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# RAT BRAIN ACETYLCHOLINESTERASE AND ITS ISOENZYMES AFTER INTRACEREBRAL ADMINISTRATION OF DFP*

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**Abstract**—Acetylcholinesterase (AChE) activity was followed in the synaptosomal and microsomal fractions of rat brains after intracerebral administration of diisopropyl phosphofluoridate (DFP). Fifteen days after DFP administration to 20-day-old rats, 47 and 41 per cent of the inhibited (phosphorylated) AChE activity was replaced in the synaptosomes and microsomes respectively. After correcting these values for ontogenic development of AChE in the young rats, post-DFP increase of AChE activity amounted to 16 and 43 per cent of controls in the microsomal and synaptosomal fractions respectively. In 58-day-old rats, 45 and 64 per cent, respectively, of the inhibited microsomal and synaptosomal AChE activity was replaced within 20 days after the administration of DFP; in the control 58-day-old rats the increase in AChE activity during the 20-day period was minimal. Both the microsomal and synaptosomal fractions showed four AChE isoenzymes. The two sets of isoenzymes differed in their DFP sensitivity and in the rate of the post-DFP return of activity; after DFP, each fraction reconstituted its characteristic quantitative isoenzyme pattern. Altogether, the early and fast post-DFP appearance of AChE activity in the synaptosomal fraction and the early reconstitution of the synaptosomal isoenzyme pattern suggest that nerve endings are capable of AChE synthesis or repair apart from the similar microsomal process.

Acetylcholinesterase (AChE; EC 3.1.1.7) is localized both post-synaptically and pre-synaptically; it may be concentrated at the pre-synaptic site in the sympathetic ganglia [1, 2] and in the central nervous system (CNS) [3–6]. There may be several functional reasons for the pre-synaptic location of AChE [1].

The pre-synaptic enzyme may be formed locally as synaptosomes were shown to be capable of protein synthesis (cf. Ref. 7), or it may be synthesized in the cell body and migrate down the axon into the nerve terminals presumably via the microtubules [8, 9]. Since the 1960s, these alternatives were studied at the periphery [10–13]. Recently, it has been suggested that the enzyme may be synthesized in the axon or the nerve terminal and that only a small portion of it may originate in the cell soma [14].

In the present study, we desired to evaluate the origin of the AChE of the brain nerve terminals. Diisopropyl fluorophosphoridate (DFP) was utilized to irreversibly phosphorylate AChE, and the subsequent recovery of AChE activity was evaluated, after DFP, in synaptosomal and microsomal fractions representing nerve terminals and soma respectively; the early appearance of AChE activity in synaptosomes as compared to the microsomes would argue for the nerve terminal synthesis of the enzyme.

The second approach involved the evaluation of the action of DFP on the isoenzymes of AChE [15–17]. As microsomal and nerve ending AChE isoenzymes show characteristic patterns and differ in their sensitivity to DFP [18], the evaluation of isoenzymes after DFP should constitute additional evidence as to the origin of the new, post-DFP enzyme.

## METHODS

Male Sprague–Dawley rats 20 and 58 days of age, weighing 25–35 and 200–250 g, respectively, were used in these experiments. All animals were bred in the laboratory.

DFP was obtained from Sigma Chemicals, St. Louis, Mo., dissolved (1 g/ml) in propylene glycol. Thirty min prior to the administration of DFP the animals were given 6 mg/kg of atropine methyl nitrate intraperitoneally. The animals were anesthetized with ether, and 10 $\mu$l of the DFP solution was injected intracerebrally with a Hamilton syringe; appropriate dilutions of DFP were prepared in propylene glycol prior to administration. Doses of 105–115 $\mu$g (3.5 mg/kg) and of 350–500 $\mu$g (2.0 mg/kg) were used in 20- and 50-day-old rats respectively; control animals were given 10 $\mu$l propylene glycol intracerebrally. Animals were sacrificed at various time intervals after the administration of DFP or of propylene glycol. The cerebral hemispheres were excised and bathed in 0.32 M sucrose in 25 mM Tris, pH 7.5, prior to homogenization. Excision was completed within 20 sec after sacrifice of the animal.

