a result of which there is increased accessibility and/or permeability of the mitochondrial membrane to the corresponding amine substrate. Bino *et al.* [18] have recently reported that Δ^9-THC has an effect on the outer membrane and cristae of isolated rat liver mitochondria.

The most interesting result of this study is the striking increase in the activity of hypothalamic MAO induced by both acute and chronic administration of Δ^9-THC. Since the hypothalamus is rich in monoaminergic neurons, the present findings, suggest that the central monoamine neurons are an important site of action of Δ^9-THC. The neuropharmacological implications of the present study in relation to the role of biogenic amines in Δ^9-THC-induced changes in brain function need further investigation.

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Modification of procaine metabolism in rat liver after administration of phenobarbital or ethyl *p*-nitrophenyl phenylphosphonothioate

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Our previous studies have shown that a liver microsomal arylamidase is responsible for the hydrolysis of several drugs possessing an amido bond such as isocarboxazid (ISOC), a monoamine oxidase (MAO) inhibitor [1, 2]. Recently, procaine (PROC) was also found to be readily hydrolyzed by this enzyme *in vitro*.* The arylamidase was known to be either induced by phenobarbital (PB) [3] or markedly inhibited by organophosphorus insecticides at doses lower than those which inhibited serum cholinesterase in our laboratory [4, 5].

The present studies were therefore undertaken to determine whether PROC metabolism *in vivo* is modified by

* K. Moroi and T. Satoh, manuscript in preparation.

Table 1. Effect of pretreatment with phenobarbital or ethyl *p*-nitrophenyl phenylphosphonothioate (EPN) on procaine metabolism in rats*

Treatment	Isocarboxazid†		Procaine†	
	Enzyme activity	(%)	Enzyme activity	(%)
Control	2·23 ± 0·19 (19)	100·0	2·74 ± 0·13 (12)	100·0
EPN (1 mg/kg, p.o.)				
2 hr	0·14 ± 0·07 (5)	5·7	0 (5)	0
4 hr	0·23 ± 0·02 (7)	9·3	0·68 ± 0·07 (8)	27·4
6 hr	0·46 ± 0·08 (7)	18·6	0·57 ± 0·01 (6)	22·9
Phenobarbital (80 mg/kg/day, 4 days)	5·08 ± 0·23 (12)	204·8	7·41 ± 0·72 (8)	298·8

* Figures in parentheses indicate number of animals used. Enzyme activity represents mean ± S.E.M. and values are expressed in μmoles product/g liver wet wt/30 min.

† Isocarboxazid and procaine were used as substrates.

