In order to study the site of denitration in blood, the rate of metabolism in human whole blood, red cells, and plasma was compared in five subjects. Whole blood metabolized GTN to GDN − 1·2 at a rate of 0·079 (± 0·003) and GDN −1·3 at a rate of 0·017 (± 0·003) μmoles/ml/min. Washed red cells showed about the same rate of denitration: GDN −1·2, 0·073 (± 0·006); GDN −1·3, 0·014 (± 0·002) μmoles/ml/min respectively. In the incubation with plasma, no metabolites could be found. Crandall[6] has reported that GTN and erythritol tetranitrate could be denitrated enzymatically by dog blood erythrocytes. However, DiCarlo and Melgar[7] reported that GTN can be metabolized by rat serum, at the temperature optimum 50–57°.

Enzymatic denitration of GTN in liver is known to be dependent on GSH.[12,13] In species so far tested where there is no plasma GSH, there is an abundance of it in the erythrocytes.[9,10] Even after addition of GSH to plasma, there was no enzymatic denitration. In contrast, in dialyzed red cells, from which the endogenous GSH has been removed, the addition of GSH restores the activity. GSH is therefore required for the metabolism of GTN in red blood cells.

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Effects of obidoxime on content and synthesis of brain acetylcholine in DFP intoxicated rats

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IN A PREVIOUS paper[1] we demonstrated that the reactivator obidoxime prevented the rise of rat brain ACh* during DFP intoxication without modification of the inhibited brain ChE activity.

Two hypotheses were suggested to explain this phenomenon: (1) the diffusion to the periphery of

*Abbreviations used: ACh, acetylcholine; ChE as used here is total capacity to hydrolyze acetylthiocholine under standard conditions; ACh synthesis refers to total ACh found in the tissue and medium after 30-min incubation period. DFP, diisopropyl phosporofluoridate; obidoxime, bis 4-hydroxyiminoethyl-pyridinium-1-methyl ether dichloride.

ACh accumulated in the brain, and the subsequent hydrolysis of ACh by obidoxime reactivated erythrocyte ChE, or (2) an effect of this oxime on ACh synthesis. This latter postulated action of the oxime could be indirect affecting a negative feedback mechanism controlling ACh concentration and the rate of synthesis, as suggested by Sharkawi and Schulman,[2] since it is known that very small amounts of the drug cross the blood brain barrier.[1,3] However, a direct action is not excluded, since Bajgar *et al.*[4] have recently demonstrated that obidoxime reactivated ChE activity in the ponto-medullar area of the mouse brain during isopropylmethyl phosphorofluoridate intoxication and therefore must have reached this section of the brain.

In this paper the effects of obidoxime treatment on ACh content and synthesis in whole rat brain and in cerebral cortex are described.

MATERIALS AND METHODS

Animals. Female rats of 150–190 g, from an outbred Wistar derived strain maintained at our Institute, were used.

Materials. Commercial preparations of ACh-chloride (Roche S.p.A., Milano, Italy) and of obidoxime (Toxogonin,® Merck A.G., Darmstadt, West Germany) were used. DFP was purchased from B.D.H. (British Drug House Ltd., England).

Drug treatment and preparation of biological material. DFP, dissolved in peanut oil (1·5 mg/ml), was given s.c. at a dose of 1·5 mg/kg. Control rats received an equivalent volume of peanut oil subcutaneously. An aqueous solution of obidoxime (5 mg/ml) was administered i.p. in a total dose of 25 mg/kg, 12·5 mg/kg 10 min before DFP treatment and the balance 15 min after DFP administration. The rats were killed by decapitation 90 min after DFP. In order to cool the brain immediately after killing, but without freezing, the decapitated heads were placed in liquid nitrogen for about 15 sec, following which the brain, without cerebellum, was removed. This procedure was used in experiments on whole brain and in experiments on cerebral cortex.

In the experiments on the whole brain one hemisphere was used for the determination of ACh content. The other hemisphere was cut, with a Stadie–Riggs tissue slicer, into slices about 0·35 mm thick and incubated immediately to determine ACh synthesis.