The subcellular fractions were isolated according

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to the method of Autilio *et al.* [19]. Synaptosomal fractions were collected and diluted in 0.32 M sucrose in 25 mM Tris buffer, pH 7.5, and centrifuged at 40,000 *g* for 30 min. The pellets obtained by the isolation procedure were rewashed in sucrose–Tris buffer, pH 7.5 (0.4 mg/kg of original tissue), and stored at −40° until use. The protein content of the fraction was measured by the method of Lowry *et al.* [20]. NADPH-dependent cytochrome *c* reductase was assayed by the method of Sottocosa *et al.* [21]. Rotenone was added to the assay medium to eliminate mitochondrial contribution to the cytochrome reductase pool [21].

Unfrozen synaptosomal pellets were fixed for electron microscopy in 0.1 M phosphate-buffered glutaraldehyde (3.125%); washing and fixing were carried out by routine procedures. AChE activity of the synaptosomal and microsomal fractions was measured by the method of Ellman *et al.* [22]; acetylthiocholine iodide was used as the substrate and quinidine sulfate (1%) was employed to eliminate any activity of butyrylcholinesterase (BuChE) that may have been present. Specific activity of AChE is defined as μmoles acetylthiocholine iodide hydrolyzed/min/mg of protein.

Triton X-100 extraction was employed for the study of microsomal and synaptosomal isoenzymes. Known amounts (1 mg protein/ml) of synaptosomal and microsomal fractions were homogenized with an equal volume of 2% Triton X-100 in sucrose–Tris buffer, pH 7.5, the final concentration of Triton being 1%. At this concentration, Triton was found to be capable of complete or nearly complete extraction of AChE and its isoenzymes. This was indicated by the absence of AChE activity in the pellet as shown by the titrimetric procedure [22] as well as by the absence in the pellet of AChE staining ([23]; cf. below). Solutions were stored at 4° for 6 hr and then centrifuged at 100,000 *g* for 30 min. Supernatant was used for the measurement of AChE activity as described above. Triton X-100 was found to be devoid of anti-AChE activity at concentrations of up to 5% [24].

Polyacrylamide disc electrophoresis was performed as modified by Juul [23]. Equal amounts of protein were always applied to each gel. Both pre-incubation and incubation solutions included suitable inhibitors when required. Ambenonium (Mytelase; *N*:*N*-bis-3 diethylamine ethyl oxamide bis-q-chlorobenzyl chloride, $5 \times 10^{-6}$ M) and DFP ($10^{-6}$ M) were employed to inhibit differentially AChE and BuChE respectively. The isoenzymes of microsomes and synaptosomes were stained for AChE employing the modification by Juul [23] of the method of Koelle [1]. A Canalco model T microdensitometer without a filter was used to determine relative distribution of AChE activity in various isoenzymes. For heavier and weaker bands, the settings were 20 and 4 $mm^2$/integration respectively. The contribution of each peak to the total AChE activity was calculated in per cent of the latter. The specific AChE activity of the applied sample was obtained by the method of Ellman *et al.* [22]; relative specific activity of each isoenzyme was calculated from per cent contribution of each peak to total AChE activity. The relative recovery of enzyme activity of microsomal and synaptosomal isoenzymes was calculated by comparing the values obtained in DFP-treated rats with controls (cf. Figs. 5 and 6).

## RESULTS

To ascertain the purity and authenticity of the synaptosomal fractions, electron microscopy analysis was employed. A typical electron micrograph of the fraction is shown in Fig. 1; as can be seen, the fraction contained intact synaptosomes with their vesicles, intraterminal mitochondria and junctional complexes. This was the general finding in the case of all the micrographs studied at present; it is in good agreement with the available descriptions of the synaptosomal fractions [25]. To further ascertain the degree of microsomal contamination of the synaptosomal fraction, the NADPH-dependent cytochrome *c* reductase was measured in the presence of rotenone. Mean reductase values were 0.255 and 0.028 unit/min/mg of protein for the microsomes and synaptosomes respectively; thus, it appeared that synaptosomes contained less than 11 per cent of microsomal contamination.

