able to incorporate [^{35}S]cysteine label into the glutathione pool, as GSH and oxidized GSH, at a relative distribution percentage that was not significantly different from control. Therefore, under the conditions of acute exposure, it is unlikely that GSH synthesis is being directly inhibited by oltipraz. Further, the fact that the oxy derivative of oltipraz did not affect the uptake of [^{35}S]cysteine into the parasites, even at 50 μM, suggests that the thione group of the drug molecule is a necessary participant in the oltipraz-induced effects on transport mechanisms.

In the present study, GSH (1 mM), cysteine (1 mM) and methionine (1 mM), but neither DTT (1 mM or 100 μM) nor 2-mercaptoethanol (1 mM or 100 μM), were able to block the *in vitro* effect of oltipraz on GSH levels occurring during incubation. The antagonism of oltipraz effects by GSH or GSH precursors may be mediated through a process of mass action on the replenishment of GSH stores. DTT and 2-mercaptoethanol, unlike the other compounds tested, are incapable of participating in the production of GSH, which may explain their inability to antagonize the effects of oltipraz. Alternatively, the inactivity of the oxy derivative of oltipraz and the rather stringent structural requirements for the maintenance of antischistosomal activity of a series of oltipraz congeners [1] suggest that oltipraz may influence a membrane receptor which possibly functions as a dipeptidase or transport carrier molecule for amino acids.

Tegumental morphology is not affected by *in vitro* treatment of the parasites with 10 μM oltipraz for 12 hr [8], indicating that structural damage to the tegument is not a contributary factor in the induction of acute oltipraz effects on membrane transport. While the structural integrity of the outer tegumental membrane was maintained, microelectrode recordings which showed depolarization of the tegument suggest that a significant redistribution of ions across the tegumental membrane had occurred. The effect(s) of a drug-induced ionic imbalance on the Na^+-driven carrier-mediated cotransport of glucose [9] may have contributed to a decrease in glucose uptake which was also observed (unpublished results). Perturbations of ionic equilibria across the tegument may also contribute to the inhibition by oltipraz of the uptake of GSH precursors. Consistent with the effects on other parameters measured, tegument potentials of worms incubated in 1 mM cysteine or 10 μM oltipraz plus 1 mM cysteine were not significantly different from control values. These results indicate that cysteine is able to prevent oltipraz-induced ionic imbalances *in vitro* without directly affecting ionic fluxes across the membrane. The oxy derivative of oltipraz did not affect the tegumental membrane potential, suggesting that the disruption of membrane conductance may contribute to the antischistosomal efficacy of oltipraz.

Acknowledgements—We are indebted to Ms. H. Cirrito and Ms. I. Mao for technical assistance with parasite preparations. We would also like to thank Dr. D. Reed for preparation of the HPLC column. These studies received financial support from NIH Grants AI-07203-08 and AI-16312-07.

Department of Pharmacology and Toxicology
Michigan State University
East Lansing, MI 48824-1317
U.S.A.

DAN D. MORRISON
DAVID P. THOMPSON
DAVID R. SEMEYN*
JAMES L. BENNETT†

REFERENCES

1. E. Bueding, P. Dolan and J. P. Leroy, *Res. Commun. Chem. Path. Pharmac.* **37**, 293 (1982).
2. R. H. Fetterer, R. A. Pax and J. L. Bennett, *Expl. Parasit.* **43**, 286 (1977).
3. P. W. Albro, *Analyt. Biochem.* **64**, 485 (1975).
4. D. J. Reed, J. R. Babson, P. W. Beatty, A. E. Brodie, W. W. Ellis and D. W. Potter, *Analyt. Biochem.* **106**, 55 (1980).
5. G. L. Ellman, *Archs. Biochem. Biophys.* **82**, 70 (1959).
6. D. R. Semeyn, R. A. Pax and J. L. Bennett, *J. Parisit.* **68**, 353 (1982).
7. D. P. Thompson, R. A. Pax and J. L. Bennett, *Parisitology* **85**, 163 (1982).
8. C. S. Bricker, J. W. Depensbusch, J. L. Bennett and D. P. Thompson, *Z. Parasitkde.* **69**, 61 (1983).
9. G. L. Uglem and C. P. Read, *J. Parasit.* **61**, 390 (1975).

* Enrolled in the Medical Scientist Training Program of the College of Osteopathic Medicine of Michigan State University.
† Author to whom all correspondence should be sent.