In the experiments on the cerebral cortex, cortical slices of approximately 100 mg were cut with a Stadie-Riggs slicer, each hemisphere providing one slice about 0·35 mm thick. A cortical slice from one hemisphere was used for the determination of ACh content, while a slice from the same area of the other hemisphere was used to determine ACh synthesis. All operations were carried out at 0°.

Determination of ACh content in the whole brain and in cerebral cortex. A whole brain hemisphere, or a cortical slice was homogenized in a Potter homogenizer containing Ringer solution and 10% TCA (1:8:1) at 0°. The homogenate was centrifuged at 12,000 *g* for 20 min, and the supernatant brought to pH 7·2 with 0·1 N NaOH just before the bioassay. All steps were carried out at 0°.

The ACh content was bioassayed on isolated strips of terminal guinea-pig ileum, as previously described.[1]

Synthesis of total ACh in the whole brain and in cerebral cortex. The conditions of incubation were the same as previously described[5] but with low K^+ concentration. Approximately 500 mg of whole brain slices were incubated in 5·0 ml of McLennan and Elliott[6] saline medium with modified K^+ (0·121 M NaCl, 0·004 M KCl, 0·024 M $NaHCO_3$, 1·2 mM $MgSO_4$, 1·3 mM $CaCl_2$, 0·4 mM KH_2PO_4) containing 0·01 M glucose and 0·2 mM eserine sulphate at pH 7·4. In the case of cerebral cortex a cortical slice of approximately 100 mg was incubated in 2·0 ml of the same medium. The tissues were incubated in an atmosphere of 95% oxygen and 5% carbon dioxide for 30 min at 37°. At the end of the incubation period 10% TCA was added to the medium. The total ACh in the tissue and medium after 30 min incubation period was assayed as described. In order to exclude any effect of eserine, present in these homogenates, on the response of isolated terminal guinea-pig ileum the same amount of eserine sulphate was added to the standard solution as a control.

RESULTS

ACh content and ACh synthesis in whole rat brain. The effects of obidoxime administered shortly before and shortly after DFP on ACh content and ACh synthesis in whole brain are shown in Fig. 1. As in the previous paper, the whole brain of rats treated with DFP alone and killed 90 min later exhibited a 58% elevation of ACh content when compared with the controls and obidoxime treatment prevented the rise in brain ACh in DFP-treated rats ($P < 0.0005$). The *in vitro* synthesis of total ACh in whole brain of rats treated with DFP alone and with obidoxime did not differ significantly from that of the control group.

ACh content and ACh synthesis in rat cerebral cortex. The effects of obidoxime administered shortly before and shortly after DFP on ACh content and ACh synthesis in cerebral cortex are shown in

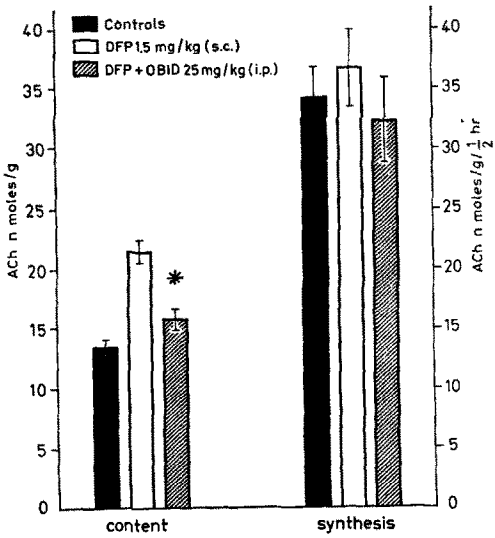

FIG. 1. Effect of obidoxime on ACh content and ACh synthesis in whole brain of DFP-intoxicated rats. The rats received 1·5 mg/kg s.c. of DFP and two OBID (obidoxime) treatments (12·5 mg/kg i.p. 10 min before and 15 min after DFP) and were killed 90 min after DFP administration. One hemisphere was used for the determination of ACh content and the other for its synthesis during the incubation. ACh was bioassayed on guinea-pig ileum. The values for ACh content are nmoles/g of fresh tissue. Total ACh synthesis was measured after 30 min incubation in a medium containing 4 mM KCl, and expressed as nmoles of ACh/g of fresh tissue per 0·5 hour. The bars give the mean ± S.E. for groups of 6 rats. The asterisk indicates a significant difference between a DFP–OBID group and the corresponding DFP group (two-tailed test, $P < 0.0005$). Black bars show ACh content and ACh synthesis after incubation in nine untreated rats not included in the balanced replicates of the experiment.