The first series of experiments was carried out with 20-day-old rats. First, the intracerebral dose of DFP capable of significant inhibition of cholinesterases was established. DFP doses of 45, 60, 75, 90, 105 and 120 μg were employed in rats of 25 g of weight, three rats/dose; total cholinesterase (AChE and BuChE) activity was measured in the brain homogenates. The inhibition of brain ChE varied from 75 ± 2.5 to 95 ± 2.3 per cent with doses of 45 and 120 μg respectively. The dose of 105–115 μg (3.5 mg/kg) was employed routinely in the subsequent experiments.

Table 1 summarizes the activity of control and DFP-treated rats at various intervals of time up to 15 days after the administration of DFP, at which time the rats were 35 days old. Four hr after injection, DFP caused an inhibition of 76 and 60 per cent, respectively, of the microsomal and nerve ending AChE. Fifteen days later, the inhibition amounted to 35 and 13 per cent respectively; this difference between the synaptosomal and microsomal AChE inhibition was statistically significant (Fig. 2). The times required for the 50 per cent return of the activities of the two enzymes (half-lives or $T_{1/2}$ times) were approximately 9 and 14 days for the synaptosomal and microsomal AChE respectively. As can be seen from Fig. 2, the recovery of enzyme activity was initiated at least as early in the nerve endings as in the microsomal fraction.

As in this series of experiments DFP was administered at the time of rapid formation of AChE [26], it was critical to differentiate between the ontogenesis of AChE on the one hand and the post-DFP AChE activity on the other. The developmental increases in AChE activity were determined in control rats for the growth period of days 20–35 (Table 1) and the increments subtracted from the values for enzyme activity of DFP-treated rats at the pertinent time intervals (Fig. 3). As can be seen, the microsomes and the synaptosomes of the DFP-treated animals exhibited, in addition to postnatal increase in AChE, 16 and 43 per cent increments of AChE activity respectively. The differences between the microsomal and