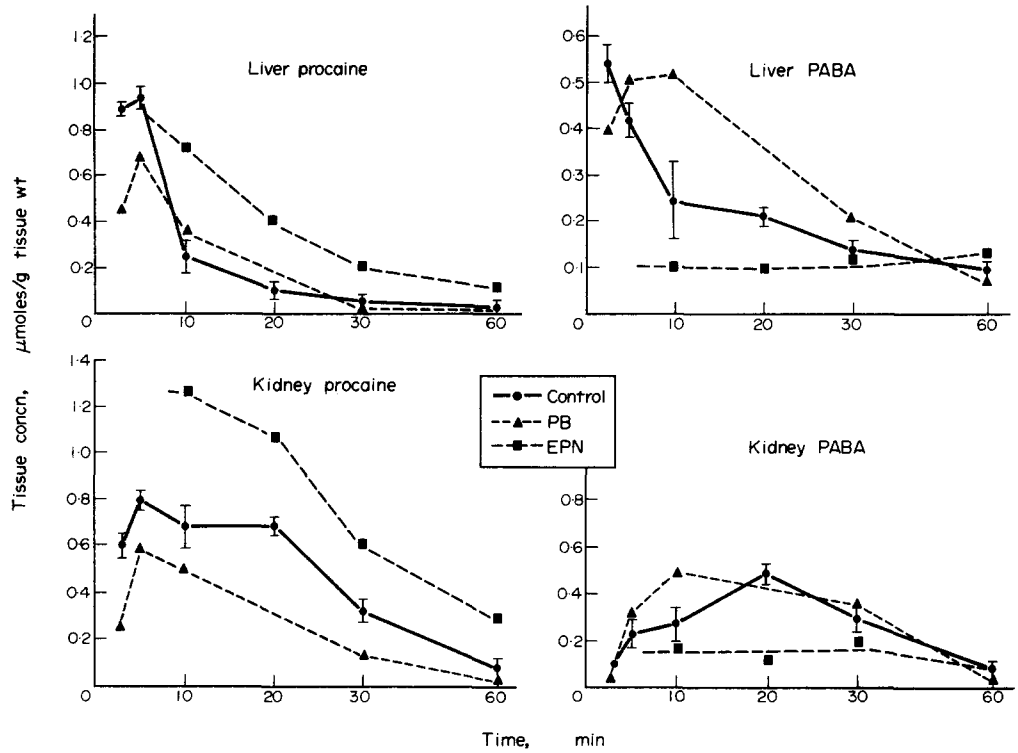

Fig. 1. Effects of pretreatment of rats with phenobarbital (80 mg/kg/day, i.p., for 4 days) or EPN (1 mg/kg, p.o., 2 hr prior to procaine injection) on the elimination of tissue procaine and formation of *p*-aminobenzoic acid (PABA). Each point represents the mean value of at least four animals; vertical lines on the figure represent S.E.M. at each point. Twenty-four hr after the last injection of phenobarbital, the animals received procaine, 100 mg/kg, i.p.

pretreatment with PB or ethyl *p*-nitrophenyl phenylphosphonothioate (EPN) or not.

PROC and PB were commercially available. PROC hydrochloride was dissolved in distilled water and administered i.p. at a dose of 100 mg/kg (injection volume, 0·1 ml/100 g of body weight) to three groups of adult male rats of the Wistar strain weighing 230–280 g. The first group received EPN (5 mg/kg, p.o.) 2 hr before PROC. The second group of rats was pretreated with PB sodium salt (80 mg/kg, i.p., daily for 4 days). A third group had no pretreatment before PROC administration.

Animals had free access to food and water *ad lib*. At various times after PROC injection, animals were sacrificed by decapitation, livers were removed and homogenized in 1·15% KCl in a glass homogenizer with Teflon pestle. Determinations of tissue concentrations of PROC and its major metabolite, *p*-aminobenzoic acid (PABA), were carried out by the method of Ting *et al.* [6]. Tissue amidase activities toward ISOC and PROC were determined as previously described [3, 4].

As shown in Table 1, the liver amidase activity of PB-treated rats was higher than that of controls for both substrates. In contrast, the enzyme activity of EPN-pretreated rats was significantly inhibited compared with that of control rats.

Figure 1 shows decay curves of tissue PROC levels and formation of PABA after injection of 100 mg/kg of PROC to rats. In the EPN-pretreated group, PROC concentrations in liver and kidney were much higher than control levels at 10 min and after, and, as expected, PABA concentrations in both tissues appeared to be lower compared with the non-pretreated group. On the other hand, an appreciable concentration of liver PABA in rats pretreated with PB was found with a maximum between 5 and 10 min after PROC injection, while the maximal PABA level in kidney of PB-induced rats was seen later and was almost equal to the PABA level of control rats 20 min after PROC administration.

Recently, there has been increasing interest in one drug or chemical affecting the metabolism of a second drug, and Sher [7] reviewed literature reports on drug–drug and drug–chemical interactions. Many of the anticholinesterase insecticides have been found to change the rates of drug metabolism both *in vivo* and *in vitro* [8]. Studies by Kampffmeyer [9] have demonstrated that the apparent biological half-life of phenacetin in dog plasma was decreased after treatment with phenobarbital.

The present studies show that the rate of PROC metabolism clearly depends on liver arylamidase activity. Our previous paper demonstrated that MAO inhibition of rat tissues by the administration of ISOC was reduced by pretreatment of rats with EPN, since most of the inhibitory effect of ISOC on MAO is the result of the hydrolysis of this drug by the liver microsomal enzyme system to its active metabolite, benzylhydrazine [4, 10, 11].

Further studies on correlation between pharmacological activities of PROC and the rate of its metabolism are now in progress.

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Action of proteolytic enzymes upon horse urinary kallikrein*

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Horse urinary kallikrein (HUK) is an enzyme that releases kallidin (lysylbradykinin) from horse plasma substrate [1]. This kallikrein [2], as well as purified kallikreins (EC 3.4.4.21) from other sources [3], possesses esterolytic activity toward synthetic *N*-substituted arginine esters. The resistance of kallikrein to proteolytic enzymes was observed for human urinary [4] and hog pancreatic kallikreins [5], but human plasma kallikrein was inactivated by several proteolytic enzymes [4].

