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PHOSPHOLIPID-INDUCED ACTIVATION OF TRYPTOPHAN HYDROXYLASE FROM THE RAT BRAINSTEM

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Abstract—Phospholipids (phosphatidylserine, phosphatidylethanolamine and lysophosphatidylcholine, but not phosphatidylinositol) and gangliosides stimulated the activity of tryptophan hydroxylase extracted from the brainstem of adult rats. This effect was pH dependent; it was much more pronounced at alkaline pH (8.3) than at pH 7.6 (pH for tryptophan hydroxylase assay under standard conditions); this resulted in a shift of the optimal pH for tryptophan hydroxylase activity from 7.6 to 7.9 when phosphatidylserine was included in the assay mixture. Another change concerned the apparent affinity of the enzyme for its cofactor, 6-MPH_4, which doubled in the presence of phosphatidylserine. These alterations did not completely resemble those induced by sodium dodecylsulfate (SDS), suggesting that the detergent properties of phosphatidylserine were not solely responsible for tryptophan hydroxylase activation. This conclusion was strengthened by the observation that phosphatidylserine and SDS exerted opposite effects on tryptophan hydroxylase from young (16 day-old) rats: at pH 7.6, SDS stimulated, whereas the phospholipid largely inhibited the enzymic activity. Pretreatment of the 35,000 *g* supernatant of an homogenate of brainstem from adult rats with phospholipase A or C resulted in the activation of tryptophan hydroxylase. This suggests that changes in the composition of endogenous phospholipids (notably the formation of lysophosphatides and the removal of possible inhibitory phospholipids) in the vicinity of tryptophan hydroxylase may alter its activity. These results are discussed in relation to the possible role of phospholipids in the control of tryptophan hydroxylase activity under physiological conditions.

The subcellular distribution of tryptophan hydroxylase (L-tryptophan-5-monooxygenase, EC 1.14.16.4), the rate limiting enzyme in the biosynthesis of serotonin (5-HT), is still the subject of controversy. On the basis of biochemical data, most authors have concluded that tryptophan hydroxylase is a soluble enzyme [1]. However, some evidence for the existence of a particulate form of this enzyme, notably in synaptosomes, has also been reported [2–4]. Recently, Hori *et al.* [5] even mentioned that about 90 per cent of tryptophan hydroxylase was found in "microsomal" and "mitochondrial" fractions prepared from bovine raphe nuclei. Owing to the preparation of a specific antibody against tryptophan hydroxylase purified from the raphe area, Joh *et al.* [6] and Pickel *et al.* [7] recently confirmed these biochemical data. Immunocytochemical studies by this group revealed that in serotoninergic neurons, tryptophan hydroxylase was in fact associated with subcellular organelles which had the size and the distribution of microtubules and small and large synaptic vesicles. Therefore, tryptophan hydroxylase could be a particulate rather than a soluble enzyme *in vivo* and the drastic homogenization procedures used to extract this enzyme for biochemical studies might disrupt its association with subcellular organelles.

The activity of membrane-bound enzymes generally depends on the local environment, notably the phospholipid components of the membrane. For instance, this has been shown for the enzymic system involved in electron transfer, monoamine oxidase, collagen galactosyl- and glucosyl-transferases, UDP galactose:ceramide galactosyltransferase, UDP glucoronyl transferase and the uptake processes for monoamines in synaptosomes [8–14].

If tryptophan hydroxylase is actually a membrane-bound enzyme, its activity might well depend on phospholipids. In the present paper, we describe the effects of various phospholipids, particularly phosphatidylserine, on the activity of tryptophan hydroxylase extracted from the brainstem of the rat. In most cases, phospholipids activated tryptophan hydroxylase from the adult rat. This activation did not entirely resemble that induced by sodium dodecylsulfate, suggesting that the detergent properties of phospholipids were not solely responsible for this effect [15]. In addition, changes in the endogenous phospholipid composition of tryptophan hydroxylase preparations by treatment with phospholipase A or C altered this enzymic activity. These results suggest that the interaction of tryptophan hydroxylase with phospholipids may play a role in the control of its activity under physiological conditions.

MATERIALS AND METHODS

Sodium dodecylsulfate (SDS, laurylsulfate), L-α-phosphatidyl-L-serine (from bovine brain) and L-α-lysophosphatidylcholine (from egg yolk, type 1) were from Sigma. Phosphatidylethanolamine (from bovine brain, Folch Fr. V), phosphatidylinositol (from bovine brain, Folch Fr. 1) and gangliosides (from bovine brain) were from Koch–Light.

Phospholipase-A (from bee venom, 1219 units/mg), phospholipase-C (from *Cl. welchii*, 7.6 units/mg) and phospholipase-D (from cabbage type 1, 14.3 units/mg) were purchased from Sigma. Phospholipase-A_2 (from snake venom, crotalus, 200 units/mg) was from Boehringer Mannheim. D,L-6-methyl-5,6,7,8-tetrahydropterin (6-MPH_4, Calbiochem), *N'*-methyl-*N*-(3-hydroxybenzyl) hydrazinium dihydrogen phosphate (NSD 1034, Smith and Nephew Research), and catalase (14,400 units/mg, Sigma) were used for tryptophan hydroxylase assays.

