REFERENCES

1. A. Sato, T. Nakajima and Y. Koyama, *Toxic. appl. Pharmac.* **68**, 242 (1983).
2. A. H. Conney, *Pharmac. Rev.* **19**, 317 (1967).
3. A. D. Alvares, D. R. Bickers and A. Kappas, *Proc. natn. Acad. Sci. U.S.A.* **70**, 1321 (1973).
4. R. Snyder and H. Remmer, *Pharmac. Ther.* **7**, 203 (1979).
5. T. Nakajima, Y. Koyama and A. Sato, *Biochem. Pharmac.* **31**, 1005 (1982).
6. A. Sato and T. Nakajima, *Toxic. appl. Pharmac.* **47**, 41 (1979).
7. O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. biol. Chem.* **193**, 265 (1951).
8. G. L. Miller, *Analyt. Chem.* **31**, 964 (1959).
9. T. Omura and R. Sato, *J. biol. Chem.* **239**, 2370 (1964).
10. Y. Y. Tu and C. S. Yang, *Cancer Res.* **43**, 623 (1983).
11. T. Nakajima and A. Sato, *Toxic. appl. Pharmac.* **50**, 549 (1979).
12. R. Kato, *Jap. J. Pharmac.* **17**, 181 (1967).
13. H. Greim and H. Remmer, *Naunyn-Schmiedebergs Arch. Pharmac.* **266**, 338 (1970).
14. M. Marselos and M. Laitinen, *Biochem. Pharmac.* **24**, 1529 (1975).

Biochemical Pharmacology, Vol. 33, No. 15, pp. 2519–2521, 1984.
Printed in Great Britain.

0006-2952/84 $3.00 + 0.00

Succinylcholine—tissue distribution and elimination from plasma in the dog

(*Received* 9 *December* 1983; *accepted* 16 *March* 1984)

According to Taylor [1] the brief duration of action of SCh** is due to a rapid enzymatic hydrolysis by pseudocholinesterase. Its presence in blood is indeed very short-lived as recently proven by chemical determinations [2]. Other means of disposition seem to have been largely neglected in the discussion of its evanescent action. The experiments reported here were undertaken to throw further light on the role of enzymatic hydrolysis as compared to redistribution in tissues and excretion.

Materials and methods

Chemicals. All chemicals were of analytical grade. The reagents were prepared as described by Nordgren *et al.* [2].

Animal experiments. Mongrel dogs were injected i.v. or i.m. with doses of SCh ranging between 2 and 106 mg/kg body weight. The dogs were pretreated with pentobarbital. Blood was collected from an indwelling catheter in heparinized tubes containing eserine (final concentration 10^{-4} M) at intervals ranging between 0.5 and 36 min after drug administration. Tissues were collected 1–45 min after administration of SCh. In some cases the tissues were embalmed by injection and soaking in FAX (Champion Chemical Co., Ontario, Canada), a commercially available glutaraldehyde embalming fluid diluted 1:8 with water. Two of the dogs received artificial ventilation from a Harvard Model 613 Dual Phase Respirator throughout the experiments. The others were left to die from the dose of SCh (no artificial respiration).

Determination of SCh. The analytical method used has been described by Forney *et al.* [3] and Nordgren *et al.* [2]. SCh is extracted from plasma or tissue homogenate (in 0.4 N perchloric acid) into dichloromethane as an ion pair with hexanitrodiphenylamine. To enable gas chromatography SCh is demethylated with sodium benzenethiolate to form the corresponding tertiary amine which is quantitated by gas chromatography–mass spectrometry using deuterium labeled SCh as internal standard.

Results and discussion

To throw further light on the importance of enzymatic hydrolysis of SCh as compared to tissue distribution and excretion, the elimination of SCh from dog plasma was studied in animals kept alive by artificial respiration and in animals that were left to die from the SCh dose. Dog plasma is known to have ChE activity [4]. There is no reason to believe that the plasma ChE activity stops when the animals die, since it is well known that the esterase activity can be kept under test tube conditions for a considerable time.

