Effect of Hydrolytic Products of Procaine on Procaine Toxicity and Metabolism in Mice.* (18550)

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During previously reported studies on the effect of cholinesterase inhibitors on the toxicity of procaine in mice(1) a preliminary test of the effect of diethylaminoethanol (DEAE) in this regard was made, since Velázquez *et al.*(2) had observed that this hydrolytic product of procaine exerts some cholinesterase inhibiting action. This test showed that pretreatment with DEAE significantly increased the toxicity of procaine in mice. Considering the possibility that this could be due to a competition between DEAE and procaine for procainesterase(3,4) we have extended the observations with DEAE and have also included para-aminobenzoic acid (PABA), the other hydrolytic product of procaine, and dimethylaminoethanol (DMAE), a close analogue of DEAE. Tests have also been carried out to determine whether the rate of disappearance of procaine *in vivo* is altered by pretreatment of mice with these three agents.

Materials and methods. In order to be able to select doses of PABA, DEAE, and DMAE which were approximate toxic equivalents in mice, and to choose the time intervals between the administration of premedicants and procaine which would permit the development of the maximum action of the former, preliminary LD_{50} determinations were made on each of these agents and the death times were observed. Healthy Carworth white mice of both sexes weighing 20-30 g were used. The PABA solution, neutralized with sodium hydroxide, was prepared in a 25% concentration for the determination of its LD_{50}, and in a 7% concentration for the premedication in the procaine toxicity tests. Both DEAE and DMAE were 5% in concentration and neutralized with hydrochloric acid. In the toxicity study 1% procaine hydrochloride was used, and in the *in vivo* hydrolysis study a 0.5% solution was employed. All drugs were made up in 0.9% saline solution. PABA, DEAE, and DMAE were administered subcutaneously, while the procaine was injected into a tail vein in all cases. The LD_{50} and its 5% fiducial limits was calculated by means of probit analysis (5).

In the study of the influence of the various subcutaneous premedications on the rate of procaine hydrolysis *in vivo*, all mice were fasted for 7 hours before receiving the procaine injection. At the end of the fasting period, 30 mg/kg of procaine was injected intravenously. At various time intervals after the procaine injection the mice were dropped into a Waring Blendor and homogenized for 60 seconds in 3 ml of 4% trichloro-acetic acid for each gram of body weight. The trichloro-acetic acid precipitated the protein and stopped the procaine hydrolysis instantly. The homogenate was then filtered. Three ml of the filtrate were used for the measurement of procaine by a spectrophotometric method(6). The procaine content of the mouse body at zero time was obtained by dropping the mouse into the homogenizer immediately after the procaine. This maneuver took only a few seconds and gave the tissues little chance to destroy the procaine.

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TABLE I. Subcutaneous Toxicity of PABA, DEAE, and DMAE in Mice.

	No. of mice	LD_{50} (g/kg)	5% fiducial limits of LD_{50} (g/kg)	Time of most deaths
25% PABA	60	4.39	4.20-4.59	7-24 hr
5% DEAE	65	1.61	1.08-2.07	15-60 min.
5% DMAE	110	2.08	2.04-2.12	15-60 min.

Results. Following the injection of lethal doses of PABA the mice became inactive, weak, and drowsy in 30 to 60 minutes and prostrate in several hours. Most of the deaths occurred in 7 to 24 hours but a few died as long as 48 hours after the injection. After the administration of DEAE the animals succumbed in 15 to 60 minutes. There were no delayed deaths. Following the injection of DMAE most of the mice died in 15 to 60 minutes, but some died after having remained prostrate for two to three days. The deaths occurring within an hour were generally preceded by convulsions. Mice dying after one day manifested no convulsions, but severe prostration and gasping were seen for many hours before death. The toxicities of PABA, DEAE, and DMAE, and the range of death times in mice following injections of these substances are illustrated in Table I.

