted with non-radioactive glyceryl 1,2-dipalmitate mixed with glyceryl 1,3-dipalmitate. Changing the proportions of the non-radioactive isomers did not affect the hydrolysis of glyceryl di[1-¹⁴C]palmitate (Expt. 1, Table 1). When glyceryl 1,3-dipalmitate alone was used as a diluent the proportion of glyceryl di[1-¹⁴C]-palmitate hydrolysed was independent of the amount of glyceryl di[1-¹⁴C]palmitate present (Expt. 2, Table 1). Thus under the conditions of assay of lipase activity, glyceryl 1,3-dipalmitate and glyceryl 1,2-dipalmitate were equivalent diluents and were hydrolysed at the same rate. Calculations of lipase activity were made assuming this to be so. The apparent lack of stereospecificity could have been due to isomerization being more rapid than hydrolysis. Glyceryl mono[1-¹⁴C]palmitate was also prepared from the hydrolysis of glyceryl tri[1-¹⁴C]palmitate with pan-

creatic lipase. As shown in Table 2 it was hydrolysed at the same rate whether mixed with glyceryl 2-palmitate or glyceryl 1-palmitate. Mattson & Volpenhein (1962) have shown that isomerization between 1- and 2-monoglycerides takes place readily.

The subcellular distributions of lipolytic activities with respect to the hydrolysis of glyceryl tripalmitate and glyceryl dipalmitate are shown in Table 3. The probable contents of the subcellular tissue fractions were confirmed by the distributions of lactate dehydrogenase, succinate dehydrogenase and acetylcholine esterase activities. The hydrolysis of glyceryl tripalmitate was low in the microsomal and supernatant fractions (specific activities relative to unfractionated homogenate were 0.39 and 0.11 respectively). There was some concentration of lipolytic activity, when glyceryl dipalmitate was the substrate, in the mitochondrial fraction (specific activity relative to unfractionated homogenate was 1.65) and there was appreciable activity in the supernatant fraction (specific activity relative to unfractionated homogenate was 0.26). The recovery of lipolytic activity from subcellular fractionation was 89 and 95% for the hydrolysis of glyceryl tripalmitate and glyceryl dipalmitate respectively.

Attention was concentrated on the lipases of isolated nerve endings (synaptosomes). Synaptosomes

Table 1. *Hydrolysis of glyceryl 1,2-dipalmitate and glyceryl 1,3-dipalmitate by osmotically ruptured synaptosomes*

Mixtures containing 2.0 μ mol of non-radioactive dipalmitin were incubated for 60 min. Results are for duplicate incubations. Expt. 1: glyceryl di[1-¹⁴C]palmitate (specific radioactivity 0.38 Ci/mol) was mixed with mixtures of glyceryl 1,2-dipalmitate and glyceryl 1,3-dipalmitate. Expt. 2: different amounts of glyceryl di[1-¹⁴C]palmitate (specific radioactivity 2.1 Ci/mol) were mixed with glyceryl 1,3-dipalmitate. Values for palmitate released were calculated by assuming identical rates of hydrolysis for glyceryl 1,2-dipalmitate and glyceryl 1,3-dipalmitate.

Expt.	Protein (mg)	Glyceryl dipalmitate			[¹⁴ C]Palmitate ester released (%)	Palmitate released (μmol/h per g of protein)
		¹⁴ C-labelled (nmol)	Non-radioactive (%)			
			1,2-	1,3-		
1	0.81	10	0	100	21.4±1.1	528±28
		10	10	90	19.3±0.7	477±18
		10	20	80	24.4±0.3	601±6
		10	30	70	19.8±0.7	490±17
		10	Mixture*		20.6±1.0	508±24
2	0.71	1.96	0	100	20.6±0.9	580±25
		3.06	0	100	24.3±0.3	685±8
		5.56	0	100	23.9±0.8	673±23
		7.86	0	100	20.1±0.6	566±17
		6.03	0	100	20.7±0.1	583±3

* Mixture of glyceryl 1,2-dipalmitate and glyceryl 1,3-dipalmitate used in all other experiments for the dilution of glyceryl di[1-¹⁴C]palmitate in the preparation of emulsions of substrate.

Table 2. Comparison of the hydrolyses of glyceryl 1-monopalmitate and glyceryl 2-monopalmitate by osmotically ruptured synaptosomes

Glyceryl mono[1-¹⁴C]palmitate (specific radioactivity 0.11 Ci/mol; 7.6×10^4 d.p.m. or 6.7×10^4 d.p.m.) was mixed with non-radioactive glyceryl 1-monopalmitate or glyceryl 2-monopalmitate respectively. Each incubation contained 0.038 mg of protein and 2 μ mol of glyceryl monopalmitate. Values are means of two independent determinations. Palmitate released was calculated by assuming identical rates of hydrolyses of radioactive and non-radioactive glyceryl monopalmitate.