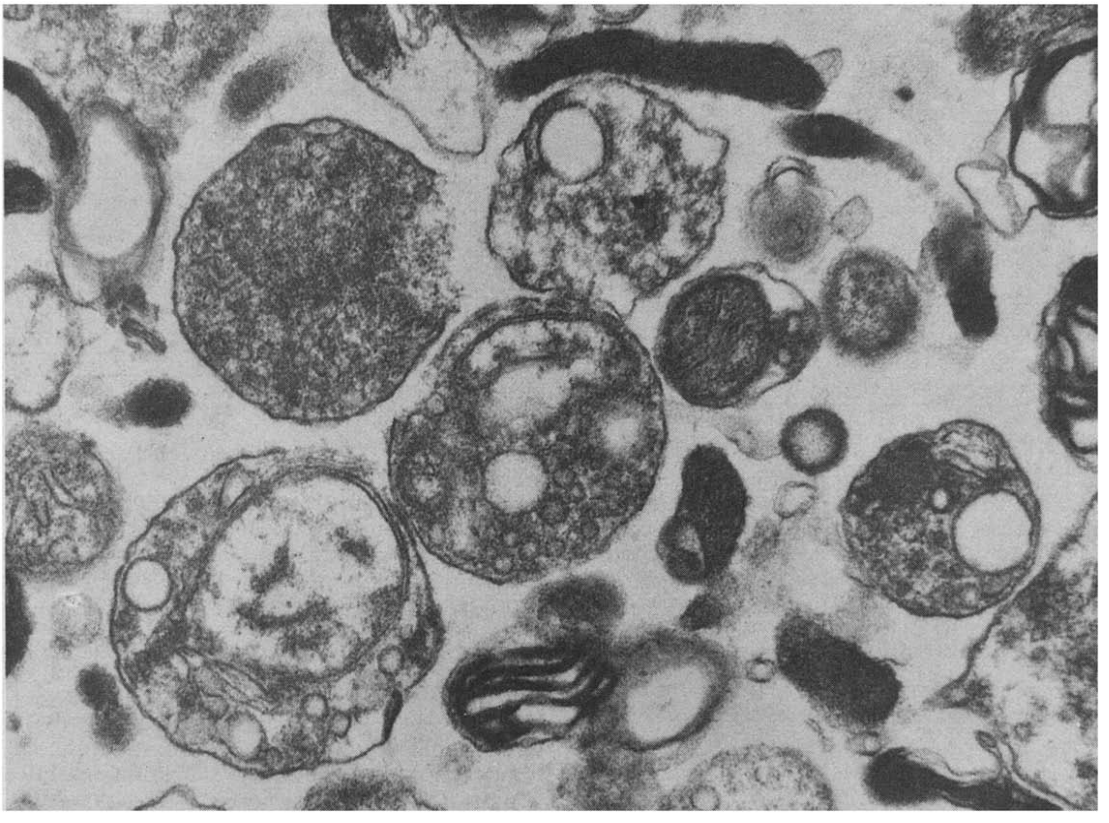

Fig. 1. Electron micrograph of the synaptosomal fraction of rat brain. Note the presence of intact synaptosomes, junctional complexes and intraterminal mitochondria and synaptic vesicles.

synaptosomal AChE activities were highly significant and, in fact, more pronounced than those between the values representing the total increases for the two enzymes (compare Figs. 2 and 3). This was due to the fact that the correction was more pronounced in the case of microsomes than in that of synaptosomes, as the former exhibited, during the 15 days in question, a much higher increase in developmental AChE than the latter (Table 1).

Studying protein synthesis in synaptosomes, Austin and Morgan [27] showed that the rate of incorporation of [$^{14}$C]leucine decreases linearly with the age of the rat. The question arises as to whether AChE may appear early in the nerve endings after DFP administration only during the period of developmental synthesis of proteins or also after the brain maturation has occurred. Fifty-eight-day-old rats were used for the pertinent study.

It was first determined that indeed AChE activity of the two fractions increased only slightly after 58 days of life. The mean specific activities of the synaptosomal and microsomal AChE were 0.125 ± 0.003 and 0.179 ± 0.004, respectively, in the case of 58-day-old rats, and 0.145 ± 0.004 and 0.184 ± 0.005, respectively, in that of 78-day-old rats (three rats/group). These data are in agreement with those of Freedman and Himwich [28].

The intracerebral dose of 350–500 μg DFP was used, depending on rat weight; this dose sufficed to produce marked inhibition of microsomal and synaptosomal AChE (cf. Fig. 4). Figure 4 shows AChE activity of microsomes and synaptosomes of the control and treated rats on days 1–20 after the administration of either DFP or propylene glycol. As can be seen, 80 and 86 per cent of microsomal and of the nerve ending AChE, respectively, was inhibited on the day

Table 1. Activity of acetylcholinesterase in subcellular fractions of rat brain after inhibition of the enzyme by DFP (3.5 mg/kg) injected intracerebrally in 20-day-old rats

| | Control | | | DFP-treated group | | | |
|---|---|---|---|---|---|---|---|
| Age of rat | No. of rats | Microsomes (sp. act. ± S. E. M.) | Synaptosomes (sp. act. ± S. E. M.) | Days after injection | No. of rats | Microsomes (sp. act. ± S. E. M.) | Synaptosomes (sp. act. ± S. E. M) |
| 20 | 6 | 0.109 ± 0.002 | 0.0986 ± 0.001 | 1 | 6 | 0.0263 ± 0.0001 | 0.0399 ± 0.0002 |
| 23 | 6 | 0.145 ± 0.003 | 0.1027 ± 0.001 | 3 | 6 | 0.0618 ± 0.0005 | 0.0478 ± 0.0004 |
| 25 | 6 | 0.187 ± 0.005 | 0.122 ± 0.002 | 5 | 6 | 0.0974 ± 0.001 | 0.0864 ± 0.0008 |
| 35 | 6 | 0.190 ± 0.004 | 0.120 ± 0.002 | 15 | 6 | 0.125 ± 0.003 | 0.104 ± 0.002 |