Biochemical Pharmacology, Vol. 36, No. 7, pp. 1171–1174, 1987.
Printed in Great Britain.

0006-2952/87 $3.00 + 0.00

Intestinal first-pass metabolism of phenacetin, acetaminophen, ethenzamide and salicylamide in rabbits pretreated with 3,4-benzo[*a*]pyrene

(*Received* 29 *May* 1986; *accepted* 26 *September* 1986)

The liver is usually considered to be the major drug-eliminating organ. However, all drugs that are administered orally must traverse the gastrointestinal wall before reaching the systemic circulation. The loss of drug as it passes through the gastrointestinal wall and the liver during the absorption process is known as the first-pass effect.

Phenacetin (PHT), used as an analgesic and an antipyretic, is metabolized extensively, the major metabolic route being O-de-ethylation to acetaminophen (NAPA). NAPA is subsequently conjugated to form a glucuronide (NAPA glucuronide, NAPAG) and a sulfate (NAPA sulfate, NAPAS). Similarly, the primary pathway for ethenzamide (ETB) metabolism is via O-de-ethylation to salicylamide (SAM), and SAM is further metabolized to SAM glucuronide (SAMG) and SAM sulfate (SAMS).

It has been reported that there is in rats an enzyme system in the mucosa of the small intestine that is capable of metabolizing PHT and that the activity of this enzyme system is increased in rats pretreated with 3,4-benzo[*a*]pyrene [1, 2] and 3-methylcholanthrene [3]. Recently, Klippert *et al.* [4] found that 3-methylcholanthrene pretreatment results in enhanced PHT disposition as was shown from decreased plasma half-life time, decreased oral availability, increased clearance, and an increase in metabolite levels. In 3-methylcholanthrene-pretreated rats the intestine contributes significantly, and predominantly over the liver, to PHT first-pass metabolism. In contrast, gut wall metabolism in control rats could not be demonstrated [4].

To provide more information on the role of intestinal

metabolism of PHT, NAPA, ETB and SAM as a determinant of their bioavailability, we have studied in rabbits the effect of induction of intestinal drug-metabolizing enzymes with 3,4-benzo[*a*]pyrene on the absorption of these drugs and their major metabolites (sulfates and glucuronides). Minor metabolites of these drugs were not determined in this study.

Materials and methods

Materials. PHT, NAPA, ETB and SAM were of pharmaceutical grade and were purchased from the Hishiyama Yakuhin Kako Co. Ltd. (Osaka, Japan), the Yamanouchi Seiyaku Co., Ltd. (Tokyo, Japan), the Kongo Kagaku Co., Ltd. (Toyama, Japan) and the Yoshitomi Seiyaku Co., Ltd. (Osaka, Japan) respectively. β-Glucuronidase was obtained from the Tokyo Zohki Kagaku Co., Ltd. (Tokyo, Japan). β-Glucuronidase/arylsulfatase was purchased from Boehringer Mannheim GmbH (Mannheim, Federal Republic of Germany). Olive oil was obtained from the Katayama Kagaku Co. Ltd. (Osaka, Japan). All other reagents used in these experiments were of the finest grade available.

Animals. Male albino rabbits, weighing approximately 2 kg, were used throughout the study. The animals were housed in an air-conditioned room and maintained on a standard laboratory diet. The rabbits were fasted for 24 hr prior to the experiments but had free access to water. After fasting, rabbits were dosed orally with 3,4-benzo[*a*]pyrene (40 mg/kg) dissolved in olive oil. The control rabbits did not receive the olive oil vehicle. Twenty-four hours after the 3,4-benzo[*a*]pyrene treatment, experiments on the intestinal absorption of drugs were carried out.