All these compounds were dissolved in 0.05 M Tris–acetate buffer (at various pH) containing 2 mM β-mercaptoethanol (Merck). Since phosphatidylserine was delivered in a chloroform–methanol (95:5) solution, it was necessary to eliminate the organic solvents to include the phospholipid in the assay mixture for tryptophan hydroxylase. For this purpose, the emulsion formed by mixing appropriate volumes of phosphatidylserine solution and Tris-acetate buffer was vigorously stirred at 25° until the complete evaporation of the organic solvents; at this stage, the phosphatidylserine Tris–acetate mixture was completely clear.

Tryptophan hydroxylase was extracted from the brainstem of rats at various ages. Adult male (250–300 g) and young male and female Sprague–Dawley rats (Charles River strain) were kept in a controlled environment (24°, 60 per cent humidity, alternative cycles of 12 hr light and 12 hr darkness, food and water *ad libitum*) for at least 8 days before sacrifice. Before weaning (on the 21st post-natal day), the litters were limited to nine newborn rats per dam.

Rats were killed by decapitation and their brainstem was removed in less than 30 sec at 4°. Tissues from 5–20 rats were homogenized in 3 volumes 0.05 M Tris–acetate buffer (pH 7.6) containing 2 mM β-mercaptoethanol using a Polytron PT 10 OD apparatus. Then, homogenates were centrifuged (35,000 *g*, 30 min, 4°) and the supernatant was generally used as the final tryptophan hydroxylase solution. On occasion, the enzyme was partially purified by ammonium sulfate fractionation (between 20 and 50 per cent) followed by Sephadex G25 or G200 (Pharmacia) gel filtration as previously described [16].

Treatments with phospholipase A or A_2 consisted in incubating the 35,000 *g* supernatant of a rat brainstem homogenate with the phospholipase for 20 min at 30° at pH 8.0 in the presence of 0.1 mM Ca^{2+}. When the fatty acids and lysophosphatides produced were removed by defatted bovine serum albumin, a procedure adapted from Graham *et al.* [13] was used [17]. Briefly, 10 mg of defatted serum albumin were first added per mg protein in the preincubated supernatant. After stirring the mixture for 5 min at room temperature, saturated ammonium sulfate (pH 7.6) was progressively added until 50 per cent S in the final solution. Tryptophan hydroxylase, but not serum albumin, precipitated in the cold (4°) and was recovered by centrifugation (35,000 *g*, 20 min, 4°) 30 min later. The pellet was dissolved in 2 ml of 0.05 M Tris–acetate buffer (pH 7.6) containing 2 mM β-mercaptoethanol. This solution was then filtered at 4° through a Sephadex G25 column (0.9 cm in diameter, 14 cm high) and the fractions corresponding to the void volume of the column were used as the tryptophan hydroxylase source.

Treatments with phospholipase C or D were similar to those used with phospholipase A except that the pHs of the preincubating mixtures were 7.6 and 6.5, respectively. After 20 min at 30°, the 35,000 *g* supernatant containing the phospholipase and 0.1 mM Ca^{2+} was cooled in a melting ice bath and aliquots (0.1 ml) were used for tryptophan hydroxylase assays. Although higher concentrations of Ca^{2+} are required for full activation of phospholipases [12, 13, 18], 0.1 mM was chosen in all cases to avoid activation of the Ca^{2+}-dependent neutral proteinase which has been shown to induce a stimulation of tryptophan hydroxylase activity [19]. Although the optimal pH for the activity of phospholipase D is 5.4 [18] pretreatment with this enzyme was performed at pH 6.5 since we observed in preliminary experiments that preincubating the 35,000 *g* supernatant for 20 min at 30° at a lower pH resulted in a marked irreversible loss of tryptophan hydroxylase activity (−60 per cent after a preincubation at pH 6.0).

Tryptophan hydroxylase was assayed according to a slight modification of the method originally described by Gál and Patterson [15, 20]. In most cases, the concentration of tryptophan was equal to 0.15 mM, that of 6-MPH_4 to 0.16 mM. The final product, 5-hydroxytryptophan (5-HTP), was measured using a spectrofluorimetric method [21]. The enzymic activity is expressed in nmoles. 5-HTP formed per mg protein and per 15 or 60 min. For the determination of the kinetic parameters (apparent K_m, V_{max}) of tryptophan hydroxylase for tryptophan, the pterin cofactor concentration was raised to 0.32 mM and tryptophan concentration varied between 20 μM and 0.5 mM. Conversely, the concentration of tryptophan was maintained at 0.5 mM and that of 6-MPH_4 varied between 50 μM and 0.32 mM in experiments designed to estimate the V_{max} and the apparent K_m of tryptophan hydroxylase for its pterin cofactor. In both cases, parameters were calculated by linear regression analysis of double reciprocal plots (Lineweaver–Burk representation).