The results are demonstrated in Fig. 1. Panel A and B show the elimination from plasma during artificial respiration. A dog was injected with SCh 2 mg/kg i.v. (panel A). A second dose of SCh (106 mg/kg) was administered i.v. 1.5 hr after the first dose (panel B). Artificial ventilation was maintained throughout the experiment. Even after the higher dose the SCh is rapidly eliminated. This was expected and is in accordance with studies in humans [1, 2].

Panel C and D show the plasma curves from two dogs receiving no artificial ventilation. The dose administered was 10 mg/kg i.v. (panel C) and 67 mg/kg i.v. (panel D), respectively. In both dogs the respirations ceased after approximately 30 sec, followed by a decrease in blood pressure and cessation of regular heart beats after about 4 min. In these dogs, the decrease in plasma SCh stopped upon circulatory failure, which indicates that the rapid disappearance of SCh from plasma is not only due to enzymatic hydrolysis. Tissue distribution and excretion also play an important role.

The initial concentration of SCh (30 sec after injection) is very high, but in all cases except one in accordance with a rapid and even distribution to the whole plasma volume. In the dog given the high dose and no artificial ventilation, the initial concentration is higher than expected, probably due to the rapid circulatory failure.

The concentration of SCh in tissues, under the above mentioned conditions, was also studied in samples collected at different times after administration (from one up to 45 min). Because of the analysis of forensic cases performed simultaneously some of the tissues were embalmed. The results are summarized in Table 1. In all cases, without comparison, the kidney has the highest levels, and the elimination seems to be rather slow (No. 1). The relative distribution between the organs studied show roughly the same relationship in all dogs, with or without artificial respiration and irrespective of the dose administered. However, the tissue concentrations seem to be lower for animals kept under artificial respiration (No. 1 and 5), even though a lower dose of SCh naturally results in lower tissue levels. I.m. administration results in considerably lower tissue levels compared to a similar i.v. dose (No. 3 and 4). After injection of 10 mg/kg i.m. cessation of respiration occurred after about one min.

The present study shows that the rapid disappearance of SCh from plasma is not only due to hydrolysis by ChE,

** Abbreviations: SCh, succinylcholine; ChE, cholinesterase.

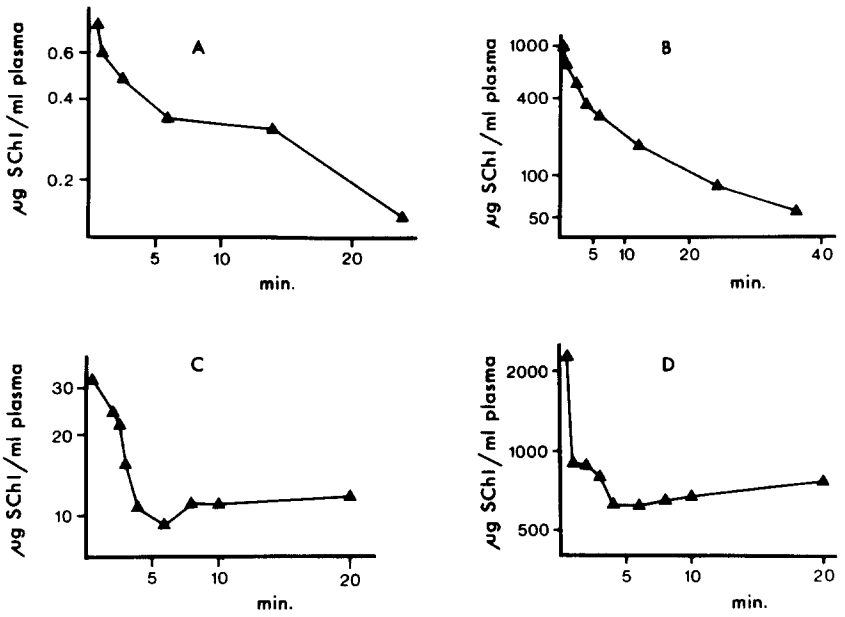

Fig. 1. Elimination of SCh iodide in dog plasma after i.v. administration of: (A) 2 mg/kg, 1.5 hr later followed by (B) 106 mg/kg. The dog was kept under artificial respiration. (C) 10 mg/kg. No artificial respiration. (D) 67 mg/kg. No artificial respiration.