HUK (sp. act., 22 TAME units/mg) was obtained by a previously described procedure [2, 6] up to the DEAE-cellulose chromatography step. The kinin-releasing activity was estimated on the isolated guinea pig ileum using heat-treated horse plasma as substrate [2, 6]. The esterolytic activity was measured after the hydrolysis of TAME or BAEE (0·004 M, pH 8·5, 37°) in a pH-stat. When these activities had to be determined in the presence of proteases that possessed esterolytic and kinin-releasing action, the proteases were previously inhibited with specific inhibitors without effect on HUK.

After 6 hr of incubation of HUK with pronase (B grade, Calbiochem.; 1:10 enzyme:substrate weight ratio in 0·05 M Tris–HCl, pH 8·0, 37°), 75 per cent destruction of both activities of kallikrein was observed (Fig. 1). No further inactivation was obtained by prolonging the incubation period, unless a subsequent addition of pronase was made, indicating an auto-inactivation of pronase. Pronase is known to be a mixture of proteases with very low specificity [7], and it was probably able to cleave one or more exposed bonds important for the activity of kallikrein.

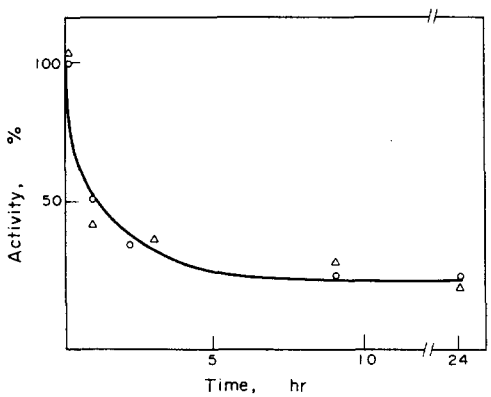

Fig. 1. Inactivation of kallikrein by pronase at pH 8·0, 37°. The concentration of HUK was 0·5 mg/ml. The activity of pronase was interrupted by EDTA, 4×10^{-3} M. —O—O—, Esterolytic activity; —△—△—, kinin-releasing activity.

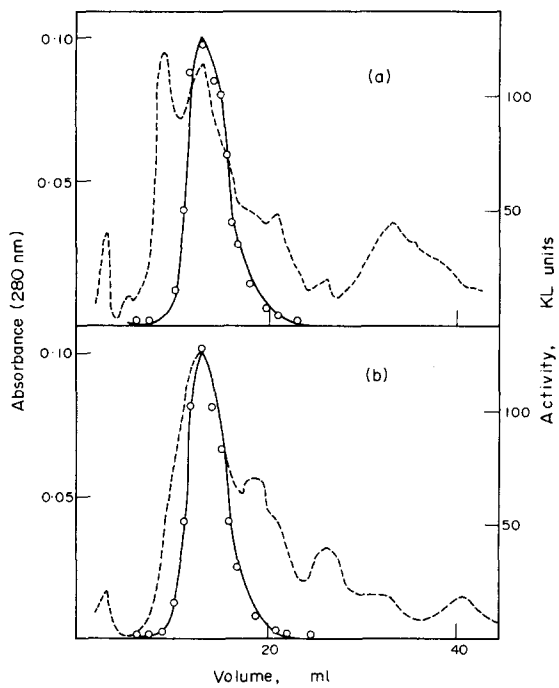

Fig. 2. Gel filtration of kallikrein. A 1·0 × 40 cm Sephadex G-75 column equilibrated with 0·1 M phosphate buffer, pH 6·0, was used. Flow rate: 8 ml/hr. Fractions of 1 ml. (a) HUK not treated by trypsin; (b) HUK treated by trypsin in a weight ratio of 1:20 for 20 hr at 37°. 0·05 M Tris–HCl, pH 8·0. (---) Represents the absorbance at 280 nm and (O—O) the kinin-releasing activity in KL units.

Trypsin (twice crystallized, Worthington Biochemical Corp.), under the same conditions described for pronase in Fig. 1, did not cause inactivation of HUK even at an enzyme:substrate weight ratio as high as 1:1. A trypsin–kallikrein incubate was gel-filtered through a Sephadex G-75 column, the elution pattern was compared to that of untreated HUK, and no difference was detected in the size of the HUK molecule (Fig. 2). Also, the proteases, chymotrypsin, pepsin, papain, carboxypeptidase B (twice crystallized enzymes purchased from Worthington Biochemical Corp.) and subtilisin (Nagarse, Nagase Co. Ltd.), under

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