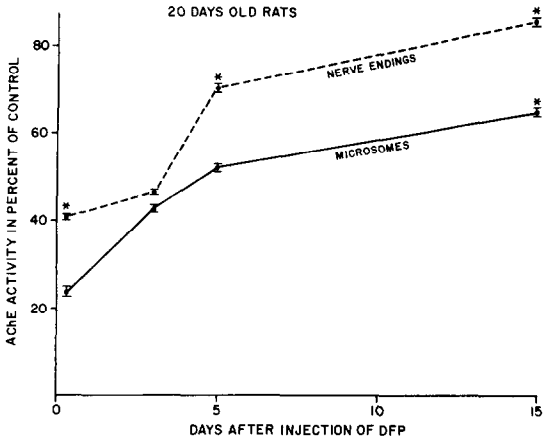


Fig. 2. Effect of DFP on AChE activity of microsomal and nerve ending fractions. DFP was administered to 20-day-old rats. Abscissa: days after intracerebral injection of DFP. Ordinate: activities of microsomal and nerve ending AChE in per cent of controls. The first two points represent the activities of microsomal and nerve ending AChE 4 hr after DFP. The points represent means ± S. E. (six rat brains/mean). Asterisks show significant differences between means, the P values varying between $\leq 0.05$ and $\leq 0.01$.

of injection; 35 per cent of the microsomal AChE and 22 per cent of the synaptosomal AChE remained inhibited on day 20 after DFP (see Fig. 4). To facilitate the comparison between the rate of increase after DFP, of the microsomal and synaptosomal enzyme, Fig. 4 also shows these increments after correcting for the slight difference between the inhibition of the synaptosomal and microsomal AChE (86 and 80 per cent respectively). Altogether, as in the case of younger animals, the synaptosomal AChE showed a more rapid rate of activity increase as compared to the microsomal enzyme; in fact, at any interval after

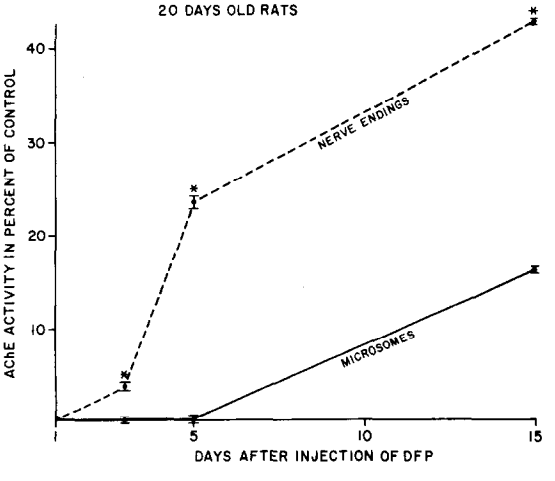


Fig. 3. Effect of DFP on microsomal and nerve ending AChE as corrected for developmental increment of AChE. DFP was given to 20-day-old rats; the subsequent inhibition of microsomal and nerve ending AChE is expressed in per cent of controls after correcting the post-DFP AChE activities for the developmental increase of the two enzymes in the control rats (cf. also text). For other explanations, see Fig. 2.

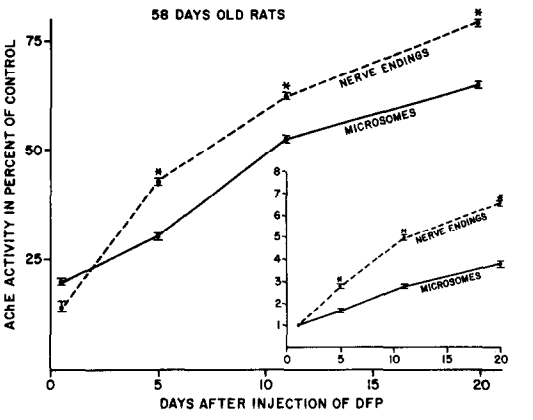


Fig. 4. Effect of DFP on microsomal and nerve ending AChE in 58-day-old rats. DFP was administered intracerebrally. Abscissa: days after injection of DFP; ordinate: activities of microsomal and nerve ending AChE in per cent of controls; notice the relatively slight difference between the initial inhibition of microsomal and nerve ending AChE (80 and 86 per cent respectively). In the inset, abscissa: days after injection of DFP; ordinate: AChE activities, the microsomal activity taken as 1.0. In the case of both figures, the points represent means ± S. E. (six rat brains/mean), the asterisks show significant differences between means, the P values varying between $\leq 0.05$ and 0.005, and the first two points represent the activities of microsomal and nerve ending AChE 4 hr after DFP.

the administration of DFP the nerve endings always showed a higher enzyme activity than the microsomes. The $T_{1/2}$ times were approximately 11 and 19 days for the synaptosomal and microsomal enzyme respectively. These values did not differ essentially from those calculated for the 20-day-old animals. Thus, the post-DFP recovery of AChE activity of the nerve endings is not an age-dependent phenomenon.

