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Stimulation of *p*-aminohippurate Transport by Slices of Rat Renal Cortex Following *in Vivo* Administration of Triiodothyronine* (33913)

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Thyroxine administration causes an increase in oxygen consumption in kidney slices (1, 2) as well as stimulating renal protein synthesis (3–5). Furthermore, thyroidectomy is known to depress uptake of *p*-aminohippurate (PAH) by rat kidney slices (6, 7). These results suggested that thyroid hormone may exert a trophic or regulatory effect on renal function. The present study was carried out to determine if thyroid hormone would act as a direct, nonspecific stimulus of renal transport in rats. The effect of *in vivo* administration of triiodothyronine (T_3) on organic ion transport was determined by measuring PAH and NMN (*N*-methylnicotinamide) uptake in renal cortical slices. Inasmuch as the growth response of the remaining kidney after unilateral nephrectomy is influenced by age (8), a comparison of the response to T_3 in both weanling and adult rats was made. The effect of T_3 on the renal uptake of PAH and NMN, when added *in vitro* to kidney slices, was also determined.

Materials and Methods. Male Sprague-Dawley rats were used in all experiments. The weanling rats weighed 50–60 g at the beginning of each experiment, while the adult rats weighed 200-220 g. DL-Triiodothyronine was dissolved in alkaline saline and injected intraperitoneally into rats in doses of 200 or 500 μg/kg once daily for 3–7 days. Control animals received alkaline saline for the same period of time.

Twenty four or 48 hr after the last injection the animals were killed by a blow on the head. The kidneys were removed immediately, weighed, and placed in ice-cold normal saline. Renal cortical slices weighing about 100 mg were prepared freehand and kept in cold normal saline until incubated. Slices were incubated in 2.7 ml of the phosphate buffer devised by Cross and Taggart (9), which contained 7.4×10^{-5} *M* PAH and 6.0×10^{-6} *M* ^{14}C-NMN. In *in vitro* studies, a solution of 1×10^{-4} *M* T_3 was diluted with distilled water so that when 0.3 ml was added to the incubation media the final concentration of T_3 was 1×10^{-6} *M* or 1×10^{-8} *M*; the pH of the media was 7.4 Control beakers received 0.3 ml of distilled water. All incubations were carried out in a Dubnoff metabolic shaker for 90 min at 25° under a gas phase of 100% oxygen. At the end of the incubation period the slices were quickly removed from the media, blotted, and weighed. Both tissue and media were treated as outlined by Cross and Taggart (9) and the S/M ratio for PAH and NMN was determined. PAH was estimated by the method of Smith *et al.* (10) while 1.0 ml of slice and media homogenate was added to 10 ml of modified Bray's solution (2.5 g of 2,-5-diphenyloxazole and 100 g of napthalene/liter of dioxane) and the amount of ^{14}C NMN was counted in a Beckman LS-100 liquid scintillation counter.

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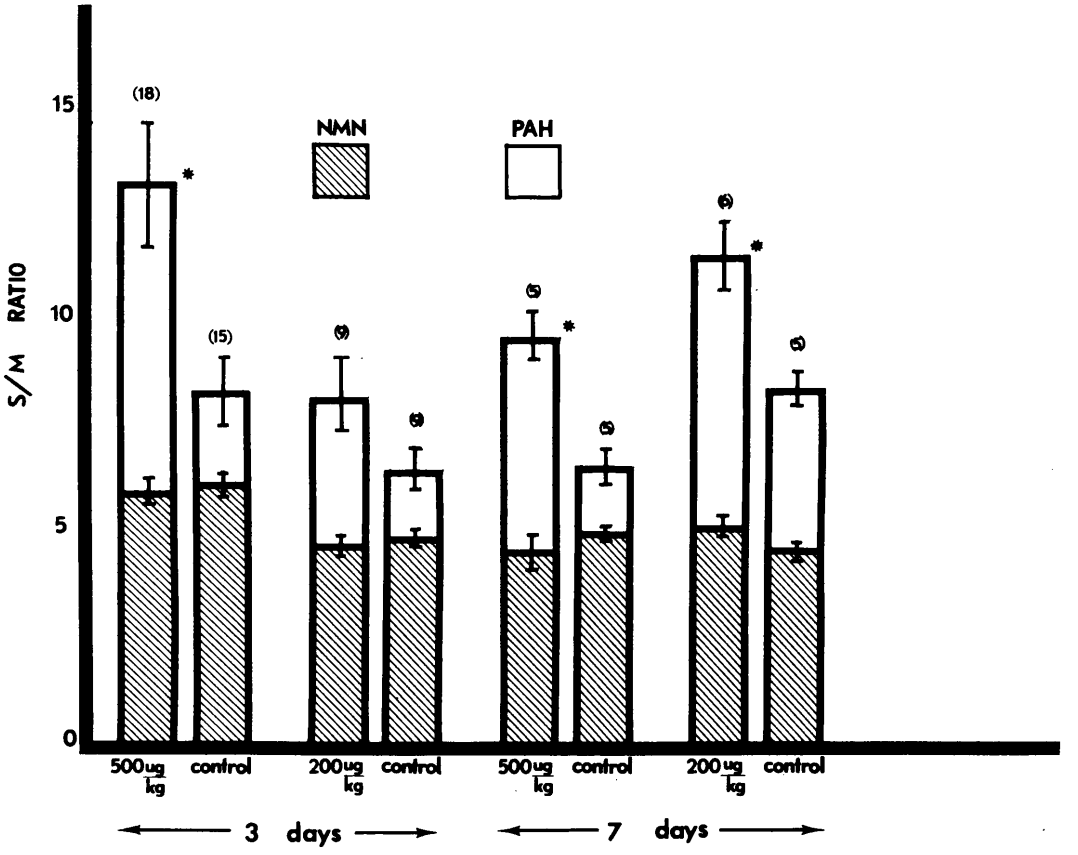

