The Diuretic Activity of Tetrahydroaminacrin in Rats[1] (34825)

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Tetrahydroaminacrin (THA, 1,2,3,4-tetrahydro-5-aminoacridine) is a potent cholinesterase inhibitor (1). Centrally, it acts as an analeptic and antihallucinogen (2, 3). Peripherally, its action is believed to be due mainly to its anticholinesterase properties (4). While testing a series of cholinesterase inhibitors for diuretic activity, we found that THA caused a marked diuresis in rats.

Experiments reported in this paper indicate that the observed diruesis in rats is not due primarily to a direct or indirect muscarinic action of THA, nor is it due to release of pituitary hormones.

Methods. Albino Sprague-Dawley rats of either sex were used in these experiments. The hypophysectomized rats were purchased from Charles River Breeding Laboratories, Inc. The rats were allowed free access to Purina Lab Chow and tap water. At the beginning of a study the rats weighed about 200 g and by the end of the same study several weeks later they had grown to about 450 g. The hypophysectomized rats, however, remained at their initial weight throughout the study.

THA was obtained from Monsanto and atropine sulfate was obtained from Matheson, Coleman, and Bell. The drugs were dissolved in 0.9% saline and administered subcutaneously in volumes less than 1 ml.

Each experiment consisted of a series of urine collections. At least 3 days separated each urine collection. The THA was administered to the rats just prior to a 25 ml/kg gavage of 0.9% saline. The rats were placed in metabolism cages and the urine was collected in graduated centrifuge tubes for 4 hr. When atropine was used, it was administered 30 min prior to the gavage.

Urine volume for the 4-hr collection period was expressed as milliliters/100 g rat weight. Urine sodium and potassium concentrations were determined with a Baird-Atomic flame photometer with an internal lithium standard. Chloride concentration was determined with a Buchler-Cotlove chloridometer. Total electrolyte excretion for the 4-hr period was expressed as milliequivalents/100 g rat weight.

Every rat received each treatment in a randomized sequence according to a Latin square plan. Prior to the first urine collection the rats were paired and randomly assigned to columns of the square. Each urine collection corresponded to a row of the square, and the treatments were randomly assigned to letters within the square. With this design, the variance due to animals, urine collections, and treatments were estimated in the analysis of variance (5).

The diuretic study was designed as a single-factor experiment at five levels. Five pair of male rats were assigned to a 5 × 5 Latin square and five pair of female rats were assigned to a second Latin square. THA was administered in doses of 1, 2, 3, 4, and 8 mg/kg. The control group received only saline. The significance of the effect of tetrahydroaminacrin was estimated by comparing the mean diuretic response of the control group with the mean of the drug responses at the various dose levels of the drug (5). The significance of the linearity of regression of the drug response on the $\log_2$ of the dose from 1 mg/kg to 8 mg/kg was estimated by regression analysis in a single-factor experiment (5).

In the atropine study 12 pair of male rats were assigned to three 4 × 4 Latin squares. The treatments, in a 2 × 2 factorial design,

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were: saline; THA, 4 mg/kg; atropine sulfate, 3 mg/kg; and THA plus atropine. The significance of the blockade of the THA response by atropine was estimated by measuring the statistical interaction between THA and atropine (5). Interaction measures the amount that the response to THA in the normal rats differs from the response to THA in the atropinized rats. More precisely,

Interaction = (THA - saline) - (THA with atropine - atropine)

In the experiment using hypophysectomized rats, 12 pair of male hypophysectomized rats were assigned to four 3 × 3 Latin squares. An experiment of the same design, using normal rats, was run in parallel as a check, although the data from the two groups were not pooled in the analysis of variance. THA was administered in doses of 2 and 4 mg/kg. The control group received saline only. The significance of the diuretic effect of THA was estimated by comparing the control response to the mean of the response of the other two dose levels (5). The significance of the differences between the means of the hypophysectomized rats and normal rats at the control dose level was estimated by Student's *t* test.