To be able to evaluate AChE isoenzymes and their patterns, it was necessary to solubilize all the isoenzymes, since the major part of enzymatic activity is membrane-bound [24]. Triton X-100 (1%) was used to solubilize the membrane-bound AChE; the electrophoretic pattern of the AChE isoenzymes obtained from the supernatant of Triton X-100-treated synaptosomal and microsomal fractions was consistent and reproducible [18].

The study was carried out in 20-day-old rats. Figures 5A and 6A show the pattern of the microsomal and synaptosomal isoenzymes after their electrophoretic separation; the bands were stained for AChE as described in Methods. In both cases, four isoenzymes can be seen. The general pattern and intensities of staining of synaptosomal and microsomal isoenzymes differed. For instance, the synaptosomal isoenzymes generally separated better than the microsomal ones; furthermore, the synaptosomal isoenzymes 3 and 1 seemed to contain higher percentages of the total synaptosomal AChE activity as compared to the microsomal isoenzymes 3 and 1 with respect to the total microsomal enzyme activity. Whether all or some of the isoenzymes of the two fractions represent common forms cannot be readily resolved (cf. Discussion); accordingly, they are referred to as microsomal isoenzymes M1–4 and synaptosomal isoenzymes S1–4.

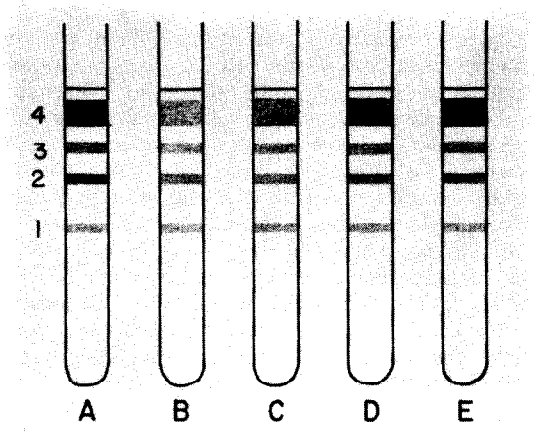


Fig. 5. AChE isoenzymes from the Triton X-100 extract of rat brain microsomal preparation. The original photographs of gels obtained in a single rat experiment were diagramatized to illustrate control pattern as well as the effect of DFP. Electrophoresis and AChE staining were carried out as described in Methods. Gel A: control isoenzyme pattern; gels B–E: isoenzyme patterns 1, 3, 5 and 15 days, respectively, after intracerebral injection of DFP (see also text).

| | M4 | M3 | M2 | M1 |
|---|---|---|---|---|
| Day 1 | 18.8 | 75 | 51 | 100 |
| Day 3 | 33.8 | 89 | 54 | |
| Day 5 | 48.3 | | 59 | |
| Day 15 | 64.2 | 100 | 75 | |

AChE activities of microsomal isoenzymes after intracerebral DFP administration. Enzyme activities in per cent of controls were determined by densitometric scans (cf. Methods and text). The values are given as means of six experiments (six rats) per value.

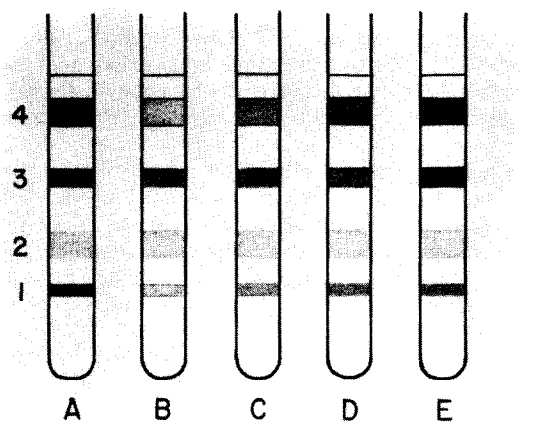


Fig. 6. AChE isoenzymes from the Triton X-100 extract of rat brain synaptosomal preparation. The original photographs of gels obtained in a single rat experiment were diagramatized to illustrate control pattern as well as the effect of DFP. Electrophoresis and AChE staining were carried out as described in Methods. Gel A: control isoenzyme pattern; gels B–E: isoenzyme patterns 1, 3, 5 and 15 days, respectively, after intracerebral injection of DFP (see also text).