Incubation time (min)	Glyceryl mono[1- ¹⁴ C]palmitate hydrolysed (%)		Palmitate released (mmol/g of protein)	
	Mixed with glyceryl 1-palmitate	Mixed with glyceryl 2-palmitate	Mixed with glyceryl 1-palmitate	Mixed with glyceryl 2-palmitate
30	9.6 $\pm$ 0.3	10.2 $\pm$ 2.4	5.1 $\pm$ 0.1	5.4 $\pm$ 1.3
60	19.2 $\pm$ 1.2	18.9 $\pm$ 1.3	10.1 $\pm$ 0.6	9.9 $\pm$ 0.7

Table 3. Subcellular distributions of triglyceride lipase and diglyceride lipase

Subcellular fractions were prepared by the method of Eichberg *et al.* (1964). Hydrolyses of glyceryl tripalmitate and glyceryl dipalmitate were measured in separate experiments involving separate fractionations of combined cortex and cerebellum. Relative specific activity is relative to that of the homogenate. Values are means of duplicate determinations.

Fraction	Probable content	Protein recovered (%)	Hydrolysis of glyceryl tripalmitate		
			(% of activity recovered)	(μ mol of palmitate released/h per g of protein)	Relative specific activity
Homogenate	—	—	—	13.4	1.00
P1	Nuclei, large myelin and cell debris	59.1	77.2	17.0	1.26
P2A	Small myelin	6.3	5.7	11.8	0.88
P2B	Nerve endings	10.2	11.1	14.2	1.06
P2C	Mitochondria	2.5	2.7	13.8	1.03
P3	Microsomal fraction	1.9	0.8	5.3	0.39
S3	Cytoplasm	19.9	2.4	1.5	0.11
Total recovery (%)		91.3	88.8	—	—
Fraction	Probable content	Protein recovered (%)	Hydrolysis of glyceryl dipalmitate		
			(% of activity recovered)	(μ mol of palmitate released/h per g of protein)	Relative specific activity
Homogenate	—	—	—	174.6	1.00
P1	Nuclei, large myelin and cell debris	42.5	49.9	194.3	1.11
P2A	Small myelin	12.9	17.1	219.3	1.26
P2B	Nerve endings	13.4	15.0	184.1	1.05
P2C	Mitochondria	4.8	8.2	288.8	1.65
P3	Microsomal fraction	3.2	3.4	178.9	1.02
S3	Cytoplasm	23.2	6.3	44.9	0.26
Total recovery (%)		100	94.7	—	—

that had been incubated for 1 h in 0.154 M-Tris-HCl buffer, pH 7.4, and collected by centrifugation retained $78 \pm 1\%$ (mean $\pm$ S.D.; four determinations) of their lactate dehydrogenase activity and $99 \pm 0\%$ of their protein. Assuming that 10% of the wet weight

of synaptosomes was protein, that 50% of the volume of the synaptosomes was occupied by cytoplasmic lactate dehydrogenase, and that the density of synaptosomes is 1.0, the calculated concentration of lactate dehydrogenase in the synaptosomes at the

Fig. 1. Effects of concentration of protein on the glyceride lipases of synaptosomes

(a) Hydrolysis of glyceryl tripalmitate. (b) Hydrolysis of glyceryl dipalmitate. (c) Hydrolysis of glyceryl monopalmitate; tissue was incubated with $1\text{ }\mu\text{mol}$ of glyceryl mono[$1\text{-}^{14}\text{C}$]palmitate for 10 min. Δ , Synaptosomes incubated in 0.154M -Tris-HCl buffer, pH 7.4; $\circ$, osmotically ruptured synaptosomes incubated in 0.05M -Tris-HCl buffer, pH 7.4.

end of the incubation was 186 ± 10 times that in the incubation medium. Thus the synaptosomes had retained a degree of intactness. Synaptosomes incubated in 0.154M buffer are referred to subsequently as intact synaptosomes. The hydrolysis of glyceryl tripalmitate by intact synaptosomes increased linearly with protein up to a protein concentration of 2.2 mg/ml , as shown in Fig. 1(a). In this range of protein concentration palmitate was released at a rate of $3.8\text{ }\mu\text{mol/h}$ per g of protein. Increase of protein beyond a concentration of 2.2 mg/ml had little effect on hydrolysis.

Osmotically ruptured synaptosomes hydrolysed glyceryl tripalmitate more rapidly than did intact synaptosomes (Table 4, Fig. 1a). The rate of hydrolysis in the experiment shown in Fig. 1(a) could be expressed as $(15.3 + 3.2p)\text{ nmol/h}$ where p was the concentration of protein in mg/ml . In an experiment with unfractionated homogenate, in the range $p = 0.8\text{--}5.7\text{ mg/ml}$, the rate of hydrolysis was $(8.0 + 11.2p)\text{ nmol/h}$. In these experiments there appeared

to be a component of lipolysis which was independent of protein concentration. This phenomenon with tissue fractions containing synaptosomes must be kept in mind in the interpretation of Table 3.