FIG. 1. PAH and NMN transport in renal cortical slices from weanling rats receiving 200 or 500 μg/kg of T_3 for 3 or 7 days: control animals received alkaline saline; all animals were killed 48 hours after the last injection. The data are presented as the mean ($\pm$SE) PAH and NMN S/M ratio obtained from duplicate determinations using the number of animals indicated in parentheses. Asterisks indicate those values that are significantly different ($p < .05$) from their respective controls.

Data obtained were analyzed statistically using Student's t test, group comparison (11). The 0.05 level of probability was used as the criterion of significance.

Results. Administration of 500 μg/kg of T_3 to weanling rats for 3 or 7 days significantly enhanced the ability of renal cortical slices to transport PAH, but did not alter NMN transport (Fig. 1). The lower dose of T_3 (200 μg/kg) stimulated PAH transport only when given for 7 days. The kidneys of weanling rats that received T_3 weighed 80–100 mg more than those from control animals. When expressed as a percentage of body weight, the increase in kidney weight became even more apparent since the T_3-treated animals weighed less than control animals at the time of sacrifice (Fig. 2).

Treatment of adult rats with 500 μg/kg of T_3 for 3 days did not significantly alter either PAH or NMN transport, nor did it significantly increase the kidney weight/body weight ratio (Fig. 3).

T_3 significantly inhibited uptake of PAH when added to renal cortical slices *in vitro* in concentrations of 1×10^{-6} *M* or 1×10^{-8} *M* (Fig. 4). Although T_3 tended to depress NMN transport, this effect was not significant.

Discussion. The results presented here suggest that the stimulatory effect of T_3 on the kidney is relatively specific for organic acid