| | S4 | S3 | S2 | S1 |
|---|---|---|---|---|
| Day 1 | 17.0 | 70 | 96 | 18.2 |
| Day 3 | 32.1 | 78 | 98 | 38.2 |
| Day 5 | 52.7 | 88 | 100 | 60.7 |
| Day 15 | 84.0 | 100 | 100 | 75.4 |

AChE activities of synaptosomal isoenzymes after intracerebral DFP administration. Enzyme activities in per cent of controls were determined by densitometric scans (cf. Methods and text). The values are given as means of six experiments (six rats) per value.

Synaptosomal and microsomal isoenzymes appeared to be differentially sensitive to the intracerebral administration of DFP, 105–115 μg (3.5 mg/kg); furthermore, post-DFP increase in the activity of the isoenzymes proceeded at different rates, as demonstrated by the quantitative densitometric evaluation of the AChE activities (cf. Methods; see Figs. 5B and 6B). In the decreasing order of their DFP sensitivity, the microsomal isoenzymes could be ranked as M4, 2 and 3, M1 being insensitive to DFP. M3 showed the fastest return of activity after DFP; the approximate half-lives were 3, 6 and 15 days, respectively, for M3, 4 and 2. Synaptosomal isoenzymes could be ranked as S4, 1 and 3 in the decreasing order of DFP sensitivity; S2 was little or not at all affected by DFP. S3 and S1 exhibited the fastest rates of return of activity after DFP; the approximate half-lives were 4.5, 5 and 8.5 days for S1, 3 and 4 respectively. Altogether, over the 15-day time period after DFP, the AChE activities of the inhibited microsomal and synaptosomal isoenzymes and their characteristic pattern gradually returned toward normal levels; there was no interchange of patterns (cf. Figs. 5A and 6A).

## DISCUSSION

AChE is inhibited irreversibly by organophosphorus anti-AChE compounds (cf. for instance Ref. 29); in the case of DFP this inhibition becomes irreversible after the "aging" process of 30–60 min, and this fact was reflected in the experimental protocol used at present. The synthesis of AChE which follows the administration of DFP was exploited in the past [12,13,30,31] to resolve the question of the cell site of origin of the new enzyme.

It should be pointed out that the dose of DFP needed to significantly inhibit brain AChE was relatively high, even though DFP was given intracerebrally (cf. Results). There may be several reasons for this, as DFP is bound irreversibly by non-ChE proteins and by proteolipids (cf. Ref. 29) and as it is relatively readily hydrolyzed enzymically [32] and in alkaline media.

The present results demonstrate clearly that both in the growing (20-day-old) and older (58-day-old) rats the post-DFP recovery of activity of the synaptosomal AChE proceeds fast and early. This was particularly pronounced in the younger rats when the post-DFP synthesis of AChE was corrected to account for rapid postnatal synthesis of the enzyme (cf. Refs. 26, 33 and 34); after DFP, 43 per cent of the control enzyme level was restituted in the synaptosomes in addition to the ontogenetic AChE.

The fast increase in the activity of synaptosomal AChE may be compatible with the concept that the enzyme is synthesized in the microsomes and carried to the nerve endings by the fast axoplasmic transport; indeed, the latter may proceed in certain peripheral

nerves at the rate of 430 mm/24 hr; however, a relatively small fraction of AChE is transported at this rate [14]. Furthermore, this explanation does not seem to account for several observations made at present. First, it is difficult to reconcile the microsomal origin of the nerve terminal AChE with the earlier appearance of the latter as compared to the former. Second, it is difficult to explain, on this basis, the fact that the activity of the synaptosomal enzyme increased faster than that of the microsomal enzyme. If the enzyme had originated in the microsomes and had accumulated in the nerve terminals, the curves depicting the increase in the activity of the two enzymes should have crossed each other at some point in time, which was not the case (cf. Fig. 3 and insert of Fig. 4). Third, the pattern of the synaptosomal and microsomal isoenzymes, their DFP sensitivity, and the rate of the reappearance of their activity after DFP differed, which again suggests their independent origin. Altogether, the present data suggest the existence of an independent mechanism for AChE synthesis in the nerve terminals; furthermore, this mechanism seems to be available both during postnatal ontogenesis and in mature animals.