It has been shown previously (see Michaelson *et al.*, 1963; Zieher & De Robertis, 1963; Maynert *et al.*, 1964) that osmotic rupture of crude preparations of nerve-ending particles can result in the release of 25–70% of bound amine (noradrenaline and 5-hydroxytryptamine). At the same time some amine remains associated with vesicle fractions (Zieher & De Robertis, 1963; Maynert *et al.*, 1964). A possible explanation of the relationships between the activity of triglyceride lipase and protein concentration, indicated above, is that lipase is activated by endogenous amine in solution. If this were so then the equilibria between bound and free amine would be such that the concentration of unbound amine was relatively unchanged.

In contrast to the hydrolysis of glyceryl tripalmitate the hydrolysis of glyceryl dipalmitate by ruptured

Table 4. *Effect of noradrenaline, 5-hydroxytryptamine and adrenaline on the hydrolysis of glyceryl tripalmitate by osmotically ruptured synaptosomes*

Expts. 1 and 2: glyceryl tri[1-¹⁴C]palmitate and noradrenaline were added to the tissue at the same time. Amount of protein was 4.60mg and 4.13mg respectively. (In Expt. 1 50mg of bovine serum albumin was present.) Expts. 3, 4 and 5: tissue was preincubated with iproniazid phosphate (final concentration 0.36mM) and amine, as described in the text. Amount of protein was 2.98mg, 2.36mg and 2.75mg respectively. Expt. 6: tissue was preincubated with tranlylcypromine (final concentration 0.50mM) and amine. Amount of protein was 3.16mg. Concentrations of amine given are those in the completely constituted incubation mixture.

Expt.	Additions	Palmitate released ($\mu\text{mol/h}$ per g of protein)	Increase in release of palmitate due to amine (%)	Significance (<i>P</i>)
1	None	4.3 ± 0.2 (5)	—	—
	Noradrenaline (1.00mM)	6.3 ± 0.2 (5)	49	0.001
2	None	9.6 ± 0.95 (4)	—	—
	Noradrenaline (0.01mM)	11.3 ± 0.2 (4)	18	0.02
	Noradrenaline (0.10mM)	11.7 ± 0.7 (4)	22	0.02
	Noradrenaline (1.00mM)	12.2 ± 0.4 (4)	27	0.01
3	None	15.2 ± 0.9 (4)	—	—
	Noradrenaline (1.00mM)	17.2 ± 0.9 (4)	13	0.001
	Iproniazid phosphate	11.2 ± 1.3 (4)	—	—
	Iproniazid phosphate+nor- adrenaline (1.00mM)	14.4 ± 1.2 (4)	28	0.01
	Iproniazid phosphate+5-hydroxy- tryptamine (1.00mM)	13.9 ± 1.2 (4)	24	0.001
4	Iproniazid phosphate	14.3 ± 0.3 (4)	—	—
	Iproniazid phosphate+adrenaline (1.00mM)	18.8 ± 0.9 (4)	31	0.001
5	Iproniazid phosphate	8.7 ± 0.5 (4)	—	—
	Iproniazid phosphate+5-hydroxy- tryptamine (1.00mM)	20.7 ± 1.01 (4)	137	0.001
	Iproniazid phosphate+nor- adrenaline (1.00mM)	9.9 ± 0.2 (4)	13	0.001
6	None	10.4 ± 0.5 (4)	—	—
	Tranlylcypromine	10.0 ± 0.8 (4)	—	—
	Tranlylcypromine+noradrenaline (1.00mM)	13.2 ± 0.8 (4)	32	0.001
	Tranlylcypromine+methylamine (1.00mM)	10.1 ± 0.5 (4)	1	Not significant

synaptosomes was proportional to the concentration of protein. In the experiment shown in Fig. 1(b) the rates of release of palmitate were $304 \mu\text{mol/h}$ per g of protein and $494 \mu\text{mol/h}$ per g of protein for intact and ruptured synaptosomes respectively. Thus osmotic rupture of the synaptosomes increased hydrolysis by 63%.

The variation of the hydrolysis of glyceryl monopalmitate by osmotically ruptured synaptosomes with concentration of protein is shown in Fig. 1(c). Dilution of the synaptosomes increased the specific activity with respect to protein. The logarithm of the rate of release of palmitate varied linearly with the logarithm of the protein concentration (Fig. 1c). The

relationship can be expressed in the form $S = Ap^{-B}$ where S is the specific activity in nmol of palmitate released/h per mg of protein, p is the concentration of protein in mg/ml, and A and B are constants. In the experiment shown A was 6×10^3 and B was 0.2.

