Correlation of Plasma Level with Spinal Cord Depressant Action of Mephenesin and its Carbamic Acid Ester.*† (21182)

PETER E. DRESEL,‡ ELIZABETH E. UNDERWOOD, AND ARTHUR P. RICHARDSON.

From the Department of Pharmacology, School of Medicine, Emory University, Ga.

The action of mephenesin on multineuron reflexes has been studied intensively by a large number of investigators. Clinical applications have been hampered by the short duration of action of this drug and the consequent need to administer relatively large doses at frequent intervals. Several attempts have been made to increase the duration of action by esterification of the primary alcohol group of mephenesin which has been shown to undergo rapid oxidation *in vivo* to the inactive lactic acid(1). One of the compounds which was synthesized for this reason is the carbamic acid ester of mephenesin (hereafter referred to as the carbamate).

The carbamate(2-4) has been shown to remain in the circulation for a significantly longer period after oral or intravenous administration. The distribution of both compounds in dogs has also been studied(4,5) and their tissue plasma ratios at high blood levels has been found to be effectively the same. Some of the pharmacologic actions of the carbamate have been described previously(6). The great similarity of action of the 2 compounds by a number of criteria was pointed out. The carbamate was shown to be slightly more potent on a molar basis by some of these criteria. There seemed to remain a possibility that the increased duration of action could be a function of potency alone. The present work is an attempt to resolve this possibility.

Methods. Analytical. Plasma levels of mephenesin and its carbamic acid ester were determined by a modification of a method of Titus, Ulick, and Richardson(7). The modification was made in order to determine the low plasma levels dealt with in this work. 4-5 ml of blood collected from the carotid artery through a polyethylene catheter were oxalated immediately in 15 ml centrifuge tubes and, if necessary, stored in the refrigerator. One ml samples of plasma were transferred to glass-stoppered centrifuge tubes of 15 ml capacity. 2.5 ml of 0.4 N NaOH were added to each sample, followed by 6.6 ml of cold Reagent (Merck) Ethyl Ether. The tubes were stoppered immediately and vigorously shaken for 8 minutes in a shaking apparatus which allowed them to be held in the horizontal position. Five ml of the ether layer were transferred to another centrifuge tube using a 10 ml syringe. The ether was evaporated to dryness in a water bath held at 42°C. Three ml of Phosphoric Acid (Baker's Analyzed) and 0.4 ml of the same diazotized sulfanilic acid reagent used in the original method(7) were added to each tube and mixed by inversion. The color reaction proceeded in a boiling water bath for 15 minutes, at the end of which the tubes were cooled in ice water. The color was read as % transmission on a Lumetron colorimeter using an M 515 filter. Each set of samples was analyzed against a blank from blood taken before the beginning of the infusion and at least 3 concentrations of a standard solution of the compound were run to establish a standard curve. Each plasma sample was analyzed in duplicate. We consider this method to be accurate to ±3-5 μg/ml plasma with sensitivity from 10-70 μg of mephenesin and from 15-80 μg of the carbamate.

Experimental. Cats of either sex weighing between 1.5 and 3.1 kg were anesthetized with Dial-Urethane§ 0.66 ml/kg. The femoral trunk and the hamstring branches of the sciatic were cut. The central end of the cut tibial nerve freed for one cm from the branching of the sciatic trunk was stimulated with

* This investigation was made possible by a grant from the Eli Lilly Research Foundation.

† Mephenesin and its ester were kindly supplied by W. A. Lott of The Squibb Institute of Medical Research.

‡ Present address: Division of General Pharmacology, Wm. S. Merrell Co., Cincinnati, Ohio.

§ Kindly supplied to us by Dr. Yonkman of Ciba Pharmaceutical Co.

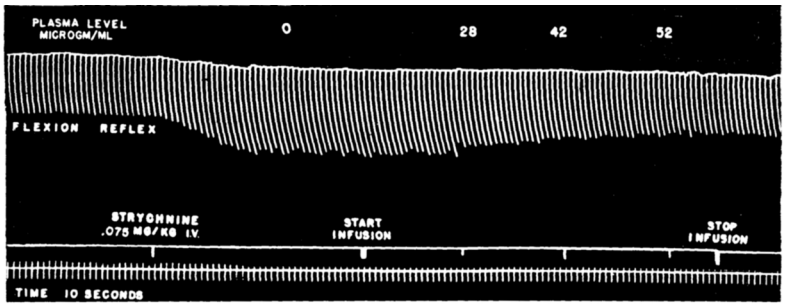

FIG. 1. Typical experiment showing effects of infusing 1.25 mg/kg/min. of mephenesin. Cat 2.0 kg. Stimulus 0.1 millisec, 12.5 v. Plasma level of 52 μg/ml should be at the time infusion was stopped (10 minutes).