All tryptophan hydroxylase assays were done in triplicate or quadruplicate. The range of variations among them was less than 4 per cent. Most experiments were repeated three times. In all cases, the rate of 5-HTP formation was proportional to both the concentration of protein in the assay mixture (0.3–2 mg/ml) and the incubation time (15 min at 37°).

Proteins were estimated with bovine serum albumin (Sigma) as the standard [22].

Statistical calculations (Student's '*t*' test) were done according to Snedecor and Cochran [23].

RESULTS

Effects of various phospholipids on the activity of tryptophan hydroxylase from the brainstem of adult rats. The addition of phospholipids to the assay mixture of tryptophan hydroxylase (pH 7.6) resulted in marked changes in the activity of this enzyme. Among the phospholipids presently studied, phosphatidylinositol was the only compound which

Table 1. Effects of phosphatidylserine on the activity of tryptophan hydroxylase in a crude extract or semi-purified preparations from the brainstem of adult rats

Enzyme source	Tryptophan hydroxylase activity No addition	Phosphatidylserine	(%)
35,000 *g* Supernatant	1.81 ± 0.03	2.68 ± 0.09	(148)
20–50% ammonium sulfate			
+Sephadex G25	2.49 ± 0.08	4.71 ± 0.13	(189)
+Sephadex G200	2.84 ± 0.07	12.91 ± 0.36	(455)

The preparation of the enzyme sources was described in Methods. Tryptophan hydroxylase was assayed at pH 7.6 with 0.15 mM tryptophan and 0.16 mM 6-MPH_4 in the absence or the presence of 0.5 mg/ml phosphatidylserine. The enzymic activity is expressed in nmoles 5-HTP formed per mg protein and per 15 min. Each value is the mean ± S.E.M. of triplicate determinations. Figures in brackets are the percent of respective control values (no addition).

inhibited the activity of tryptophan hydroxylase (Fig. 1); lysophosphatidylcholine, phosphatidylethanolamine and phosphatidylserine all activated tryptophan hydroxylase in the 35,000 *g* supernatant of a rat brainstem homogenate. As shown in Fig. 1, phosphatidylserine was the most active compound; half maximal stimulation of tryptophan hydroxylase was reached with 0.2 mM phosphatidylserine. Other brain lipids, namely gangliosides, also stimulated tryptophan hydroxylase activity; their effect was in fact more pronounced than that of phosphatidylserine in the range of lower concentrations (Fig. 1).

The partial purification of tryptophan hydroxylase by ammonium sulfate fractionation and Sephadex gel filtration apparently resulted in less than doubling its specific activity (Table 1). In fact, this enzymic activity decreased by about 75 per cent in the crude 35,000 *g* supernatant maintained at 6° for the time required for the purification procedures (20 hr) so that the specific activity of the enzyme eluted from Sephadex G200 was actually 6–7 times higher than that found in the original crude extract. As shown in Table 1, the activation of tryptophan hydroxylase by phosphatidylserine was not only detected but indeed more pronounced on the semi-purified than on the crude preparation of the enzyme.

Effect of pH on the activity of tryptophan hydroxylase in the absence or the presence of phospholipids. Increasing the pH of tryptophan hydroxylase assay from 7.6 to 8.3 resulted in a marked increase of the stimulatory effects of various phospholipids and gangliosides. This was particularly striking in the case of phosphatidylinositol which exerted an inhibitory effect at pH 7.6 and activated the enzyme at pH 8.3 (Table 2).

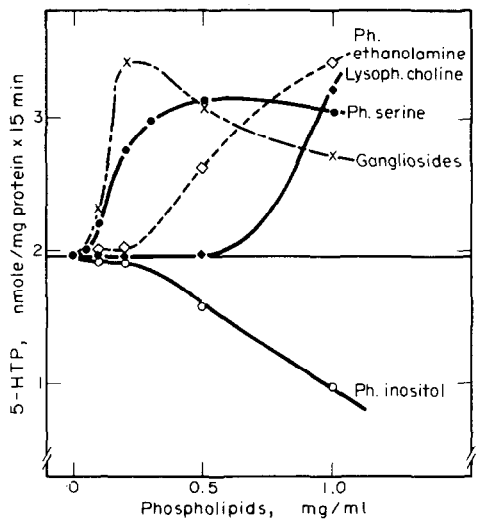

Fig. 1. Effects of various phospholipids on the activity of rat brainstem tryptophan hydroxylase. The enzyme source was the 35,000 *g* supernatant of an homogenate of brainstem from adult rats. Tryptophan hydroxylase was assayed at pH 7.6 with 0.15 mM tryptophan and 0.16 mM 6-MPH_4 in the absence or the presence of various concentrations of phosphatidylinositol (○), gangliosides (×), phosphatidylserine (●), lysophosphatidylcholine (◆) or phosphatidylethanolamine (◇). The enzymic activity is expressed in nmoles. 5-HTP formed per mg protein and per 15 min. Each point is the mean of quadriplicate determinations.