Similar conclusions were reached with respect to the origin of post-DFP AChE in the amphibian [10,14,31,32] and in the mammalian peripheral nervous system. For instance, Koenig [31] was able to demonstrate post-DFP synthesis all along the axon of the cut hypoglossal and cervical sympathetic nerves; he proposed that the axonal AChE and other proteins which are synthesized in the perikaryon become constituents of the axoplasm, whereas axolemma-bound proteins and enzymes such as AChE are synthesized by local axonal mechanisms.

The nature of the AChE isoenzymes observed in the present study deserves a special consideration; particularly, do the microsomal and nerve ending isoenzymes represent distinct and separate forms? First, it must be stressed in this context that the contamination of the nerve ending fraction by the microsomal material was minimal, as indicated by the low nerve endings level of the cytochrome *c* reductase after rotenone pretreatment (cf. Methods).

The heterogeneity of the mammalian AChE [15–18,26,35,36] including that of the synaptosomal and mitochondrial enzymes [36,37] is well documented; and in fact the S1–4 and M1–4 isoenzymes described at this time may represent different and distinct forms. This may be consistent with the differences in the pattern of the isoenzymes of the two fractions (cf. Figs. 5 and 6), with their differential sensitivity to DFP, and with the differences in the rates of the increments in their post-DFP activity. Finally, the differences in the half-life times of the various isoenzymes may support the notion of their distinctiveness. It should be added in this context that the return of activity may represent the repair of the molecule rather than the synthesis; synthesis *de novo* of the whole structure after the DFP phosphorylation may not be necessary.

On the other hand, some of the evidence presented earlier from this laboratory seems to suggest that only four identical isoenzymes are present in both fractions; thus, the four isoenzymes in question may be common to both sources. The measurement of the mobilities of the isoenzymes would be pertinent although not decisive in this context: the differences may be small so that the use of markers for the measurement of the mobilities would not be helpful.

The isoenzymes in question may actually constitute aggregates of the nomomeric form with the molecular weight of 80,000 [38, 39]; the microsomal and nerve ending isoenzymes described at this time may represent the dimers which constitute the smallest active unit. Microsomal and synaptosomal isoenzymes 4 may represent high concentrations of the dimers. Furthermore, in the cell, these isoenzymes may be present in the cellular compartment readily accessible to DFP. On the other hand, the other isoenzymes seemed less sensitive to the inhibitor; they may represent dimers attached to the fragments of the cell membrane. Such attachment appears likely on the basis of difficulties encountered in solubilizing AChE in media not containing detergents [40]. Furthermore, *in vivo*, the membrane-bound AChE forms may be less responsive to DFP as their complexing with the membrane may induce conformational changes and provide constraints which would decrease their capacity to combine with DFP. In fact, these forms of AChE may be also limited in their capacity to hydrolyze ACh, and this concept is consistent with the recent suggestion that cytoplasmic rather than vesicular ACh may be important for transmission [41].

The half-life of the microsomal and synaptosomal isoenzymes 4 was similar to that of the whole enzyme; thus, their high activity suggests that the isoenzymes 4 contribute most to the total AChE activity of the brain neurons. Highly variable data were reported for the half-life of AChE. Depending on the species, site and inhibitor employed, half-lives varied from 3 hr [16] to 1–5 days [42,43], to 11 or even 16 days [44,45]. Our own figures are intermediate between half-times for the post-DFP recovery of total AChE of the rat brain [44] and the post-Soman recovery of synaptosomal and microsomal total AChE of the cat brain [43]. Indubitably, some isoenzymes show a rapid turnover. This may be related to the relatively fast return of function after DFP (cf., for instance Ref. 46); these isoenzymes may also serve as precursors or "building blocks" with respect to isoenzymes characterized by a slow turnover value.

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