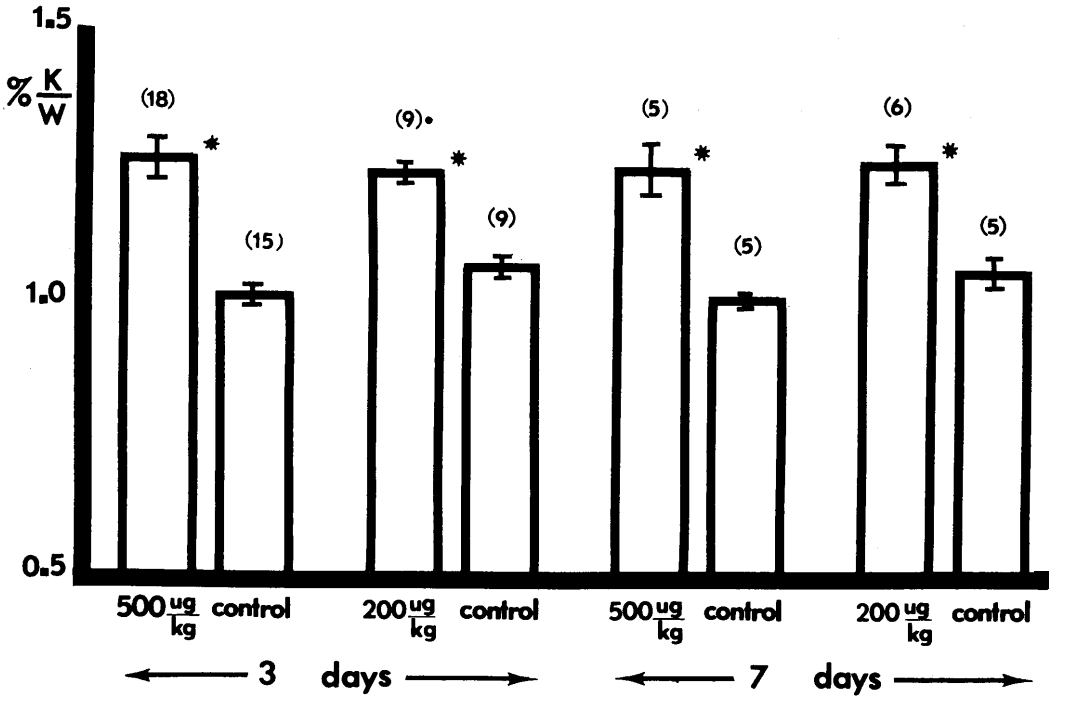

FIG. 2. Effect of T_3 administration to weanling rats on kidney weight: rats received 200 or 500 μg/kg of T_3 for 3 or 7 days, while control animals received alkaline saline; all animals were killed 48 hours after the last injection. The results are expressed as the percentage of kidney weight/body weight (±SE). The numbers in parentheses indicate the number of animals in each group. In all cases T_3 treatment caused a significant increase in kidney weight.

transport since the amount of PAH taken up per gram of slice was markedly increased in treated animals. Due to the increase in kidney weight produced by T_3, the apparent stimulation of renal transport would be even greater if the results were expressed as the amount of PAH taken up per kidney. This suggests that T_3 caused a marked increase in the activity of the specific enzymes responsible for organic transport, or that it specifically stimulated the synthesis of new transport enzymes. The ability of T_3 to stimulate PAH transport in weanling but not adult rats also suggests that T_3 stimulates the formation of new organic acid transport sites. Cell division and growth occur more rapidly in young animals and should, therefore, be easier to stimulate than in adult animals where these processes are occuring at a slower rate.

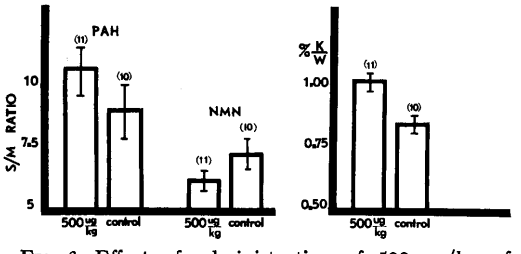

FIG. 3. Effect of administration of 500 μg/kg of T_3 for 3 days to adult rats on PAH and NMN transport in renal cortical slices, and on kidney weight: control animals received alkaline saline; all animals were killed 24 hr after the last injection. The numbers in parentheses indicate the number of animals in each group. In all cases there was no significant difference between treatment and control effects.

Farah *et al.* (6) reported that thyroidectomy depressed the uptake of PAH by kidney slices, but that administration of 10 μg of thyroxine/day for 5 days to adult rats had no effect on PAH transport. Nepomuceno and Little (7) observed that *in vitro* uptake of PAH was depressed if rats were given 10 μg of thyroxine/day for 2 weeks or longer. The use of T_3 in place of thyroxine and the use of

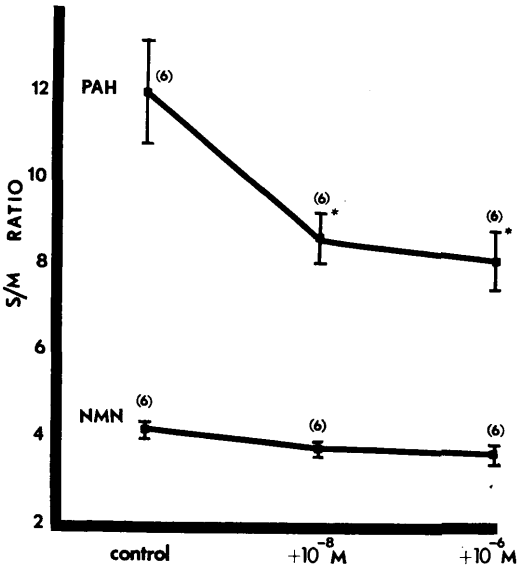

FIG. 4. Effect of T_3 (10^{-8} *M* or 10^{-6} *M*) on PAH and NMN transport in renal cortical slices from weanling rats when added *in vitro*: values represent the mean ± SE; the numbers in parentheses indicate the number of animals used in each group; asterisks indicate significant difference from control ($p<.05$).

weanling rats represent significant differences from the procedures used by the above workers and may explain the stimulation of PAH transport observed in the present study. T_3 is 3–6 times as potent as thyroxine (12, 13) and exerts its effects more rapidly. The depressant action of thyroid analogues on PAH uptake upon prolonged administration may be related to the thyrotoxicosis eventually produced. Furthermore, when weanling rats were used in this investigation, 48 hr were allowed to elapse between the last injection and the slice study to ensure that sufficient time was allowed for metabolism and excretion of T_3.