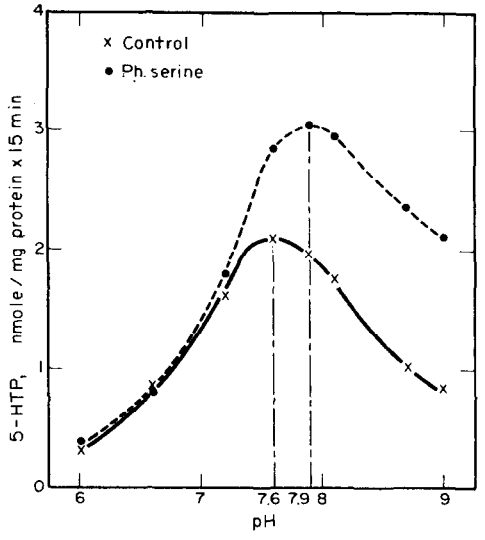

Fig. 2. Effects of pH on the activity of tryptophan hydroxylase in the absence or the presence of phosphatidylserine. The enzyme source was the 35,000 *g* supernatant of a brainstem homogenate from adult rats. Tryptophan hydroxylase was assayed at various pH with 0.15 mM tryptophan and 0.16 mM 6-MPH_4 in the absence or the presence of 0.25 mg (/mg protein) of phosphatidylserine. The enzymic activity is expressed in nmole. 5-HTP/mg protein for 15 min. Each point is the mean of triplicate determinations.

Table 2. Effects of various phospholipids on tryptophan hydroxylase activity at pH 7.6 or 8.3

Addition	Tryptophan hydroxylase activity pH 7.6	(%)	pH 8.3	(%)
None	2.19 ± 0.06	(100)	1.40 ± 0.05	(100)
Phosphatidylinositol	1.74 ± 0.05	(79)	1.96 ± 0.05	(140)
Lysophosphatidylcholine	2.07 ± 0.06	(95)	2.03 ± 0.04	(145)
Phosphatidylethanolamine	2.94 ± 0.10	(134)	2.85 ± 0.06	(204)
Gangliosides	3.41 ± 0.08	(156)	4.22 ± 0.08	(301)
Phosphatidylserine	3.25 ± 0.08	(148)	4.27 ± 0.02	(305)

The enzyme source was the 35,000 *g* supernatant of a brainstem homogenate from adult rats. Tryptophan hydroxylase was assayed at pH 7.6 or 8.3 with 0.15 mM tryptophan and 0.16 mM 6-MPH_4 in the absence or the presence of 0.5 mg (per mg protein) of each phospholipid. Each value is the mean ± S.E.M. of triplicate determinations of 5-HTP formed (in nmoles) per mg protein and per 15 min. Figures in brackets are the percent of respective control values (no addition).

As a result of the more pronounced activation of tryptophan hydroxylase in the range of alkaline pH, the optimal pH for this enzymic activity was shifted from 7.6 to 7.9 by the addition of phosphatidylserine to the assay mixture (Fig. 2).

Effects of preetreatment with phospholipase A, C or D on the activity of tryptophan hydroxylase. The 35,000 *g* supernatant of a rat brainstem homogenate was preincubated in the presence of phospholipase A and Ca^{2+} (0.1 mM). As shown in Table 3, the activity of tryptophan hydroxylase at pH 8.3 in this pretreated mixture was higher than that found in the crude supernatant preincubated without Ca^{2+} and the phospholipase A. A similar finding was obtained with another phosphatide acyl-hydrolase preparation, phospholipase A_2 (Table 3). In the absence of Ca^{2+}, pretreatment with phospholipase A or A_2 stimulated tryptophan hydroxylase activity but to a lesser extent (+ 52 per cent with phospholipase A; + 39 per cent with phospholipase A_2).

The direct addition of phospholipase A to the assay mixture (24 units/mg protein in the assay mixture) for tryptophan hydroxylase with no preincubation also induced an increase in this enzymic activity at pH 8.3 (+ 34 per cent) but not at pH 7.6.