The variations of the rates of hydrolysis of glyceryl tripalmitate and glyceryl dipalmitate by osmotically ruptured synaptosomes with concentration of substrate are shown in Fig. 2. The hydrolyses exhibited features of saturation kinetics. The apparent K_m values of glyceryl tripalmitate and glyceryl dipalmitate were $1.5 \mu\text{mol/ml}$ and $0.5 \mu\text{mol/ml}$ respectively. The corresponding apparent maximum velocities were 38 and $630 \mu\text{mol}$ of palmitate released/h per g

Fig. 2. Effect of concentration of substrate on triglyceride lipase and diglyceride lipase of osmotically ruptured synaptosomes

Values are means of duplicate determinations. (a) Triglyceride lipase; amount of protein was 3.15 mg. (b) Diglyceride lipase; amount of protein was 0.45 mg, and incubations were done for 30 min.

of protein, respectively. Concentrated emulsions were relatively unstable, and substrate concentrations of 1.0 μmol of glyceryl tripalmitate/ml and 2.0 μmol of glyceryl dipalmitate/ml were used for determinations of lipolytic activity. Although these concentrations did not correspond to zero-order kinetics they were such that hydrolyses were relatively insensitive to substrate concentration. In one experiment with brain homogenate containing 4.9 mg of protein the rates of release of palmitate from emulsions containing 1.0, 2.0 and 3.0 μmol of glyceryl tripalmitate/ml respectively were 14.1 ± 0.6 , 14.0 ± 0.5 and $14.1 \pm$

0.7 μmol/h per g of protein (mean $\pm$ average error for duplicate determinations).

The release of [1-¹⁴C]palmitate from glyceryl tri-[1-¹⁴C]palmitate in the presence of osmotically ruptured synaptosomes was linear for 80 min. When the monoamine oxidase inhibitor, iproniazid phosphate, was present the release was linear for 150 min (Fig. 3). The hydrolyses of glyceryl dipalmitate and glyceryl monopalmitate were linear for 120 min and 60 min respectively.

The rates of hydrolysis were in the increasing order glyceryl tripalmitate, glyceryl dipalmitate, glyceryl monopalmitate, as has been found in other tissues. With glyceryl tripalmitate and glyceryl dipalmitate absolute rates of hydrolysis are unknown, since the partial esters of glycerol produced could have been hydrolysed further during the incubation producing palmitate. The order of the rates of hydrolysis was such that the first step in the hydrolysis of a pure substrate was rate-limiting, and thus determined the characteristics of the hydrolysis.

The variations of the rates of hydrolyses of the substrates with pH are shown in Fig. 4. The pH optima for the hydrolyses of glyceryl tripalmitate and glyceryl dipalmitate were between 7 and 8. The pH optimum for the hydrolysis of glyceryl monopalmitate was close to 8. The pH optimum of triglyceride lipase was the same as that reported by Carter (1967) for rat hepatic lipase, and close to that reported by Gorin & Shafir (1964) for adipose tissue. It was similar to that of hormone-sensitive lipase in adipose tissue (Vaughan *et al.*, 1964; Hollett & Auditore, 1967) and was slightly lower than that reported for hepatic microsomal lipase by Guder *et al.* (1969). It was easily distinguishable from the lysosomal lipase (pH optimum 4.2) of rat liver and kidney (Mahadevan & Tappel, 1968), and the lipase (pH optimum 4.8) of polymorphonuclear leucocytes (Elsbach & Kayden, 1965). The pH optimum of monoglyceride lipase was close to that reported by Vaughan *et al.* (1964) and Gorin & Shafir (1964) for adipose tissue.

Stimulation by hydrolysis by amines

Noradrenaline stimulated the hydrolysis of glyceryl tripalmitate by ruptured synaptosomes (Expts. 1 and 2, Table 4). In the presence of iproniazid phosphate noradrenaline and 5-hydroxytryptamine stimulated the hydrolysis of glyceryl tripalmitate by both intact and ruptured synaptosomes (Table 5). With ruptured synaptosomes iproniazid phosphate itself decreased hydrolysis which could then be increased by noradrenaline, 5-hydroxytryptamine or adrenaline (Table 4). In another experiment iproniazid phosphate (0.36 mM) decreased the hydrolysis of glyceryl dipalmitate by ruptured synaptosomes from 366 ± 23

Fig. 3. Time-course of glyceride lipases of osmotically ruptured synaptosomes

(a) Triglyceride lipase; amount of protein was 3.08mg. (b) Triglyceride lipase; amount of protein was 3.35mg. Tissue was preincubated for 20min with 1.80mM-iproniazid phosphate before the addition of glyceryl tri[1- ^{14}C]palmitate. (c) Diglyceride lipase; amount of protein was 0.53mg. (d) Monoglyceride lipase; amount of protein was 0.034mg; and of glyceryl mono[1- ^{14}C]palmitate was 1 μmol .

