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SHORT COMMUNICATIONS

Induction of tyrosine α-ketoglutarate transaminase by primycin in rat liver

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Primycin is a highly toxic antibiotic with a bacteriostatic effect on Gram-positive bacteria [1]. Its chemical structure was determined by Aberhardt *et al.* [2]. Earlier literary data, in an attempt to clarify its mode of action, indicated that primycin inhibits DNA dependent RNA synthesis [3].

In 1967, Vályi-Nagy *et al.* [3] demonstrated that primycin inhibits the glucocorticoid mediated induction of tryptophan 2,3-dioxigenase (EC 1.13.11.11) in rat liver, and concluded by these indirect experiments that primycin, like actinomycin D, inhibits the transcription of the DNA.

Recent experiments seem to indicate that the biological effect of primycin could primarily be attributed to its effect on the membrane [4].

It is well documented, that in rat liver, tyrosine aminotransferase responds similarly to tryptophan pyrrolase to glucocorticoids [8]. Therefore, similar mechanisms may operate in the regulation of both tyrosine aminotransferase and tryptophan pyrrolase synthesis.

As a working hypothesis, therefore, we sought to investigate the effect of primycin on the induction of tyrosine aminotransferase *in vivo*.

We found that primycin administered in subtoxic doses causes a 4–5 fold increase in tyrosine aminotransferase (TAT) activity in rat liver, while it is practically ineffective on the dexamethasone induced synthesis of the enzyme. Table 1 illustrates the changes in TAT level 6 hr after treatment of animals. As shown, the response of the enzyme to primycin is lower than the response to dexamethasone, but the differences in enzyme level between control and treated animals are significant ($P < 0.05$). A slight inhibition was observed when primycin and dexamethasone were given together. In comparison to dexamethasone induced TAT level, this slight inhibition does not seem significant ($P > 0.05$). The inductive effect of primycin is dose-dependent; a marked increase in TAT activity could already be observed at 0.025 mg/kg, and at 0.250 mg/kg enzyme activity reached a maximum. This value was attained without further increase up to doses of 1.0 mg/kg (Fig. 1). TAT induction could also be observed in adrenalectomized rats after primycin injection.

Actinomycin D and cycloheximide inhibited the induction of TAT by primycin, an indication that the induction involves *de novo* protein synthesis (Table 1).

An increase in tyrosine aminotransferase enzyme protein was further supported by the results obtained upon immunotitration tests (Fig. 2) where we observed a 4–5 fold increase in TAT enzyme level in the liver extract of induced animals (Fig. 2, panel A). Using the same method we were able to prove that the basal and the induced enzymes are both products of the same gene (Fig. 2, panel B).

The contradicting effect of primycin on tyrosine aminotransferase and tryptophan pyrrolase seems to disprove in principle the view taken by Vályi-Nagy *et al.* [3] that this antibiotic inhibits transcription of DNA into mRNA. These opposing effects on the other hand, and the fact that primycin causes a 4–5-fold induction in TAT, seem to exclude the possibility that primycin caused induction process is mediated through the adrenal–hypophyseal system.

The findings presented above suggest the following hypothesis.

(1) Primycin stimulates the TAT enzyme formation by an

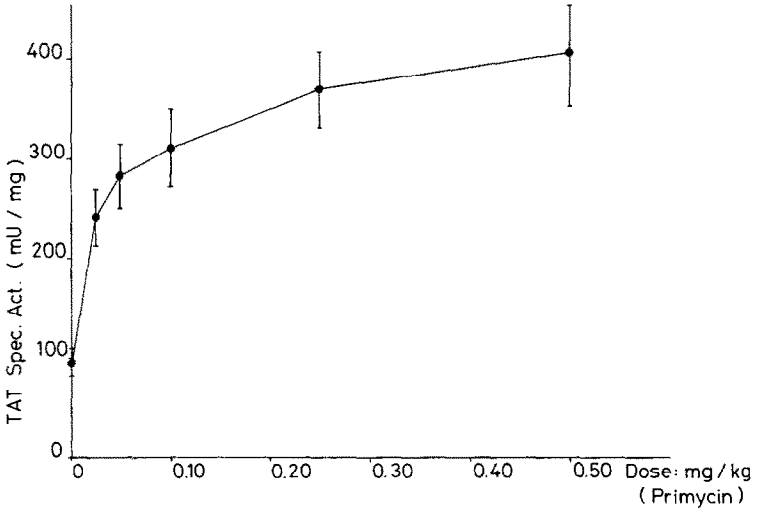

Fig. 1. Dose response of TAT induction by primycin. Primycin in different doses were administered in a single i.p. injection to male Wistar albino rats of 120–150 g body weight at 8 a.m. Animals were killed 6 hr after the treatment. Preparation of cytosols, and determination of TAT activity was the same as indicated under Table 1.