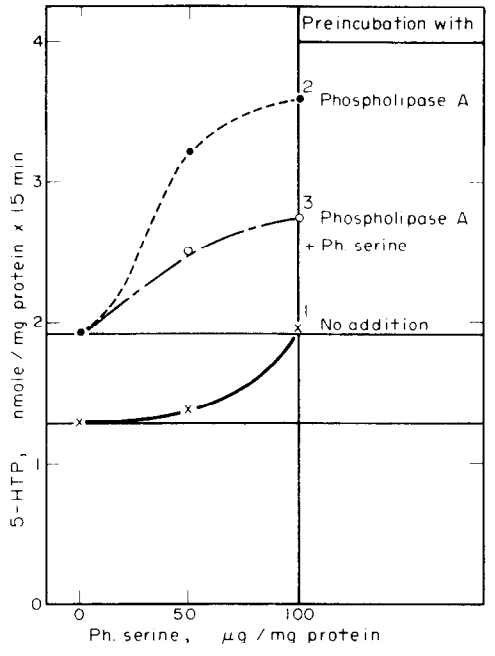

Fig. 3. Effects of low concentrations of phosphatidylserine on the activity of tryptophan hydroxylase following pretreatment with phospholipase A. The crude supernatant (35,000 *g*) of the homogenate of brainstem from adult rats was preincubated at pH 8.0 for 20 min at 30° with 0.1 mM $CaCl_2$ in the absence (1, ×) or the presence of phospholipase A (6 units/mg protein in the supernatant) alone (2, ●) or with phosphatidylserine (3, ○, 50 or 100 µg/mg protein in the supernatant). Tryptophan hydroxylase was then assayed at pH 8.3 on aliquots (0.1 ml) of the preincubated preparations with 0.15 mM tryptophan and 0.16 mM 6-MPH_4. Phosphatidylserine was added in the assay mixtures corresponding to "1" and "2" preparations so that its final concentrations were equal to those already occurring in case "3". Each point is the mean of triplicate determinations of nmol. 5-HTP formed per mg protein and per 15 min.

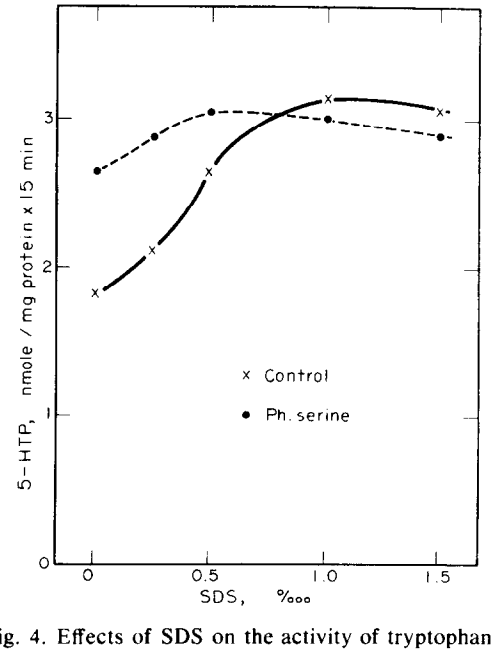

Fig. 4. Effects of SDS on the activity of tryptophan hydroxylase in the absence or the presence of phosphatidylserine. The enzyme source was the 35,000 *g* supernatant of the homogenate of brainstem from adult rats. Tryptophan hydroxylase was assayed at pH 7.6 with 0.15 mM tryptophan and 0.16 mM 6-MPH_4 in the presence or the absence of phosphatidylserine (0.25 mg/mg protein in the assay mixture) and SDS as indicated on the Fig. Each point is the mean of triplicate determinations of 5-HTP formed (in nmoles) per mg protein and per 15 min.

Table 3. Effects of preincubation with phospholipase A, A_2, C or D on the activity of tryptophan hydroxylase

	Tryptophan hydroxylase activity			
	pH 7.6	(%)	pH 8.3	(%)
1 Control	1.73 ± 0.05	(100)	1.01 ± 0.02	(100)
Phospholipase A	—		1.78 ± 0.04	(176)
Phospholipase A_2	1.76 ± 0.06	(102)	1.67 ± 0.03	(165)
Phospholipase A_2 + serum albumin	—		0.95 ± 0.02	(94)
2 Control	1.96 ± 0.06	(100)	1.33 ± 0.04	(100)
Phospholipase C	1.52 ± 0.06	(78)	1.82 ± 0.06	(137)
3 Control	1.15 ± 0.03	(100)	0.86 ± 0.02	(100)
Phospholipase D	0.92 ± 0.01	(80)	0.90 ± 0.01	(105)

The 35,000 *g* supernatant of an homogenate of brain stem from adult rats was preincubated for 20 min at 30° at pH 8.0 (exp. 1), pH 7.6 (exp. 2) or 6.5 (exp. 3) with 0.1 mM Ca^{2+} in the absence (control) or the presence of phospholipase A (12 units/mg protein in the supernatant), phospholipase A_2 (4 units/mg protein), phospholipase C (3.8 units/mg protein) or phospholipase D (7.2 units/mg protein). In the experiment 1, defatted serum albumin (10 mg/mg protein in the supernatant) was added at the end of this period and tryptophan hydroxylase was recovered by ammonium sulfate precipitation as described in Methods. Tryptophan hydroxylase was assayed at pH 7.6 or 8.3 on aliquots (0.1 ml) of the preincubated supernatants (or of the serum albumin-treated preparation) with 0.15 mM tryptophan and 0.16 mM 6-MPH_4. Each value is the mean ± S.E.M. of triplicate determinations of 5-HTP formed in nmoles per mg protein and per 15 min. Figures in brackets are the percent of respective control values.

When the reaction products of phospholipolysis were removed on defatted serum albumin, the stimulating effect of phospholipase A_2 pretreatment was no longer detected (Table 3).