(four determinations) to $264 \pm 12 \mu\text{mol}$ of palmitate released/h per g of protein (decrease of 28%; $P < 0.001$). For these reasons in later experiments tranilcypromine was used. At the concentration used (0.50mM) tranilcypromine was without effect on the hydrolyses of glyceryl tripalmitate and glyceryl

dipalmitate by ruptured synaptosomes (Tables 4 and 7 respectively), or by preparations of synaptic membranes. In the presence of tranilcypromine nor-adrenaline stimulated the hydrolysis of glyceryl tri-palmitate by homogenate, synaptosomes and mitochondria (Table 6). It diminished slightly hydrolysis

by the microsomal fraction. With synaptosomes methylamine was without effect on the hydrolysis of glyceryl tripalmitate (Table 4) indicating that stimulations were not due to non-specific effects of amine groups.

Noradrenaline, 5-hydroxytryptamine and adrenaline, in the presence of either tranlycypromine or iproniazid phosphate, stimulated the hydrolysis of glyceryl dipalmitate by osmotically ruptured synaptosomes (Table 7). The relative stimulation was small (15–33%), but the stimulation expressed as μmol of palmitate released/h per g of protein was approx. 10 times the stimulation of the hydrolysis of glyceryl tripalmitate.

No stimulations of the hydrolysis of glyceryl tripalmitate were detected with 1.00mM-glutamate, 1mM- γ -aminobutyrate or 1mM-acetylcholine in the presence of 0.10mM-eserine. The hydrolysis of glyceryl dipalmitate was not stimulated by acetylcholine (0.10mM) in the presence of eserine (0.10mM) and the hydrolysis of glyceryl monopalmitate was not stimulated by 1.00mM-noradrenaline or -5-hydroxytryptamine.

The experiments with synaptosomes were complicated by multiple pools of bound and unbound endogenous amines, and by mitochondrial monoamine oxidase, and possibly by catecholamine *O*-methyltransferase. As indicated above it is likely that the endogenous amines contribute to the stimulation of the hydrolysis of glyceryl tripalmitate. Further experiments were performed on isolated synaptic membranes, and the results are shown in Table 8. Noradrenaline and 5-hydroxytryptamine stimulated the hydrolysis of glyceryl tripalmitate by all fractions of membranes. The greatest stimulations (142 and 70%) were obtained with the fraction [M1 (0.9); see Rodriguez de Lores Arnaiz *et al.* (1967)] which had the highest acetylcholinesterase activity, the lowest succinate dehydrogenase activity, and was low in lactate dehydrogenase activity. The vesicle fraction exhibited very high lipolytic activity when this was calculated with respect to protein content. This may have been due in part to the effect of endogenous amine. The lipolytic activity of vesicles was diminished by added 5-hydroxytryptamine.

The effect of different concentrations of noradrenaline, in the presence of tranlycypromine, on

the hydrolysis of glyceryl tripalmitate by synaptic membranes was investigated. The results from a typical experiment are shown in Fig. 5(a). In all experiments lipolysis was inhibited by 10mM-noradrenaline and stimulated in the range 0.1–1mM-noradrenaline. In some experiments 1 μM -noradrenaline decreased hydrolysis. The effect of the concentration of noradrenaline on the hydrolysis of glyceryl dipalmitate by synaptic membranes is shown in Fig. 5(b). There was stimulation in the concentration range 1 μM –1mM. Decrease of hydrolysis by 10mM-noradrenaline was not detected.

Aging of synaptic membranes diminished lipolytic activity. In one experiment combined M1 (0.9) and M1 (1.0) fractions, freshly prepared, hydrolysed glyceryl tripalmitate at a rate of $31.3 \pm 1.8 \mu\text{mol}$ of palmitate released/h per g of protein (mean $\pm$ s.d., three determinations). After storage of the membranes in water at 0°C for 17h the activity had declined to $25.2 \pm 0.9 \mu\text{mol}$ /h per g of protein. During further incubation at 37°C for 2h the activity declined further to $18.2 \pm 1.0 \mu\text{mol}$ /h per g of protein. Membranes aged at 0°C when incubated with 0.5mM-pargyline had an activity of $29.2 \pm 3.0 \mu\text{mol}$ /h per g of protein, which in the presence of 0.1mM-noradrenaline was increased to $41.8 \pm 2.5 \mu\text{mol}$ /h per g of protein. Thus lipolytic activity decreased by aging could be restored by noradrenaline.