Table 1. Induction of hepatic tyrosine aminotransferase by dexamethasone and primycin. Effect of actinomycin D and cycloheximide on induction of hepatic tyrosine transaminase by dexamethasone, primycin and to the combination of dexamethasone and primycin

Treatment with actinomycin D or cycloheximide	Second treatment			
	None (control)	Dexamethasone (20 mg/kg)	Primycin (0.5 mg/kg)	Dexamethasone plus primycin
None	117.5 ± 18	527.4 ± 76	414.7 ± 107	451.2 ± 87
None†	41.0 ± 16	400.0 ± 111	362.0 ± 70	395.0 ± 102
Actinomycin D	112.8 ± 16	119.3 ± 22	99.8 ± 22	86.1 ± 23
Cycloheximide	73.6 ± 16	93.2 ± 11	81.2 ± 21	83.0 ± 19

Male, intact and adrenlectomized Wistar-strain albino rats (body weight 120–150 g), received i.p. injections of either actinomycin D (0.5 mg/kg) or cycloheximide (0.5 mg/kg) at 6.30 a.m. Second treatment with dexamethasone (20 mg/kg body weight), primycin (0.5 mg/kg body weight) and the combination of the two drugs was performed at 8 a.m. All drugs were dissolved in 1,3-propylene glycol. Control animals received 0.1 ml of 1,3-propylene glycol. The animals were killed 6 hr after the last injection, and TAT activity determined in liver cytosol fractions by the slight modification of the Diamondstone procedure [5] according to Granner *et al.* [6]. TAT activity is expressed as nmoles of product formed/mg protein/min. The values are the means (±S.D.) of individual determinations of the livers from 6 to 9 animals. Student's *t* test was used for statistical comparison ($P < 0.05$).

† Adrenalectomized rats: after operation drinking water was substituted by a 1% salt solution. Induction was carried out on the third day after operation. Procedure of induction and enzyme activity determination is described above.

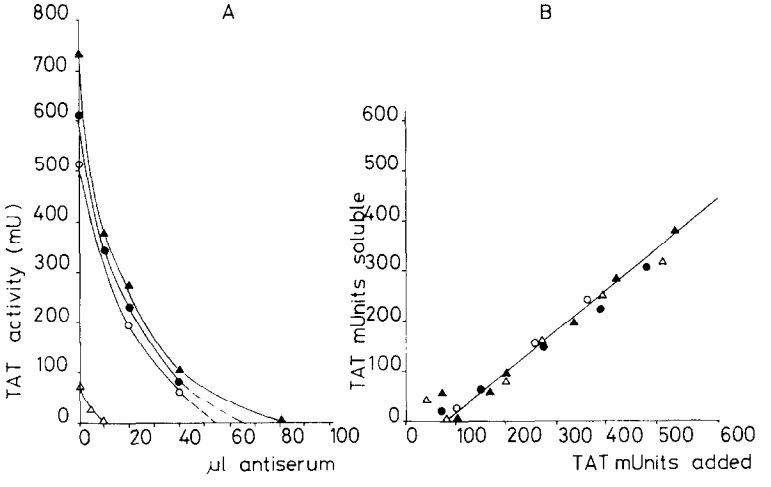

Fig. 2. Immunotitration of TAT enzyme: TAT enzyme level in liver extracts of control and induced animals (panel A). Control: △—△; dexamethasone treated: ▲—▲; primycin treated: ○—○—○; combination of dexamethasone and primycin: ●—●—●. Immunological identity of TAT enzyme originating from liver extracts of control and induced animals (panel B). Control: △—△; dexamethasone: ▲—▲; primycin: ○—○—○; primycin plus dexamethasone: ●—●—●. The TAT antiserum was prepared and immunochemical titrations carried out as described by Granner *et al.* [9].

actinomycin D-sensitive process presumably involving RNA synthesis.

(2) TAT induction by primycin in adrenalectomized rats proceeds in the same manner as in intact animals. This observation clearly indicates that the induction does not depend on the concentration of endogenous steroids; however, it cannot altogether be excluded that the presence of steroid is required.

Further discussion of the phenomenon of this type of TAT induction will be presented when the inductive effect of primycin could be reproduced on hepatoma tissue cultures and/or primary cultures of hepatocytes.