As shown in Table 3, similar pretreatment with phospholipase C resulted in a slight inhibitory effect on tryptophan hydroxylase activity at pH 7.6. Assaying this enzyme at pH 8.3 converted this effect into a definite activation (Table 3). When preincubation with phospholipase C was realized with no added Ca^{2+}, tryptophan hydroxylase activity remained unchanged; this emphasized the well-known Ca^{2+} requirement for phospholipase C activity [24].

Preincubation with phospholipase D, in the presence or the absence of 0.1 mM Ca^{2+}, induced only very discrete changes in tryptophan hydroxylase activity in the 35,000 *g* supernatant of the brainstem homogenate prepared from adult rats (Table 3).

As shown in Fig. 3, pretreatment with phospholipase A increased the activation of tryptophan hydroxylase by phosphatidylserine. Indeed, at a concentration of 50 μg/ml (containing 1 mg protein), phosphatidylserine activated tryptophan hydroxylase in the phospholipase A-pretreated supernatant by 68 per cent at pH 8.3 whereas it exerted no effect in the 35,000 *g* supernatant preincubated with no phospholipase. The stimulatory effect of the phospholipid did not result from the formation of lysophosphatidylserine since it was in fact less pronounced when phosphatidylserine was already added for the preincubation with phospholipase A (Fig. 3).

Were the detergent properties of phospholipids involved in their stimulatory effects on tryptophan hydroxylase activity? Comparison with the activation induced by sodium dodecyl sulfate

(a) Effects of SDS in the absence or the presence of phosphatidylserine. In agreement with previous findings, the addition of SDS to the assay mixture induced an activation of tryptophan hydroxylase [15]. As shown in Fig. 4, the optimal concentration of the anionic detergent was equal to 0.01% (w/v). In the range of lower concentrations of SDS, the stimulatory effects of both the detergent and phosphatidylserine were roughly additive. However, in the presence of 0.01% SDS, phosphatidylserine did not further activate the enzyme and indeed a slight decrease was even induced by the addition of the phospholipid (Fig. 4).

(b) Effects of SDS and phosphatidylserine on the activity of tryptophan hydroxylase extracted from the brainstem of rats at various ages. On the 2nd postnatal day, tryptophan hydroxylase activity in

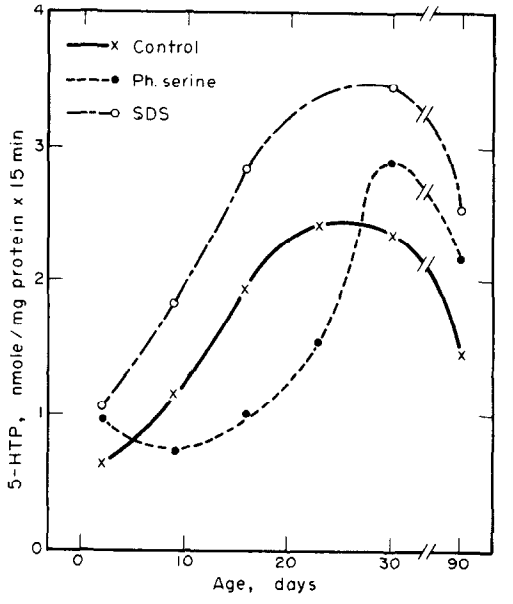

Fig. 5. Effects of phosphatidylserine or SDS on the activity of tryptophan hydroxylase at various ages. The enzyme source was the 35,000 *g* supernatant of brainstem homogenates. At each age, tryptophan hydroxylase was assayed at pH 7.6 with 0.12 mM tryptophan and 0.16 mM 6-MPH_4 in the absence or the presence of phosphatidylserine (0.5 mg/mg protein in the assay mixture) or SDS (0.01%). Each point is the mean of triplicate determinations of 5-HTP formed (in nmoles) per mg protein and per 15 min.

Table 4. Effects of SDS or phosphatidylserine on the activity of tryptophan hydroxylase extracted from the brainstem of adult or 16 day-old rats

		Tryptophan hydroxylase activity				
		No addition	SDS	(%)	Phosphatidylserine	(%)
pH 7.6	Adult rats	1.95 ± 0.03	3.37 ± 0.10	(173)	3.02 ± 0.09	(155)
	16 day-old rats	2.50 ± 0.05	3.91 ± 0.13	(156)	1.22 ± 0.04	(49)
pH 8.3	Adult rats	1.33 ± 0.02	3.42 ± 0.12	(257)	3.50 ± 0.09	(263)
	16 day-old rats	1.74 ± 0.04	3.98 ± 0.15	(229)	2.59 ± 0.10	(149)

The enzyme source was the 35,000 *g* supernatant of brainstem homogenates. Tryptophan hydroxylase was assayed at pH 7.6 or 8.3 with 0.15 mM tryptophan and 0.16 mM 6-MPH_4 in the absence or the presence of 0.01% SDS or phosphatidylserine (0.5 mg/mg protein in the assay mixture). Each value is the mean ± S.E.M. of triplicate determinations of 5-HTP formed in nmoles per mg protein and per 15 min. Figures in brackets are the percent of respective control values.

the brainstem was about 44 per cent of that found in the same area in adult rats. It increased progressively during the first three postnatal weeks and reached, at the end of this period, a level about 64 per cent higher than that found in 3 month-old rats (Fig. 5). Surprisingly, phosphatidylserine (50 μg–1 mg/ml in the assay mixture) inhibited at pH 7.6 tryptophan hydroxylase from 9 day-, 16 day- and 23 day-old rats, in contrast to that observed on the enzyme from 2 day-old and adult animals (Figs 1, 5). The effects induced by 500 μg/ml of phosphatidylserine are reported in Fig. 5.