Noradrenaline and 5-hydroxytryptamine are putative transmitter substances in brain (Curtis, 1969). Adrenaline is found in the brain of lizard and frog (Brodie & Bogdanski, 1964). Assuming transmitter functions for noradrenaline and 5-hydroxytryptamine the possible relevance of the observations described above to synaptic transmission *in vivo* would depend on whether the concentrations of unbound noradrenaline and 5-hydroxytryptamine during transmissions are similar to those present in the experiments described. It would also depend on whether the hydrolyses of endogenous glycerides are stimulated in a manner similar to that shown for exogenous glycerides. The concentration of transmitter in the synaptic cleft during transmission is not known. The following calculations are an attempt to obtain an estimate. The mean equivalent spherical diameter, d , of synaptosomes from guinea-pig brain prepared by the methods used in this investigation has been

Fig. 4. Variation of the activities of glyceride lipases with pH

Buffers (0.05M) used were: pH 3, 4 and 5, potassium hydrogen phthalate-HCl; pH 6 and 7, sodium phosphate; pH 7.4, 8 and 9, Tris-HCl. (a) $\circ$, Triglyceride lipase of osmotically ruptured synaptosomes; amount of protein was 3.54mg; Δ , triglyceride lipase of homogenate; amount of protein was 4.18mg. (b) Diglyceride lipase of osmotically ruptured synaptosomes; amount of protein was 0.642mg, and the incubation time was 30min. (c) Monoglyceride lipase of osmotically ruptured synaptosomes; amount of protein was 0.053mg, and the incubation time was 20min.

Table 5. Comparison of the effects of noradrenaline and 5-hydroxytryptamine on intact and osmotically ruptured synaptosomes

Synaptosomes containing 2.0–3.5 mg of protein were preincubated with iproniazid phosphate in Tris-HCl buffer, pH 7.4, and then incubated with glycyl tri[1-¹⁴C]palmitate. Final concentrations of iproniazid phosphate and amine were 0.36 mM and 1.00 mM respectively. With intact synaptosomes the final concentration of buffer was 0.154 M. Values are means of four incubations. The lipolytic activities of the controls containing osmotically ruptured synaptosomes were significantly higher than those containing intact synaptosomes (Expt. 1, 47%; $P < 0.01$; Expt. 2, 57%; $P < 0.01$).

Expt.	Amine	Palmitate released ($\mu\text{mol/h}$ per g of protein)					
		Intact synaptosomes			Osmotically ruptured synaptosomes		
		(a)	(b)	$\frac{(b)-(a)}{(a)}$ (%)	(a)	(b)	$\frac{(b)-(a)}{(a)}$ (%)
1	Noradrenaline	Control 14.5 $\pm$ 1.0	Amine present 21.1 $\pm$ 0.8	45	Control 21.3 $\pm$ 2.2	Amine present 28.0 $\pm$ 1.3	31
2	5-Hydroxytryptamine	9.5 $\pm$ 0.5	11.3 $\pm$ 0.1	19	14.9 $\pm$ 0.6	21.0 $\pm$ 1.6	41
				Significance (P)			Significance (P)
				0.001			0.01
				0.05			0.01

Table 6. *Effect of noradrenaline on the hydrolysis of glyceryl tripalmitate by different subcellular fractions of guinea-pig brain*

Subcellular fractions were prepared from combined cerebral cortex and cerebellum by the procedure of Eichberg *et al.* (1964). Experimental fractions were preincubated for 20min with tranlycypromine and for 10min with 1 mM-noradrenaline as described in the text, before the addition of glyceryl tripalmitate. The control fractions were preincubated for 20min with tranlycypromine only. Values are means of three independent incubations.

Fraction	Protein (mg)	Palmitate released ($\mu\text{mol/h}$ per g of protein)		(b)–(a) (%)	Significance (P)
		(a) Control	(b) Noradrenaline present		
Homogenate	1.58	13.2 $\pm$ 0.3	15.9 $\pm$ 0.4	+20	0.01
Synaptosomes	3.02	9.9 $\pm$ 0.1	12.3 $\pm$ 0.3	+25	0.001
Mitochondria	2.34	15.9 $\pm$ 0.5	18.3 $\pm$ 0.7	+15	0.02
Microsomal fraction	2.30	6.7 $\pm$ 0.2	5.9 $\pm$ 0.2	–12	0.02

shown to be 0.50 μm (Clementi *et al.*, 1966; Whittaker, 1968). Thus assuming the synaptosomes to be spheres

the mean volume of a synaptosome is given by $\frac{\pi}{6} \cdot d^3$ and is 6.55×10^{-14} ml. The content of noradrenaline in mouse brain synaptosomes, again isolated by the same procedures as that used in the present work, has been reported as 10.5 $\mu\text{g/g}$ of protein (Kuriyama & Speken, 1970). Assuming that the density of a synaptosome is 1.0, and 10% of its wet weight is protein, then the concentration of noradrenaline is 0.0062 $\mu\text{mol/ml}$ of synaptosomes. Applying this value to guinea-pig synaptosomes the mean concentration per synaptosome is $6.20 \times 10^{-9} \times 6.55 \times 10^{-14} = 4.06 \times 10^{-22}$ mol. Only a fraction, f , of the preparation will consist of synaptosomes containing noradrenaline and the concentration in these synaptosomes will be $4.06 \times 10^{-22}/f$ mol per synaptosome. If one-tenth of this were discharged during transmission the mean amount of noradrenaline in the synaptic gap would be $4.06 \times 10^{-23}/f$ mol per synaptosome engaged in transmission.