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Effect of the route of administration on microsomal enzyme induction following repeated administration of methadone in the mouse

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The metabolic inactivation of methadone is largely a result of the hepatic microsomal *N*-demethylating enzyme systems in both the rat [1, 2] and mouse [3], since the metabolic products of this pathway lack analgetic potency [4]. The induction of microsomal enzyme activity has been observed after repeated methadone administration following the oral route in mice [5] and rats [6–8] and intraperitoneal injection in mice [9]. On the other hand, at least one report found a lack of induction and a significant inhibition of microsomal enzyme activity in rats following as long as 60 days of repeated daily intubation with 50 mg/kg of this narcotic [10]. We suspect that differences in induction vary with the strain of rat employed. Thus far, this situation does not seem to be as extreme in mice [10].

Alvares and Kappas [1] reported that no increase in methadone *N*-demethylase activity could be demonstrated after intraperitoneal administration of 20 mg/kg of methadone daily in rats for 14 days. Peters [11] also reported that daily i.p. injections of 5 mg/kg of methadone to rats chronically did not significantly increase the activity of the methadone *N*-demethylase in liver microsomes. However, more recently the induction phenomenon was observed following intraperitoneal administration of methadone in mice, even with extremely low doses for long periods of time [9].

Although tolerance to the analgetic effect of methadone has been reported after prolonged subcutaneous administration in rats [12], Masten *et al.* [5] found only a slight increase in the activity of methadone *N*-demethylase in mice after 6 days of 20 mg/kg via this route. Thus, the development of CNS cellular tolerance to methadone is most likely the explanation of the former observation [13].

To date, no study has investigated the effects of the three routes of administration on the induction of microsomal metabolism in the same strain and species of rodent. Thus, we sought to investigate the effects of methadone administration upon its own *N*-demethylation by hepatic microsomal enzymes in mice receiving this narcotic by the three different routes (oral, intraperitoneal, and subcutaneous) employing identical dosage regimens for 6 days. We also wished to determine the effect of repeated methadone administration on aniline hydroxylation (a Type II substrate) and aminopyrine *N*-demethylation (a Type I substrate) *in vitro* [14], as well as on the metabolism of pentobarbital (a Type I substrate) *in vivo* [14], employing sleeping time.

Animals. Male albino ICR mice (25–30 g), obtained from Harlan Industries, Cumberland, IN, were used for both *in vivo* and *in vitro* phases of this study. These animals were housed in clear plastic mouse cages (five or six per cage) which were cleaned every other day to avoid the accumulation of ammonia, a potential inhibitor of microsomal metabolism [15]. A 12-hr light–dark cycle was maintained, while the ambient temperature was kept at 23 ± 1°. The mice were allowed free access to food (Purina Laboratory Chow, Ralston–Purina Co., St. Louis, MO) and water. SAN-I-CEL bedding (Paxton Processing Co., Whitehouse Station, NJ) was used throughout the study. After a 2-week acclimation period in our animal facilities, mice were employed in the experiments as described.

Drugs and dosage. Methadone hydrochloride and sodium pentobartbital were purchased from the Mallinckrodt Chemical Works, St. Louis, MO, and Abbott Laboratories, North Chicago, IL, respectively. Methadone, administered by oral intubation, was dissolved in water, whereas the parenteral solutions were dissolved in saline. The volumes of solution administered via the oral and the parenteral routes were 0.02 and 0.01 ml/g body weight, respectively. In the control groups, the appropriate vehicle was substituted for each drug administered.

The mice received either 3.13, 6.25, 12.5 or 25.0 mg/kg/day of methadone hydrochloride for 6 days by three different routes of administration: oral, intraperitoneal and subcutaneous. Two additional dosages 35.0 and 50.0 mg/kg/day were employed only via the oral route since these regimens were lethal to mice when administered repeatedly by either of the other two routes. All mice were taken from the same shipment of animals with the exception of the mice used for *in vitro* work for oral dosages of 25.0, 35.0 and 50.0 mg/kg. Each group consisted of eight to twelve mice.

Administration of drugs or vehicle took place between the hours of 1:00 and 2:00 p.m. each day; body weights were recorded at this time.

Tissue preparation and assays. For all *in vitro* work, mice were weighed and killed 24 hr after the sixth daily dose of methadone. This length of time was shown to be adequate for significant microsomal induction via the oral route with methadone [5]. Death via cervical dislocation was followed by decapitation.

The livers were removed (excising the gall bladder), blotted dry and weighed. The liver was then homogenized immediately with 3 vol. of ice-cold 0.05 M Tris–HCl–0.15 M KCl buffer, pH 7.4. After centrifugation, the 12,000 *g* supernatant fraction was used for the enzyme assays. The metabolic