Table 1. Tissue levels of SCh iodide

Dog No.	Tissue	Dose (mg/kg)	Route of administration	Time after administration (min)	μg SCh I/g
1	Kidney	2 + 106*	i.v.	13*	1175†‡
	Kidney			38*	803†‡
	Liver			43*	5†‡
	Diaphragm			45*	65†‡
	Spleen			42*	107†‡
2	Kidney	67	i.v.	25	2950
	Liver			25	14
	Diaphragm			25	203
3	Kidney	10	i.v.	2	219‡
	Kidney			10	306‡
	Liver			1	0.91‡
	Liver			10	0.52‡
4	Kidney	10	i.m.	4	2.6‡
	Kidney			8	1.6‡
	Liver			4	0.022‡
	Liver			8	0.014‡
5	Kidney	100 + 33§	i.v. + i.m.§	15§	1108†‡
	Liver			19§	10†‡
	Diaphragm			25§	45†‡
	Heart			30§	54†‡
	Inj. site muscle			37§	30†‡

* 2 mg/kg, 1.5 hr later followed by 106 mg/kg. The time for the autopsy refers to the second injection.
† The dog was kept under artificial respiration.
‡ The tissues were embalmed with FAX.
§ 100 mg/kg i.v., 5 min later followed by 33 mg/kg i.m. The time for the autopsy refers to the i.v. injection.

which has been generally accepted. SCh is also extensively distributed to various tissues. The elimination from tissues is much slower than from plasma. Studies are in progress to throw further light on the quantitative importance of tissue distribution as compared to enzymatic hydrolysis at different doses of SCh.

Acknowledgements—This work was supported by grants from the Swedish Medical Research Council 00199 and the Wallenberg Foundation. We would like to thank Dr. S. Britton for valuable help with the animal experiments and Mrs. E. Manzoor for excellent technical assistance.

†*Department of Toxicology*
Karolinska Institutet
Box 60 400
S-104 01 Stockholm
Sweden, and
‡*Department of Pathology*
Medical College of Ohio
C.S. 10008
Toledo, Ohio 43699
U.S.A.

INGRID NORDGREN*†
KATHLEEN BALDWIN‡
ROBERT FORNEY, JR.‡

* To whom correspondence should be sent.

REFERENCES

1. P. Taylor, in *The Pharmacological Basis of Therapeutics*, (Eds. A. G. Gilman, L. S. Goodman and A. Gilman), 6th Edn. pp. 227, 230. Macmillan, London (1980).
2. I. Nordgren, R. Forney, Jr., T. Carroll, B. Holmstedt, I. Jäderholm-Ek and B.-M. Pettersson, *Archs. Toxicol. Suppl.* **6**, 339–350 (1983).
3. R. Forney, Jr., T. Carroll, I. Nordgren, B.-M. Pettersson and B. Homstedt, *J. anal. Toxicol.* **6**, 115–119 (1982).
4. W. N. Aldridge, in *Biochemists' Handbook* (Ed. C. Long), p. 276. E. & F. N. Spon, London, (1961).

Biochemical Pharmacology, Vol. 33, No. 15, pp. 2521–2523, 1984.
Printed in Great Britain.

0006-2952/84 $3.00 + 0.00

Cytochrome P-450-dependent lipid peroxidation in reconstituted membrane vesicles

(*Received* 18 *January* 1984; *accepted* 16 *March* 1984)

The flavoprotein NADPH-cytochrome P-450 reductase has mainly been regarded as responsible for producing reactive oxygen species such as the hydroxyl radical [1] or the superoxide anion radical [2], which have been proposed to act as initiators of liver microsomal lipid peroxidation: effective rates of peroxidation have been reached in systems where the reductase serves as electron donor to ADP-Fe^{3+} complexes. The hydroxyl radical [3], the perferryl ion [4, 5] and more recently a ferrous-dioxygen-ferric chelate [6] were proposed as initiating oxygen species. No role for cytochrome P-450 in the initiation step has hitherto been presented. However Svingen *et al.* [4, 7] have suggested a role for cytochrome P-450 in the lipid hydroperoxide-dependent initiation, whereby the peroxidative capacity of cytochrome P-450 was suggested to promote this type of initiation by producing peroxide-derived products such as peroxy or alkoxyradicals, hydroxyl radicals [7, 8] or singlet oxygen [9] that may contribute to chain propagation.