With these data on hand, the LD_{50} of intravenous procaine was determined on mice premedicated with PABA, DEAE, or DMAE. The dose of each premedicant used, the interval of time allowed between its administration and the injection of procaine, and the procaine toxicity results obtained are indicated in Table II. In each case the dose of premedicant was approximately one third to one fourth the LD_{50} of the premedicant and the time interval allowed before the injection of the procaine was that which had been observed necessary to elapse before deaths began to occur following administration of the LD_{50} of the premedicant. An additional test of the toxicity of procaine was carried out on a group of mice which had been pretreated with PABA only 30 minutes before receiving the procaine. The doses of the premedicants employed produced no noticeable toxic effects on the animals. The tabulated results show that both DEAE and DMAE diminished the LD_{50} of intravenous procaine by approximately 35%, while PABA, given either 30 minutes or 7 hours before the procaine, had no influence upon the toxicity of the latter drug.

In the study of procaine hydrolysis *in vivo*, 60 mice were used in the control group and in each of the groups receiving PABA, DEAE, or DMAE. The curves in Fig. 1 relate time after injection with the percentage of the total amount of injected procaine recovered in the whole mouse homogenate. The differences between the results obtained with the control group and with the premedicated groups were all statistically non-significant.

Discussion. It could not be demonstrated that pre-treatment by DEAE, PABA, or DMAE had any effect on the rate of disappearance of intravenously administered procaine from the whole mouse body. Thus it may be presumed that if procainesterase plays an important role in the *in vivo* hydrolysis of procaine, this role is not interfered with in any way by the hydrolytic products of procaine or by DMAE. This finding indicates that the increase in the sensitivity of mice

TABLE II. Toxicity of Intravenous Procaine in Mice After Subcutaneous Premedication by PABA, DEAE, and DMAE.

Subcut. premedicant	No. of mice	Time interval between premedication and procaine	LD_{50} of procaine (mg/kg)	5% fiducial limits of LD_{50} (mg/kg)	Significance of diff. from control LD_{50}
Control	50	—	50.7	47.7-54.2	
7% PABA, 1 g/kg	46	7 hr	50.7	42.0-63.4	Non-sig.
7% PABA, 1 g/kg	50	30 min.	53.1	49.4-56.2	Non-sig.
5% DEAE, .5 g/kg	85	15 min.	32.5	30.9-34.0	Highly sig.
5% DMAE, .5 g/kg	40	15 min.	34.2	32.5-36.5	Highly sig.

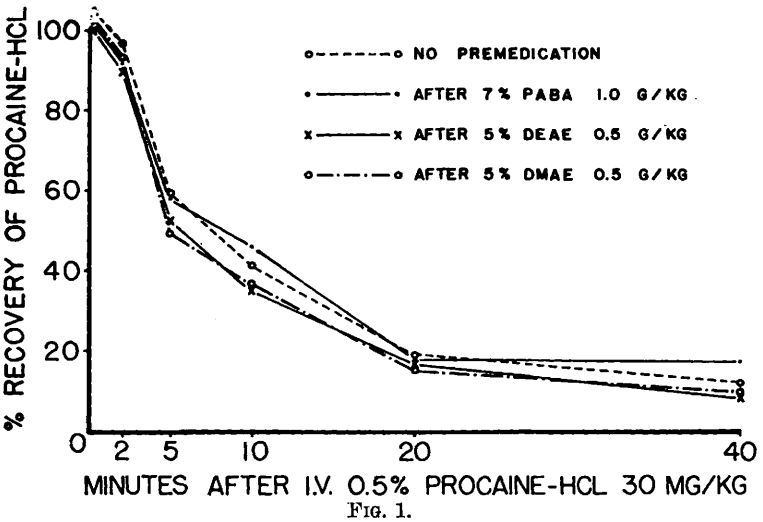

FIG. 1.