In contrast to these age-dependent effects of phosphatidylserine, SDS (0.01%) activated tryptophan hydroxylase from newborn young as well as from adult animals (Fig. 5).

Since the phospholipid-induced inhibition of tryptophan hydroxylase seen at pH 7.6 could be reversed at pH 8.3 (see above), the effects of phosphatidylserine as a function of age were also analyzed at this alkaline pH. As shown in Table 4, the marked inhibitory effect of phosphatidylserine on the enzyme from 16 day-old rats was converted into a significant activation when the assay pH was changed from 7.6 to 8.3. Similar findings were obtained on tryptophan hydroxylase from 9 and 23 day-old animals. In all cases, however, the activation induced at pH 8.3 by the phospholipid was much lower on the enzyme from young animals than that found with the adult enzyme (Table 4). In addition, in 16 day-old, but not in adult rats, the maximal stimulatory effect of 0.01% SDS was more pronounced than that induced by the optimal concentration of the phospholipid (Table 4).

(*c*) *Kinetic parameters of tryptophan hydroxylase in the absence or the presence of phosphatidylserine or SDS.* In the presence of SDS (0.01%), the apparent affinities of tryptophan hydroxylase from adult rats for both tryptophan and 6-MPH_4 were significantly increased (Table 5). Moreover, the V_{max} of the enzyme was also enhanced, this effect being significant only when 6-MPH_4 was the variable (in the presence of 0.5 mM tryptophan) (Table 5).

In adult as well as in 16 day-old rats, the apparent affinity of the enzyme for its pterin cofactor but not that for tryptophan was increased by the addition of phosphatidylserine to the assay mixture. The V_{max} of tryptophan hydroxylase from adult rats was slightly increased in the presence of phosphatidylserine (Table 5). By contrast, the inhibitory effect of the phospholipid on the enzyme from 16 day-old rats (see b, Fig. 5) resulted from a marked reduction in its V_{max} (Table 5).

These changes in the kinetic parameters of tryptophan hydroxylase in the crude 35,000 *g* supernatant were also observed on the enzyme semi-purified

Table 5. Effects of SDS or phosphatidylserine on the kinetic parameters of tryptophan hydroxylase from adult or 16 day-old rats

		TRP		6-MPH_4	
		K_m	V_{max}	K_m	V_{max}
Adult rats	Control	234 ± 14 (17)	23.8 ± 1.3	202 ± 17 (15)	28.1 ± 2.0
	SDS	110 ± 5* (5)	27.0 ± 3.0	102 ± 11* (5)	46.4 ± 5.2*
	Phosphatidylserine	223 ± 13 (6)	30.8 ± 3.2	114 ± 13* (6)	32.0 ± 4.6
16 day-old rats	Control	260 ± 21 (3)	29.7 ± 1.8	162 ± 19 (4)	35.9 ± 3.1
	Phosphatidylserine	272 ± 26 (3)	10.7 ± 3.1*	78 ± 10* (4)	11.1 ± 4.2*

The determinations of apparent K_m (in μM) and V_{max} (in nmoles 5-HTP formed per mg protein and per hr) were performed as described in Methods. SDS (0.01%) or phosphatidylserine (500 μg per mg protein in the assay mixture) was added with no preincubation. Each value is the mean ± S.E.M. of determinations made in 3–17 experiments (number of experiments indicated in brackets).

* $P < 0.05$ when compared with respective control values.

by ammonium sulfate fractionation and Sephadex G25 gel filtration (data not shown).

DISCUSSION

Phospholipids have been shown to alter the activity of numerous enzymes in the brain and other organs. This applies notably to membrane-bound enzymes (see Introduction). However, this also appears to be true in the case of "soluble" enzymes such as phenylalanine hydroxylase and tyrosine hydroxylase [25–28]. The present results reveal that the activity of tryptophan hydroxylase can be largely changed by phospholipids. Thus, the addition of various phospholipids generally leads to an activation of the enzyme from the brainstem of adult rats. A similar finding has already been noted on the conversion of tryptophan into 5-HTP by rat liver phenylalanine hydroxylase, thus, lysophosphatidylcholine dramatically increased tryptophan hydroxylation when tetrahydrobiopterin was used as the cofactor [26]. However, the same group failed to detect any effect of various phospholipids on the purified tryptophan hydroxylase from the rabbit hindbrain using 6-MPH_4 or tetrahydrobiopterin as the cofactor [29]. This is surprising since the effective concentrations of phospholipids found in the present study were in the same range as those previously used [29], furthermore, the partial purification of the enzyme from the rat brain did not suppress but even increased the stimulatory effect of phosphatidylserine. Species differences between the rat and the rabbit might be involved. Indeed, Raese *et al.* [28] have already noted such species differences concerning the effect of lysophosphatidylcholine on striatal tyrosine hydroxylase: this phospholipid activated the enzyme from the rat but not that from bovine.