Absorption studies. *In situ* rabbit intestinal sacs with complete mesenteric venous blood collection were prepared as reported by Barr and Riegelman [5]. The technique of collecting all venous blood draining from the region of absorption was developed to provide an *in vivo* preparation with intact circulation. The usefulness of this preparation is that all free drug and drug metabolites which are absorbed into the capillary blood can be collected in the venous effluent and not reach the general circulation. Animals were anesthetized with pentobarbital, given intravenously, via an ear vein. Additional pentobarbital was administered as necessary during the experiment to maintain anesthesia. After complete anesthesia, a midline incision was made, and the mid-ileal portion of the intestine (5–8 cm) was cut. The intestinal lumen was washed with 0.9% NaCl, and both sides of the mid-ileal portion of intestine were ligated to prepare a closed sac. This portion was selected because of its accessibility and suitable vasculature to facilitate cannulation. The mesenteric vein was cannulated with a polyethylene tubing (SP 45, i.d. 0.58 mm, o.d. 0.96 mm, Natsume Seisakusho Co., Ltd., Tokyo, Japan). The coagulation of blood was prevented by the intravenous administration of heparin (1000 I.U.). All venous blood was collected in centrifuge tubes and assayed for free drug and its metabolites. The amounts of free drug and its metabolites in the blood were estimated by multiplying the volume of blood collected by the concentration of free drug and its metabolites in the blood. The drug solution (3 ml) was administered by direct injection into the intestinal sac by syringe. The drug was dissolved in the pH 7.2 buffer solution reported by Schanker and Tocco [6]. The blood lost from the mesenteric vein was replaced continuously by an intravenous infusion, via ear vein, of 0.9% NaCl (0.67 ml/min). The isolated intestine was kept warm by a lamp and moist by frequent application of 0.9% NaCl to a paper covering the intestine. Results were compared statistically using Student's *t*-test.

Analytical methods. ETB, SAM, SAMG and SAMS were separately quantitated from blood by the spectrofluorometric assay method described in previous papers [7, 8]. A Shimadzu RF-510 spectrofluorometer (Shimadzu Co., Ltd., Kyoto, Japan) was used. PHT was measured spectrophotometrically with a Hitachi 200-10 spectrophotometer (Hitachi Co., Ltd., Tokyo, Japan) [9]. NAPA, NAPAG and NAPAS were separately quantitated from blood by the spectrofluorometric assay method described in a previous paper [10]. Glucuronide and sulfate were analyzed after the hydrolysis of the sample with β-glucuronidase or β-glucuronidase/arylsulfatase at 37° for 24 hr.

Results and discussion

The effects of induction of intestinal drug-metabolizing enzymes with 3,4-benzo[*a*]pyrene on the absorption of PHT, NAPA, ETB and SAM were examined in rabbits using *in situ* intestinal sacs with complete mesenteric venous blood collection. The appearance of both free drug and its metabolites into the mesenteric blood was measured directly by cannulating the mesenteric vein of exposed rabbit intestine and collecting all venous blood draining from the absorbing region.

Table 1 shows the appearance of PHT, NAPA, NAPAG and NAPAS in the mesenteric venous blood after injection of PHT into the intestinal lumen. In control rabbits, about 87 and 13% of PHT absorbed appeared as the unchanged form and metabolites respectively. By the oral pretreatment with 3,4-benzo[*a*]pyrene, the total amount absorbed was not changed compared to control. 3,4-Benzo[*a*]pyrene pretreatment resulted in enhanced PHT metabolism as was shown from the decreased appearance of PHT and the increased appearance of NAPA and NAPAG in mesenteric venous blood. Pantuck *et al.* [2] reported that oral administration of 3,4-benzo[*a*]pyrene to rats increases the O-de-ethylation of PHT by subsequently prepared everted intestinal sacs and results in an increase in the amount of NAPA and a decrease in the amount of PHT transferred from the mucosal to the serosal side of the sacs *in vitro*. However, NAPAG and NAPAS were not determined. Welch *et al.* [11] demonstrated, also in rats, that oral pretreatment with 3-methylcholanthrene decreases the plasma half-life of PHT after intravenous administration and reduces the systemic bioavailability of PHT after oral administration. By comparing the plasma

Table 1. Appearance of PHT and its metabolites in mesenteric venous blood after injection of PHT

Treatment	Total amount absorbed (% of dose)	PHT	NAPA (% of total amount absorbed)	NAPAG	NAPAS
Control	87.0 ± 5.1 (5)	86.7 ± 3.1	5.1 ± 3.7	5.5 ± 1.0	2.7 ± 1.9
Benzo[*a*]pyrene	89.5 ± 7.7*‡ (4)	54.5 ± 5.9†	27.0 ± 6.2†	13.5 ± 5.2‡	5.0 ± 0.4*

Dose: 3 ml of 200 μg/ml solution of PHT. Blood collection: 0–120 min. Results are expressed as the mean ± SD. Numbers in parentheses represent number of experiments.

*–‡ Statistical significance: * not significant; † $P < 0.001$; and ‡ $P < 0.02$.