Results. In the diuretic study, THA increased sodium and chloride excretion and urine volume above control levels, but did not alter potassium excretion. The increase in chloride excretion closely paralleled the increase in sodium excretion. These results are illustrated in Fig. 1. Sodium and chloride excretion and urine volume appeared to increase linearly with the $\log_2$ of the dose.

The appearance of the rats administered the diuretic doses of THA did not differ from the appearance of the control rats. However, administration of 16 mg/kg resulted in salivation and muscle fasiculations.

In rats pretreated with atropine, THA increased sodium and chloride excretion and urine volume above control levels. The data are given in Table I. Although atropine pretreatment depressed sodium and chloride excretion and urine volume in all of the rats, there was no statistically significant interaction between THA and atropine. That is, the THA-induced increase in sodium and chloride excretion and urine volume in the atropinized rats was not significantly different from the increase in the nonatropinized rats. Neither atropine nor THA had any effect on potassium excretion.

In the hypophysectomized rats, adminis-

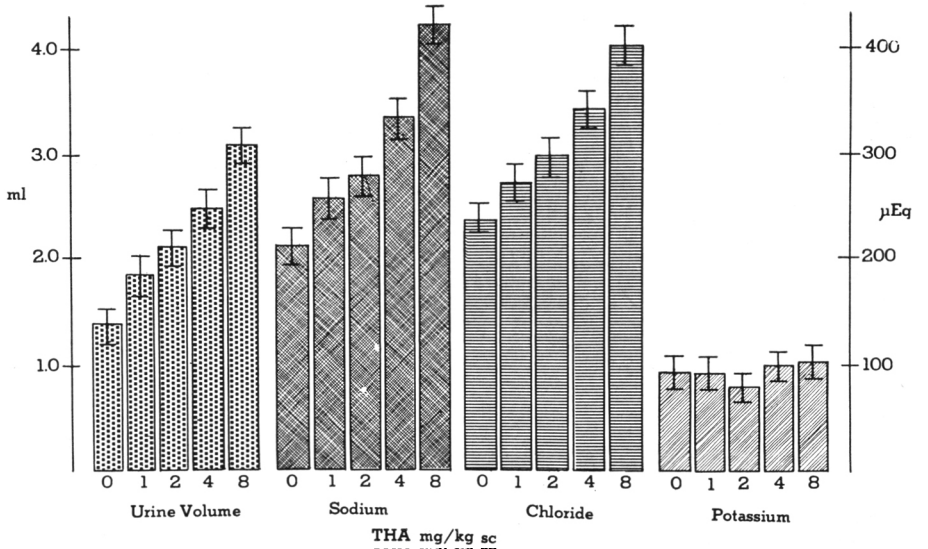

FIG. 1. Urine and electrolyte excretion in rats treated with tetrahydroaminacrin. Each value represents the mean of 10 observations. The water and electrolyte values (4-hr collection) are based on 100-g body weight.

TABLE I. The Diuretic Response of Rats to Tetrahydroaminacrin (THA) Before and After Atropine Pretreatment.

Treatment	Urine volume (ml)	Electrolyte excretion (mEq)		
		Sodium	Chloride	Potassium
Pretreated with saline				
Saline	2.03[a]	266	270	90.8
THA[b]	3.03[c]	393[c]	364[c]	107.0
Diuretic response[d]	1.00	127	94	16.2
Pretreated with atropine				
Atropine[e]	1.83	205	228	87.7
THA and atropine	2.39[b]	257[b]	282[b]	107.0
Diuretic response	0.56	52	54	20.0

[a] Each value is the mean of eight observations. The water and electrolyte values (4-hr collection) are based on 100-g body weight.

[b] Tetrahydroaminacrin at a dose of 4 mg/kg, sc.

[c] Significantly different from saline ($p = .01$).