It was recently found that cytochrome P-450 itself produces reactive oxygen radicals [10–14]. These findings suggested that there might exist a cytochrome P-450-dependent lipid peroxidation mechanism utilizing active oxygen species as initiators. Since most studies concerning reconstituted systems and lipid peroxidation have been performed in the presence of high concentrations (>100 μM) of chelated iron, it was considered of importance to evaluate any role for cytochrome P-450 in lipid peroxidation using reconstituted membrane systems fortified with NADPH in a system free from exogenous iron and iron chelators.

Materials and methods. Desferoxamine was from CIBA. Diethylenetriaminepentaacetic acid (DETAPAC) was purchased from Sigma, whereas EDTA was from Fluka. Chelex 100 was obtained from Bio-Rad. Microsomal phospholipids were extracted from liver microsomes of phenobarbital-treated rabbits according to Bligh and Dyer [15] and stored under nitrogen in sealed tubes at −20°. Electrophoretically homogeneous preparations of NADPH-cytochrome P-450 reductase and cytochromes P-450 LM2 and P-450 LM4 from liver microsomes of phenobarbital-treated rabbits were prepared according to methods of Yasukochi and Masters [16] and Haugen and Coon [17], respectively. The ethanol and benzene-inducible form of rabbit liver microsomal cytochrome P-450 (P-450 LMeb) was purified essentially as previously described [18]. The specific contents of the enzyme preparations used were: P-450 LM2, 10.5–13 nmole/mg; P-450 LMeb, 11–14.3 nmole/mg; P-450 LM4, 14.0 nmole/mg; NADPH-cytochrome P-450 reductase, 20–22 nmole of flavin per mg of protein.

Unilamellar phospholipid vesicles containing microsomal phospholipids, cytochrome P-450 and NADPH-cytochrome P-450 reductase in a molar ratio of 1200:1:0.4 were prepared by the cholate gel filtration technique [19] in Chelex 100-treated 15 mM potassium phosphate buffer, pH 7.4, containing 50 mM KCl.

Reaction mixtures contained vesicles corresponding to 0.1 nmole of cytochrome P-450 in chelexed 15 mM potassium phosphate buffer, pH 7.4, containing 50 mM KCl in a final volume of 1 ml. Preincubation was performed for 15 min at 30° and the incubations were started by the addition of 10 μl 10 mM NADPH and terminated by the addition of 0.25 ml 40% trichloroacetic acid and 0.125 ml of 5 M HCl. The incubation time was generally 30 min. Lipid peroxidation products were measured using the thiobarbituric acid assay [20]. The extinction coefficient used was 156 mM^{-1} cm^{-1}. Control incubations were carried out by adding NADPH after the addition of trichloroacetic acid.

Results and discussion. Incubation of membrane vesicles containing cytochrome P-450 LM2 and NADPH-cytochrome P-450 reductase in a chelexed, EDTA-free reaction system fortified with NADPH, resulted in a time-dependent production of TBA-reactive substances (Fig. 1a). The process was linear for at least 15 min. Linearity was also reached in response to increasing amounts of vesicles (Fig. 1b). About half the rate of lipid peroxidation was registered using membranes containing the ethanol and benzene-inducible form of rabbit liver microsomal cytochrome P-450 (P-450 LMeb), whereas the cytochrome P-450 LM4 fraction was almost equally effective as P-450 LM2 in catalyzing the generation of TBA-reactive material in the reconstituted membranes (Table 1). When vesicles devoid of cytochrome P-450 were used, only 5% of the rate of lipid peroxidation was detected (Table 1). In order to evaluate the kind of cytochrome P-450 participation in the reaction, vesicles devoid of P-450, but only containing P-450 reductase, were supplemented with boiled preparations of P-450 LM2. In this case, an inhibition of the rate of formation of TBA-reactive material, compared to P-450-containing vesicles, was registered (not shown in the figure).

Membrane vesicles were prepared having variable amounts of cytochrome P-450 LM2, whereas the content of NADPH-cytochrome P-450 reductase was kept constant.