In addition to these species differences, the present study reveals that phospholipids may exert different effects on tryptophan hydroxylase depending on the age of animals of a given species. Thus, phosphatidylserine activated the enzyme from 2 day-old and adult rats whereas it inhibited that extracted from rats between 5–27 days of age. These differences might be related to alterations in the composition of phospholipids in brain during development [30, 31]. The particular composition of phospholipids interacting with the enzyme should influence the effects of the exogenous phospholipid added to the enzyme solution. This was also supported by the fact that phospholipase A pretreatment increased the efficiency of phosphatidylserine as an activator of tryptophan hydroxylase from adult rats. This happened when the products (lysophosphatides and free fatty acids) were kept in the enzyme solution but not when they were adsorbed on serum albumin (unpublished observations). Therefore, the changes in endogenous phospholipids in the environment of tryptophan hydroxylase may explain these age-related and (perhaps) the species differences noted above.

Since phospholipids have detergent properties, and detergents, notably SDS [15] and cholate (unpublished observations), are potent activators of tryptophan hydroxylase, one may argue that the effects of phospholipids were only related to their detergent properties. Such an hypothesis could be supported by the lack of additive effects of phosphatidylserine and SDS on tryptophan hydroxylase activity and by the similar shift of the optimal pH for this enzymic activity induced by the phospholipid and the detergent [19]. However, these observations were not sufficient to claim that phospholipids and SDS similarly interacted with the enzyme since (1) the stimulatory effect of phosphorylating conditions (in the presence of ATP, Mg^{2+} and Ca^{2+}, [16]), on tryptophan hydroxylase activity was also not additive with that of phosphatidylserine and (2) a similar shift in the optimal pH for tryptophan hydroxylase activity was induced by activating this enzyme with Ca^{2+}, trypsin [19] and phosphorylating conditions [16]. Extensive studies on these various mechanisms activating tryptophan hydroxylase allowed to conclude that they are in fact totally unrelated [32]. In the particular case of phosphorylating conditions, the tryptophan hydroxylase activation involved a Ca^{2+}-dependent protein kinase [16]. Therefore it was likely by chelating Ca^{2+} that phosphatidylserine prevented the activation of tryptophan hydroxylase by phosphorylating conditions [33].

Other observations strongly suggested that the effects of phospholipids were in fact not (at least solely) related to their detergent properties. Thus, the changes in the kinetic parameters of tryptophan hydroxylase induced by phosphatidylserine were not similar to those induced by SDS. In the latter case, the apparent affinities of the enzyme for both tryptophan and its pterin cofactor were increased whereas the addition of phosphatidylserine only resulted in an increase of the apparent affinity for the cofactor. Another difference between phosphatidylserine and SDS was revealed by analyzing their effects on tryptophan hydroxylase extracted from rats at various ages. Thus, SDS stimulated the enzyme whatever the age of rats whereas the effects induced by phosphatidylserine were markedly age-dependent (see above).

In conclusion, it appears that phospholipids did not interact with tryptophan hydroxylase in exactly the same way as the anionic detergent, SDS.

If endogenous phospholipids are involved in the control of tryptophan hydroxylase activity under physiological conditions, alterations in their composition in the enzyme solution should alter its activity. The present data revealed that this occurred by treatments of the 35,000 *g* supernatant of an homogenate of brainstem from adult rats (tryptophan hydroxylase solution) with phospholipases A and C. Similar observations have been made by several authors in the case of phospholipid-dependent membrane bound enzymes [10–14]. According to Raese *et al.* [28], the addition of lysolecithin to soluble striatal tyrosine hydroxylase altered the kinetics of this enzyme in such a way that they closely resembled those of the "membrane-bound" tyrosine hydroxylase. Since, as discussed in the Introduction, a "membrane-bound" tryptophan hydroxylase may also exist in brain, further studies on its biochemical characteristics would permit to conclude, as in the case of tyrosine hydroxylase, if the interaction of phospholipids with soluble tryp-

tophan hydroxylase converted this enzyme into a "membrane-bound-like" form.

Neurotransmitters, depolarizing stimuli and psychotropic drugs have been shown to alter the turnover of various phospholipids in tissues including brain [34–39]. Since changes in the rate of tryptophan hydroxylation occur under these conditions (by depolarizing stimuli and treatments with these psychotropic drugs), this suggests that phospholipids may play a role in the control of tryptophan hydroxylase activity *in vivo*.

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