[d] The diuretic response is the increase in water and electrolyte in response to THA.

[e] Atropine sulfate USP at a dose of 3 mg/kg, sc.

tration of THA increased sodium excretion and urine volume above control levels but did not alter potassium excretion. These results are listed in Table II.

The hypophysectomized rats maintained a normal appearance throghout the experiment although they did not gain weight. Untreated hypophysectomized rats excreted the same amount of urine as untreated normal rats, but they excreted significantly less potassium than normal rats. The osmolality of the urine from the untreated hypophysectomized rats was 483 ± 76 mOsm and the osmolality of the urine from the normal rats was 767 ± 33 mOsm.

TABLE II. The Diuretic Response of Rats to Tetrahydroaminacrin (THA) After Hypophysectomy.

Treatment	Urine volume (ml)	Electrolyte excretion (mEq)	
		Sodium	Potassium
Hypophysectomized rats			
Saline	2.07[a]	249	74
THA, 2 mg/kg	2.55[b]	371[b]	85
THA, 4 mg/kg	2.71[b]	450[b]	82
Normal rats			
Saline	1.83	258	183
THA, 2 mg/kg	2.04[b]	317[b]	172
THA, 4 mg/kg	3.29[b]	430[b]	207

[a] Each value is the mean of 12 observations. The water and electrolyte values (4-hr collection) are based on 100-g body weight.

[b] Significantly different from saline ($p = .05$).

Discussion. THA caused a diuresis in rats. This diuretic response to THA was characterized by an apparent parallel increase in sodium and chloride excretion with a concomitant increase in urine volume.

Pretreatment with atropine failed to completely block the diuretic response to THA. Although the diuretic response to THA was reduced in the atropinized rats, there was no significant evidence of interaction between THA and atropine. This is interpreted to indicate that a direct or indirect muscarinic action of THA is not the principal cause of the diuresis as was the case with arecoline (6), a parasympathomimetic alkaloid, and physostigmine (7), a potent anticholinesterase agent. The diuretic and saluretic response to arecoline (6) and the saluretic response to physostigmine (7) were completely blocked by atropine at doses lower than those used in this study.

Although there was no significant interaction between THA and atropine, the diuretic response to THA in the atropinized rats was smaller than the response in the nonatropinized rats. The smaller diuretic response to THA in the atropinized rats could be due

simply to a physiological antagonism between the diuretic response to THA and the antidiuretic effect of the high dose of atropine.

In preliminary experiments in the dog and the chicken, THA, which was infused directly into the blood supplying one of the kidneys, did not cause a unilateral diuresis. There were no consistent changes in glomerular filtration rate, effective renal plasma flow, or apparent tubular excretion fraction. Carbachol administered in the same animals did cause a marked unilateral diuresis that was blocked with atropine. These effects of carbachol are consistent with effects of other direct-acting cholinomimetics on the kidney (8, 9). The apparent lack of a direct renal effect of THA in the dog or chicken in these preliminary experiments does not exclude the possibility that THA may have a direct renal effect in the rat. However, these experiments do suggest that THA may be acting at some extrarenal site in the rat to induce diuresis.

Considering the central nervous system activity of THA, a proposed extrarenal mechanism of the diuresis involving oxytocin release seemed particularly attractive. Williams and Carter suggested that a part of the diuretic response to cholinergic agents in rats that is not blocked by atropine could be due to the release of oxytocin (6). However, hypophysectomy failed to alter the diuretic response to THA suggesting that the diuresis in response to THA does not involve a release of a pituitary hormone.

Summary. THA administered subcutaneously caused a dose-related diuresis in rats. This diuretic response was probably not due to a muscarinic action of THA, as the diuresis was not blocked by atropine. Preliminary experiments in the dog and the chicken indicate that in these species there was little if any direct renal effect. The diuretic response to THA in rats does not appear to involve release of a pituitary hormone since hypophysectomy did not abolish the diuretic effect of THA.

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