Fig. 2. The ACh content of cerebral cortex in control animals was about 30 per cent lower than that of the whole brain.

Cerebral cortex of rats treated with DFP alone and killed 90 min later exhibited a 63 per cent elevation of ACh content when compared with the controls which was again prevented by obidoxime ($P < 0.001$). The *in vitro* synthesis of the total ACh in the cerebral cortex was not affected by DFP treatment or DFP plus obidoxime treatment.

DISCUSSION

The present results on ACh content in the whole brain and in cerebral cortex of DFP and DFP-obidoxime-treated rats confirm and extend the data obtained in our previous work,[1] that is the increase of ACh content seen in the early phase of DFP intoxication is prevented by obidoxime.

At the outset of this work we presumed, on the basis of some results reported by Bajgar,[7] that there might be some differences in ACh content between cerebral cortex and whole brain, following DFP and DFP-obidoxime treatment. However, it appears from these results that the increase in ACh content of DFP-treated rats was the same for total brain and cerebral cortex (158 and 163 per cent of control values respectively) as was the decrease in ACh levels due to obidoxime treatment (118 and 110 per cent of control values respectively).

We were not able to demonstrate any statistically significant difference in the synthesis of total ACh by DFP or DFP-obidoxime treated rats in whole brain or in cerebral cortex *in vitro*. It is noteworthy that Sharkawi and Schulman[2] have demonstrated that the amount of ^{14}C–ACh formed by cortex slices from morphine-treated rats, exhibiting a 45 per cent elevated ACh content, was markedly less than that formed by slices from untreated animals when incubated in 4 mM KCl medium, that is in experimental conditions similar to ours.

It appears that obidoxime antagonizes the rise of brain ACh without modifying the effects of DFP on brain ACh hydrolysis[1] or affecting its synthesis.

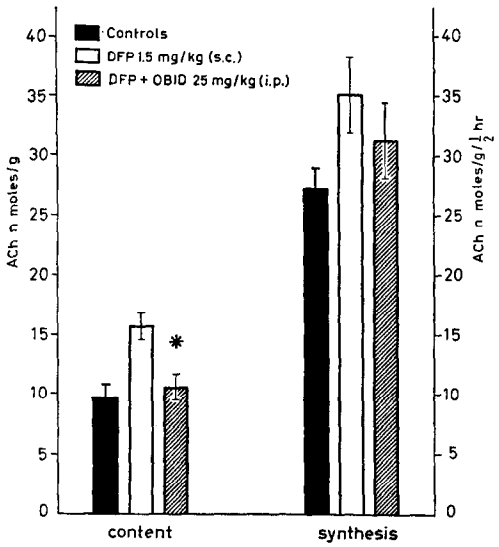

FIG. 2. Effect of obidoxime on ACh content and ACh synthesis in cerebral cortex of DFP-intoxicated rats. Drug treatment, ACh content and ACh synthesis were determined and expressed as described in Fig. 1. The bars give the mean ± S.E. for groups of nine rats. The asterisk indicates a significant difference between a DFP–OBID group and the corresponding DFP group (two-tailed test, $P < 0.001$). Black bars show ACh content and ACh synthesis in ten untreated rats not included in the balanced replicates of the experiment.

Obidoxime, in spite of its inability to influence ACh hydrolysis and ACh synthesis in brain can modify the ACh content in brain through its peripheral action, since earlier work by this laboratory[1] demonstrated a significant reactivation of peripheral ChE's in erythrocytes and serum by obidoxime.

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