Huang and Knoefel (14) reported that various halogenated tyrosine derivatives are secreted in the kidney and depress T_m PAH, indicating that these compounds are transported by the same system as PAH. In addition, Nepomuceno and Little (15) suggested that there may be substrate competition for the organic acid transport system between PAH and iodothyronine compounds. The results of the *in vitro* studies presented here suggest that T_3 is transported as an organic acid since the addition of T_3 *in vitro* inhibited PAH transport but had little effect on NMN uptake. The ability of T_3 to stimulate PAH transport when given *in vivo* while having no effect on NMN uptake also suggests that T_3 is transported as an organic acid and stimulated PAH transport by a mechanism involving substrate stimulation. This would be analogous to substrate stimulation of hepatic drug metabolizing enzymes by the barbiturates (16).

Summary. Administration of T_3 to weanling rats caused a marked increase in PAH transport in renal cortical slices but had no effect on NMN transport. When added directly to slices *in vitro*, T_3 inhibited PAH transport. The results therefore indicate that T_3 is transported as an organic acid and specifically stimulates the acid transport system, not by a generalized metabolic effect, but directly by substrate stimulation.

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1-Methyl-1-nitrosourea Depression of Brain Nicotinamide Adenine Dinucleotide in the Production of Neurologic Toxicity (33914)

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1-Methyl-1-nitrosourea (MNU) has been shown to be both a potent carcinogenic and antitumor compound (1, 2). These effects have been attributed to the liberation of diazomethane, a highly reactive agent capable of alkylating protein, RNA, DNA, and inhibiting the incorporation of amino acids into protein (3, 4). Recent investigations demonstrated that MNU can produce a rapid dose-related depression in liver nicotinamide-adenine dinucleotide (NAD) concentrations (5, 6). During the course of these studies there appeared a transient neurologic syndrome which was temporally related to the acute lowering of brain NAD levels. This communication correlates these findings with measurements of drug concentration in the acid-soluble fraction of brain, and with brain NAD glycohydrolase activity and histology.

Methods. Male albino mice, Swiss strain, weighing 20–25 g were used for all studies, and were maintained on Purina laboratory chow pellets and water *ad libitum*. 1-Methyl-1-nitrosourea, NCS-23909, and nicotinamide (Calbiochem) were prepared in distilled water. Streptozotocin, NSC-85998, a diabetogenic compound composed of the union of glucosamine and MNU at the carbon 2 position of the glucose moiety (7), was dissolved in 0.005 *M* citrate buffer, pH 4.0. The drugs were administered at a volume of 1 ml/100 g of body weight intravenously via the tail vein, or intraperitoneally. The NAD content of brain was assayed enzymatically using alcohol dehydrogenase (Sigma) after the organ was homogenized 1:5 weight:volume in 0.6 *N* perchloric acid at 4°, and the supernate was neutralized using 3 *N* KOH (8). The NAD glycohydrolase activity of brain homogenate was assayed using the method of Kaplan as modified by Waravdekar (9). The concentration of MNU in the acid-soluble fraction of brain, liver, and serum was measured colorimetrically using a modification of the Forist method for streptozotocin (10, 6). For histologic study of brain, mice were anesthetized with ethyl ether, and 10% formalin was perfused through the heart. Sections of brain and spinal cord were rapidly removed and fixed in formalin and embedded in paraffin. The cresyl violet, Weil-Weigert, and Klüver–Barrera stains were used for the study of Nissl granules, nerve cell morphology, and myelin sheaths (11). For comparison, the brains of four normal mice were prepared in the same manner.

Results. Within 2–5 hr after the intravenous injection of MNU, 100 mg/kg, 60% of the mice demonstrated episodes of tonic seizure activity characterized by a straightening of the spine and stiffening of the tail, while the fore and hind limbs were thrust posteriorly (Fig. 1). Each attack had a duration of 10–20 sec following which the animal would remain refractory to further seizure activity for a period of 5–15 min. The syndrome appeared spontaneously or could be elicited by introducing tactile or auditory stimulation. By 5 hr after the single adminis-

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