From electron microscopy of tissue sections it is known that synaptic gaps in rat brain cerebellar cortex are of the order of 20nm wide (Palay, 1958). If nerve endings of guinea-pig brain *in situ* are considered to be spheres two-thirds surrounded by a gap 20nm wide, then the volume of a single gap is given by $\frac{2}{3} \cdot \frac{\pi}{6} \cdot (d + 4 \times 10^{-6})^3 - d^3$ ml. Substituting $d = 0.5 \times 10^{-4}$ cm this volume = 1.13×10^{-14} ml. The mean concentration of noradrenaline in such a gap would thus be $(4.06 \times 10^{-23})/(f \times 1.13 \times 10^{-14})$ mol/ml = $3.6/f$ μM . Thus if f were equal to 0.3 its concentration would be 0.012mM. Stimulation of lipolysis by synaptosomes and synaptic membranes has

been observed by the addition of 0.01 mM-noradrenaline (Table 4, Expt. 2 and Fig. 5). The reported concentration of 5-hydroxytryptamine in mouse brain synaptosomes is 40 $\mu\text{g/g}$ of protein (Kuriyama & Speken, 1970). The same calculation applied to 5-hydroxytryptamine gives a concentration of 0.044mM. Stimulation of lipolysis was obtained by the addition of 1mM-5-hydroxytryptamine (Tables 4, 5, 7 and 8). The above calculations, which are necessarily tentative and approximate because of the assumptions and approximations made, support the hypothesis that endogenous concentrations of amines can influence enzymic activity in synaptic membranes.

In adipose tissue the hydrolysis of endogenous glycerides is stimulated by noradrenaline (see, e.g., Vaughan *et al.*, 1964), or adrenaline (Rizack, 1961; Vaughan *et al.*, 1964; Strand *et al.*, 1964; Rubinstein *et al.*, 1964; Hollett & Auditore, 1967; Mosinger, 1969; Wade *et al.*, 1970) and, in the presence of a monoamine oxidase inhibitor or phosphodiesterase inhibitor, by 5-hydroxytryptamine (Bieck *et al.*, 1966). Hormone stimulation of the hydrolysis of exogenous triglyceride and diglyceride by a partially purified lipase from adipose tissue has been reported (Huttunen *et al.*, 1970). Thus lipases in brain behave similarly with respect to stimulation by amines to the lipases in adipose tissue. In adipose tissue stimulation is mediated by cyclic AMP (Rizack, 1964; Butcher *et al.*, 1965). In brain the stimulation is located in preparations of synaptic membranes (Table 8). Adenylate cyclase is concentrated in synaptosomes and synaptic membranes (De Robertis *et al.*, 1967; Krishna *et al.*, 1970). Moreover noradrenaline increases the concentration of cyclic AMP in cortical slices of rat brain (Krishna *et al.*, 1970; Rall &

Table 7. *Effects of noradrenaline, 5-hydroxytryptamine and adrenaline on the hydrolysis of glyceryl dipalmitate by osmotically ruptured synaptosomes*
 Samples were preincubated with monoamine oxidase inhibitor (tranlycypromine or iproniazid phosphate) and amine as described in the text. Amount of protein was 0.68 mg and 0.64 mg for Expts. 1 and 2 respectively. Concentrations in the finally constituted mixtures were: tranlycypromine, 0.5 mM; iproniazid phosphate, 0.36 mM; noradrenaline, 1.0 mM; 5-hydroxytryptamine, 1.0 mM; and adrenaline, 1.0 mM.

Expt. 1					Expt. 2				
Additions	Palmitate released ($\mu\text{mol/h}$ per g of protein)	Increase in release of palmitate due to amine (%)	Significance (P)	Additions	Palmitate released ($\mu\text{mol/h}$ per g of protein)	Increase in release of palmitate due to amine (%)	Significance (P)		
None	355 $\pm$ 9	—	—	Iproniazid phosphate	303 $\pm$ 8	—	—		
Tranlycypromine	360 $\pm$ 13	—	—	Iproniazid phosphate + noradrenaline	355 $\pm$ 8	17	0.001		
Tranlycypromine + noradrenaline	450 $\pm$ 10	25	0.001	Iproniazid phosphate + 5-hydroxytryptamine	366 $\pm$ 8	21	0.001		
Tranlycypromine + 5-hydroxytryptamine	479 $\pm$ 13	33	0.001	Iproniazid phosphate + adrenaline	347 $\pm$ 8	15	0.001		
Tranlycypromine + adrenaline	409 $\pm$ 10	14	0.001						