Table 2. Appearance of ETB in mesenteric venous blood after injection of ETB

Treatment	Total amount absorbed (% of dose)	ETB	SAM	SAMG	SAMS
		(% of total amount absorbed)			
Control	83.7 ± 9.9 (3)	100	0	0	0
Benzo[*a*]pyrene	82.7 ± 5.7* (4)	100	0	0	0

Dose: 3 ml of 200 μg/ml solution of ETB. Blood collection: 0–120 min. Results are expressed as the mean ± SD. Numbers in parentheses represent number of experiments.
* Statistical significance: not significant.

levels of PHT in the portal circulation with those in the peripheral circulation, following the oral administration of PHT, it was concluded that the reduction in the bioavailability of PHT observed in 3-methylcholanthrene treated rats was caused by a marked increase in the metabolism of PHT during its first pass through the liver. In the present study, enhanced metabolism of PHT in the intestinal wall was found in rabbits pretreated with 3,4-benzo[*a*]pyrene orally.

To clarify the effect of enzyme induction on the intestinal absorption of PHT in detail, the appearance of NAPA and its metabolites in mesenteric venous blood after injection of NAPA in the intestinal lumen was examined. Both control and 3,4-benzo[*a*]pyrene-pretreated rabbits showed almost complete absorption of NAPA. No effect was found on the metabolism of NAPA by the pretreatment with 3,4-benzo[*a*]pyrene compared to control (data not shown). These results indicate enzyme induction for O-de-ethylation of PHT, not conjugation of glucuronic acid and sulfuric acid to NAPA, by pretreatment with 3,4-benzo[*a*]pyrene.

The primary pathway for ETB metabolism is via O-de-ethylation to SAM, and SAM is further metabolized to SAMG and SAMS. In a previous report [8], it was confirmed in rats that the first-pass metabolism of ETB is not due to the intestine but entirely due to the liver in which O-de-ethylation to SAM occurred at first, followed by exclusive conjugation with sulfuric acid and that little SAM enters into the systemic circulation. As shown in Table 2, ETB was well absorbed and O-de-ethylation in the intestinal wall was negligible in control rabbits. On pretreatment with 3,4-benzo[*a*]pyrene, metabolism of ETB was not observed. It is suggested that ETB, in contrast to PHT, is not O-de-ethylated to SAM in rabbit intestinal wall.

SAM was reported to exhibit the first-pass effect during intestinal absorption in rabbits [5, 12] and rats [13]. We have shown in a previous paper [7] that SAM is conjugated in the intestinal wall of rabbits and rats predominantly with glucuronic acid and much less with sulfuric acid. Both the intestinal wall and liver account for the first-pass metabolism of SAM in the dog [14]. Recently, Waschek *et al.* [15, 16] suggested that in dogs SAM undergoes sulfation in organs other than the liver and the intestinal wall. The appearance of SAM and its metabolites in mesenteric venous blood after injection of SAM into the intestinal lumen was examined. The effect of pretreatment with 3,4-benzo[*a*]pyrene was hardly observed in the intestinal metabolism of SAM (data not shown). From the results described above, it is suggested that the metabolic enzyme for sulfation and glucuronidation is not induced in rabbit intestine by pretreatment with 3,4-benzo[*a*]pyrene.

3,4-Benzo[*a*]pyrene is one of the polycyclic hydrocarbons present in cigarette smoke. Pantuck *et al.* [17] demonstrated that the low concentration of PHT in the plasma of smokers following oral administration of PHT is due to increased metabolism of PHT, either in the gastrointestinal tract or during the first few passes through the liver. Furthermore, studies in rats indicate that exposure to cigarette smoke or treatment with 3,4-benzo[*a*]pyrene enhances the *in vivo* metabolism of PHT and that treatment with 3,4-benzo[*a*]pyrene stimulates enzymes in the intestinal mucosa that O-de-ethylate PHT to NAPA [1]. Stimulation of intestinal PHT O-de-ethylase activity in rats treated with 3-methylcholanthrene or exposed to cigarette smoke has been reported by Welch *et al.* [3]. 3,4-Benzo[*a*]pyrene and other polycyclic hydrocarbons have been found in a number of smoked and cooked foods [18]. Charcoal-broiled beef contains 3,4-benzo[*a*]pyrene and other polycyclic hydrocarbons and has been shown to stimulate the oxidative metabolism of 3,4-benzo[*a*]pyrene by rat liver and placenta [19].

It is well known that intestinal metabolism plays a role in determining the bioavailability of orally administered drugs. Further investigation is required to clarify the mechanism of induction of intestinal drug-metabolizing enzymes.