Disappearance of intravenously administered procaine in control mice and in mice pretreated subcutaneously with PABA, DEAE, and DMAE. (Each point represents the average of 10 determinations on 10 mice).

to the lethal action of intravenous procaine following pretreatment with apparently non-toxic doses of DEAE and DMAE may be the result of the additive actions of these materials and procaine. These additive actions seem most likely to be on the cardiovascular system. Following rapid intravenous injection procaine causes death primarily through its effect on the heart(7-9). Therefore, premedication by relatively small doses of drugs having an effect on the heart or vascular system could conceivably lower the threshold for the toxic dose of intravenous procaine. Observations by other workers indicate that DEAE and DMAE possess actions which might be of some value in interpreting the influence of these agents on the toxicity of intravenously administered procaine. DEAE has been shown to cause inhibition of the sympathetic vasoconstrictor reflex in man (10), dilatation of peripheral vessels in mammals(11), and hypotension(12,13). Both procaine and DEAE inhibit cholinesterase but PABA does not(2). DMAE may slightly lower the blood pressure in the dog(14), and in toxic doses this agent produces auriculoventricular dissociation(15), pulmonary edema and death(16).

PABA has little effect on the cardiovascular system, and would not be expected to exert any influence on the intravenous procaine toxicity in mice. The subcutaneous LD_{50} of 25% PABA (neutralized with sodium hydroxide) in mice was found to be 4.39 g/kg which is very close to the intravenous LD_{50} demonstrated by Scott and Robbins(17) and by Richards(18) for rats injected intraperitoneally. It is significant that PABA, DEAE, and DMAE are all less toxic than procaine which has a subcutaneous

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LD_{50} of 968 mg/kg in mice(19).

The present results are of some interest in the light of the work of Richards and Kueter(20) who observed that the incidence of procaine convulsions in guinea pigs was reduced by the prior administration of either DEAE or PABA, or of both of these substances simultaneously. They presented evidence favoring competitive inhibition as the mechanism of this phenomenon. They observed however that PABA and DEAE had no inhibitory effect on the local anesthetic action of procaine, nor on procaine-induced convulsions in mice. Our results further fail to show in mice any evidence of competition between procaine and these substances for procainesterase, or for any other receptor substance or structure which may be involved in the gross lethal action of intravenously administered procaine.

Summary. The subcutaneous LD_{50}s of 25% PABA, 5% DEAE, and 5% DMAE in mice were found to be 4.39, 1.61, and 2.08 g/kg respectively. Subcutaneous premedication by 1 g/kg of 7% PABA did not alter the LD_{50} of intravenous procaine in mice, while subcutaneous premedication by 0.5 g/kg of either 5% DEAE or 5% DMAE lowered the LD_{50} about 35%. Neither PABA, DEAE, nor DMAE changed the rate of disappearance of procaine from the whole body of the mouse. It is concluded that the influence observed to be exerted by DEAE and DMAE on the toxicity of intravenous procaine is probably due to their additive toxic effects on the cardio-vascular system, and not to the inhibition of procainesterase *in vivo*.

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Phosphocreatine and Adenosine Triphosphate Content of Rat Tissues after Adrenalectomy and Cortisone Treatment (18551)

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In studies on the metabolic effects of adrenalectomy and hormone treatment, the question frequently arises as to whether adrenalectomized animals lack an adequate supply of readily available energy. One index of their energy supply is the adenosine triphosphate (ATP) and phosphocreatine content of the tissue. Data on the ATP and phosphocreatine content of rat tissue from adrenalectomized animals are somewhat inconsistent(1). Part of this confusion is due to lack of correction for salt balance and part is probably due to earlier inadequate methods for measuring ATP. It was therefore of interest to determine the ATP and phosphocreatine content of tissues of animals under various conditions of treatment. Using rats maintained on saline we have found no significant alteration in the phosphocreatine or ATP content of muscle after adrenalectomy or after treatment with cortisone. All data presented are on animals starved for a period of 24 hours before analysis, since this condition might be likely to result in altered phosphocreatine. No difference was found between well-fed or starved animals.

Methods. A. *Preparation of animals.* The rats, weighing approximately 150 g were adrenalectomized bilaterally in the laboratory of Dr. H. C. Stoerk to whom we are indebted for this material. After adrenalectomy, the animals were supplied an adequate diet and

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