Table 8. *Effect of amines on the hydrolysis of tripalmitin by synaptic membranes and vesicles*

For noradrenaline, values are means $\pm$ s.d. of three independent incubations containing 0.54–1.97 mg of protein. For 5-hydroxytryptamine, values are means of four incubations containing 0.32–0.64 mg of protein. Relative specific activity = specific activity of fraction/specific activity of homogenate. For the naming of fractions see Rodriguez de Lores Arnaiz *et al.* (1967).

Fraction	Probable content	Palmitate released ($\mu\text{mol/h}$ per g of protein)		$\frac{(b)-(a)}{(a)}$ (%)	Signifi- cance (<i>P</i>)	Relative specific activity		
		(a) Control	(b) Amine present			Acetyl- choline esterase	Succinate dehydro- genase	Lactate dehydro- genase
M1 (0.9)	Synaptic membranes	10.6 $\pm$ 1.0	25.6 $\pm$ 7.8	+142	0.05	2.95	0.10	0.08
M1 (1.0)	Synaptic membranes	13.3 $\pm$ 0.7	20.3 $\pm$ 0.3	+53	0.05	2.02	0.41	0.04
M1 (1.2)	Synaptic membranes + mitochondria	9.7 $\pm$ 0.5	13.2 $\pm$ 0.8	+36	0.001	0.42	1.21	0.07
M2A	Vesicles	51.3 $\pm$ 14.8	26.3 $\pm$ 10.0	-49	Not significant	0.55	0.00	0.00
M1 (0.9)	Synaptic membranes	17.4 $\pm$ 1.7	29.6 $\pm$ 3.3	+70	0.001			
M1 (1.0)	Synaptic membranes	22.3 $\pm$ 0.6	30.4 $\pm$ 2.0	+36	0.001			
M1 (1.2)	Synaptic membranes + mitochondria	23.9 $\pm$ 1.9	27.6 $\pm$ 0.4	+15	0.01			
M2A	Vesicles	79.4 $\pm$ 7.8	40.9 $\pm$ 2.5	-48	0.001

Fig. 5. Effect of different concentrations of noradrenaline on triglyceride lipase and diglyceride lipase of synaptic membranes

Combined synaptic-membrane fractions M1 (0.9) and M1 (1.0) (see Table 8 and the text) containing 1.10mg of protein were preincubated with tranylcypromine and noradrenaline before the addition of substrate. Final concentration of tranylcypromine in the fully constituted mixture was 0.5mM. Values are means of three independent incubations. Bars through the points indicate $\pm$ S.D. (a) Hydrolysis of glyceryl tripalmitate; (b) hydrolysis of glyceryl dipalmitate.

Sattin, 1970), slices of mouse and rabbit brain (Kakiuchi & Rall, 1968a), slices of rabbit cerebellum (Kakiuchi & Rall, 1968b), and particulate preparations of rat cerebellum (Klainer *et al.*, 1962). It is likely that the stimulation of lipases in brain by amines is mediated by cyclic AMP as it is in adipose tissue.

It has been reported previously that noradrenaline, 5-hydroxytryptamine and adrenaline stimulate the production of unesterified fatty acid in intact synaptosomes, and that noradrenaline and 5-hydroxytryptamine stimulated their production in preparations of synaptic membranes (Price & Rowe, 1972). The results reported here are thus consistent with endogenous glycerides contributing to the amine-stimulated production of unesterified fatty acids.

If amines stimulate the hydrolysis of endogenous glycerides then the concentrations of triglyceride and

diglyceride in nerve endings are controlled by the local concentrations of noradrenaline and 5-hydroxytryptamine. These changes together with the concomitant production of unesterified fatty acids could affect membrane properties during synaptic transmission. In particular the production of diglyceride from triglyceride, and to a lesser extent the hydrolysis of diglyceride, would be under hormonal control. In brain, diglyceride is a precursor for the synthesis of phosphatidylcholine and phosphatidylethanolamine, which are synthesized by the Kennedy pathway (Rossiter & Strickland, 1959; Strickland *et al.*, 1963; Ansell & Spanner, 1967, 1968). As a substrate for diglyceride kinase (Hokin & Hokin, 1959) it is precursor to phosphatidic acid and the inositol phosphatides. Moreover, it is formed from the action of phospholipase C, the presence of which has been reported in brain (Roitman & Gatt, 1963; Ansell & Spanner, 1966; Illingworth & Glover, 1969). Thus hormonal control of glyceride lipases during synaptic transmission could also influence phospholipid metabolism.

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