In summary, 3,4-benzo[*a*]pyrene pretreatment resulted in enhanced PHT metabolism as was shown from the decreased appearance of PHT and the increased appearance of NAPA and NAPAG in mesenteric venous blood. However, no effect of 3,4-benzo[*a*]pyrene on the metabolism of NAPA was found. These results indicate enzyme induction for O-de-ethylation of PHT by pretreatment with 3,4-benzo[*a*]pyrene. In control rabbits, ETB was not metabolized in the intestinal wall. In addition, the effect was hardly detectable in the metabolism of ETB and SAM after pretreatment with 3,4-benzo[*a*]pyrene. It is suggested that ETB, in contrast with PHT, is not O-de-ethylated to SAM in rabbit intestinal wall.

Acknowledgement—We wish to thank Hisako Ogata for skilled technical assistance.

Faculty of Pharmaceutical Sciences
Nagasaki University
Nagasaki 852, Japan

JUNZO NAKAMURA*
TADAHIRO NAKAMURA
SAMIR KUMAR PODDER
HITOSHI SASAKI
JUICHIRO SHIBASAKI

REFERENCES

1. E. J. Pantuck, K-C. Hsiao, A. Maggio, K. Nakamura, R. Kuntzman and A. H. Conney, *Clin. Pharmac. Ther.* **15**, 9 (1974).
2. E. J. Pantuck, K-C. Hsiao, S. A. Kaplan, R. Kuntzman and A. H. Conney, *J. Pharmac. exp. Ther.* **191**, 45 (1974).
3. R. M. Welch, J. Cavallito and A. Loh, *Toxic. appl. Pharmac.* **23**, 749 (1972).
4. P. J. M. Klippert, R. J. J. Littel and J. Noordhoek, *J. Pharmac. exp. Ther.* **225**, 153 (1983).
5. W. H. Barr and S. Riegelman, *J. pharm. Sci.* **59**, 154 (1970).
6. L. S. Schanker and D. J. Tocco, *J. Pharmac. exp. Ther.* **128**, 115 (1960).

* Author to whom correspondence should be addressed.

7. J. Shibasaki, R. Konishi, M. Koike, A. Imamura and M. Sueyasu, *J. Pharmacobio-Dynamics* **4**, 91 (1981).
8. J. Shibasaki, R. Konishi, M. Takemura, E. Fukushima, J. Nakamura and H. Sasaki, *J. Pharmacobio-Dynamics* **7**, 804 (1984).
9. B. B. Brodie and J. Axelrod, *J. Pharmac. exp. Ther.* **94**, 22 (1948).
10. J. Shibasaki, R. Konishi, K. Yamada and S. Matsuda, *Chem. pharm. Bull., Tokyo* **30**, 358 (1982).
11. R. M. Welch, C. R. Hughes and R. L. Deangelis, *Drug Metab. Dispos.* **4**, 402 (1976).
12. W. H. Barr and S. Riegelman, *J. pharm. Sci.* **59**, 164 (1970).
13. K. Iwamoto, Y. Arakawa and J. Watanabe, *J. Pharm. Pharmac.* **35**, 687 (1983).
14. R. Gugler, P. Lain and D. L. Azarnoff, *J. Pharmac. exp. Ther.* **195**, 416 (1975).
15. J. A. Waschek, G. M. Rubin, T. N. Tozer, R. M. Fielding, W. R. Couet, D. J. Effeney and S. M. Pond, *J. Pharmac. exp. Ther.* **230**, 89 (1984).
16. R. M. Fielding, J. A. Waschek, D. J. Effeney, A. C. Pogany, S. M. Pond and T. N. Tozer, *J. Pharmac. exp. Ther.* **236**, 97 (1986).
17. E. J. Pantuck, R. Kuntzman and A. H. Conney, *Science* **175**, 1248 (1972).
18. G. M. Badger, R. W. L. Kimber and T. M. Spotswood, *Nature, Lond.* **187**, 663 (1960).
19. Y. E. Harrison and W. L. West, *Biochem. Pharmac.* **20**, 2105 (1971).

Biochemical Pharmacology, Vol. 36, No. 7, pp. 1174–1176, 1987.
Printed in Great Britain.

0006-2952/87 $3.00 + 0.00

Involvement of calmodulin-dependent phosphorylation in the activation of brainstem tryptophan hydroxylase induced by depolarization of slices or other treatments that raise intracellular free calcium levels

(*Received* 2 *September* 1986; *accepted* 23 *September* 1986)

Several lines of evidence now indicate that both crude supernatant and purified preparations of brainstem or midbrain tryptophan hydroxylase, the rate-limiting enzyme in serotonin synthesis [tryptophan-5-monooxygenase; L-tryptophan, tetrahydropterin:oxygen oxidoreductase (5-hydroxylating), EC 1.14. 16.4], can be activated by calcium–calmodulin-dependent protein kinase [1–3]. Tryptophan hydroxylase also becomes activated as a result of *in vivo* electrical stimulation [4] or drug-induced increases in firing of serotonergic neurons in the brain.* In both cases a phosphorylation reaction has been implicated in the activation process which is sensitive to frequency of stimulation and is readily reversible. Other studies on slices of brainstem revealed that potassium depolarization produces a calcium-dependent activation of the enzyme [5]. Indeed a variety of manipulations that raise intracellular levels of free calcium (A23187, metabolic inhibitors, methylxanthines) activate this enzyme [6–8]. A role for calmodulin, in addition to that of calcium, in mediating the activation of tryptophan hydroxylase is supported by the inhibitory effects of haloperidol and fluphenazine, antipsychotic drugs, on the activation process induced in the slices [7, 8]. The present experiments were designed to explore further the involvement of calmodulin-sensitive phosphorylation in the activation of tryptophan hydroxylase that results from treatments to brainstem slices.

Methods

The procedures used in this study have already been outlined in detail in previous reports [5, 6]. Brainstems (diencephalon, midbrain and pons-medulla) from male Sprague–Dawley rats were cut into 250 μm slices with a Dupont–Sorvall MT-2 tissue chopper, and each sliced brainstem was incubated at 25° in oxygenated medium (150 mM NaCl, 6 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, 10 mM glucose and 10 mM Tris acetate buffer, pH 7.4) to which various deletions, substitutions or additions were made as indicated in Results. The slice preparations were exposed to the treatment medium for 10 min. Calcium-free control medium containing 0.1 mM ethylene glycol bis (β-aminoethylether)-*N*,*N*′tetraacetic acid (EGTA) was used in experiments with 3-isobutyl-1-methylxanthine (IBMX) and guanidine. The drugs W-7 [*N*-(6-aminohexyl)-5-chloro-1-naphthalenesulfonamide hydrochloride] (Sigma Chemical Co., St Louis, MO) and polymixin B (United States Biochemical Corp., Cleveland, OH) were preincubated with the slices in control medium for 10 min in addition to being included in the incubation medium along with the calcium-mobilizing agents. Low speed supernatant extracts (30,000 *g* for 30 min) of tryptophan hydroxylase were prepared from brainstem slices that had been pelleted by brief centrifugation. The tissue was homogenized in 2 vol. of 0.05 M Tris-acetate buffer, pH 7.4, by means of a Tissumizer (45 sec at setting 50) (Tekmar, Cincinnati, OH) instead of a glass homogenizer. The supernatant fraction was made 2 mM with dithiothreitol, subjected to gel filtration, and assayed for tryptophan hydroxylase in the presence of 50 μM D,L-6-methyl-5,6,7,8-tetrahydropterin (6-MPH_4) and 200 μM L-tryptophan [5, 6]. All additions to the enzyme assay such as alkaline phosphatase or phosphorylating conditions are indicated in the Results. The 5-hydroxytryptophan (5-HTP) formed in the assay was isolated and quantitated by HPLC with electrochemical detection [4]. Enzyme activities are the mean ± SEM of at least four experiments and are expressed in pmoles 5-HTP formed per mg protein per min. The significance of differences in enzyme activity between the various treatment groups was determined by ANOVA and the Newman–Keuls test.

Results and discussion

Table 1 shows that the increases in tryptophan hydroxylase activity induced by the four different treatments to the slices (potassium depolarization, ionophore A23187, the methylxanthine, IBMX or the metabolic inhibitor, guanidine) were all reversed by incubation of the enzyme extract with alkaline phosphatase prior to addition of the assay reactants. In contrast, no change in activity of enzyme from control slices occurred with alkaline phosphatase. Incubation of the enzyme extracts under phosphorylating conditions produced only a slight further increase in the activity of enzyme from the treated slices, so that this

* M. C. Boadle-Biber, J. N. Johannessen, N. Narasimhachari and T-H. Phan, manuscript submitted for publication.