shielded electrodes. Recordings were made on a smoked drum with a spring tension lever attached to the cut tendon of the tibialis anticus muscle. Stimuli were delivered at the minimum rate of the Grass Model S-4-A stimulator used (one per 9.5 sec.). The response to supramaximal stimulation as measured by excursion of the writing lever was used in all the experiments reported here. The voltage was greater than 2 x maximal, ranging from 12-25 volts at 0.1 msec. duration. The leg was fixed in position by using a clamp on the femur and another on the paw. Infusions and injections were made into the jugular vein cannulated with a long piece of polyethylene tubing reaching the Engineering Specialties Constant Infusion Machine Model 4C.‖ This pump allows reproducible rates from 0 to approximately 13 ml/min. with an accuracy of ±1%. It was calibrated by timing repeatedly at various settings the delivery of 2 ml of saline into a Normax burette. Infusions were done at one ml/kg/min., varying concentrations of the drugs being used. Infusion rates ranged from 1.0-1.75 mg/kg/min. for the carbamate and from 1.25-1.65 mg/kg/min. for mephenesin. The dead space of the polyethylene tubing was corrected for by introducing a small air bubble into it and starting the timing of the infusion when this bubble reached the animal. There was no danger of accidentally changing the position of the animal during the experiment. The only time the experimentor came close to the animal was when the clamp on the carotid was removed to allow a blood sample to be taken.

After allowing a control period of 5-15 minutes, strychnine sulfate 0.075-0.10 mg/kg was injected. If the increase in excursion was unsuitable, which occurred with only very few of the animals, another ½ of this dose was administered. After allowing sufficient time for the strychninized animal to equilibrate, a control blood sample was taken and the infusion started. Blood samples were taken every 3 minutes thereafter until the infusion was terminated. Ten to 20 seconds were needed to collect the blood. Control experiments without infusion showed that the strychnine effect lasted for considerably longer than any infusion period reported herein.

Results. A typical record obtained by the method described is seen in Fig. 1. Seven animals were used with each of the drugs. The interpretation of the experiments was made as follows: 1. The mean of 10 heights of contraction was calculated for the control period, the control period after strychnine, and at the times blood samples were taken. 2. The increase in height of contraction over control after the injection of strychnine was arbitrarily equated to 100%. The reduction in twitch height could then be assigned a value of "increment in arbitrary per cent." 3. A plot of % increment (see 2) *vs.* plasma level of the compound was made for each experiment and a smooth curve was fitted. 4. Values of plasma levels at arbitrary 90%, 80%, 70%, etc. increment were read from this curve. 5. The means of values from No. 4 for all 7 experiments for each drug were determined, expressed in μg/ml, and converted to

‖ Obtained from Engineering Specialties, Madeira, Ohio.

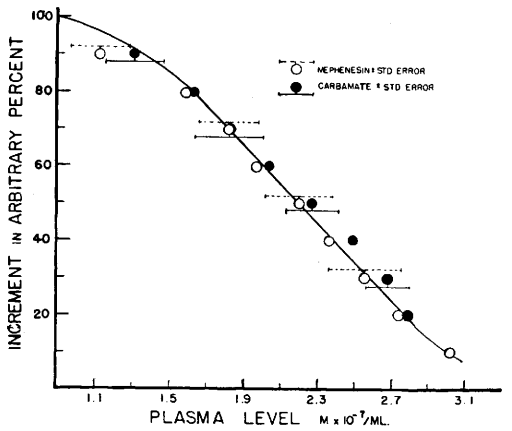

FIG. 2. Plot of mean plasma levels of mephenesin and its carbamate against increment in flexion reflex after strychnine (see text).

Moles x 10^{-7}/ml. These values were then plotted as in No. 3 to obtained the curve seen in Fig. 2.

Knowing that the plasma level ± its standard error at the 50% increment point for the carbamate is 2.27 ± 0.13 M x 10^{-7}/ml and that for mephenesin 2.20 ± 0.11 M x 10^{-7}/ml there is obviously no need for further statistical evaluation to see that the 2 compounds are equal in potency on a molar basis. On a microgram basis the carbamate is significantly less potent than the parent compound.

Discussion. These results cannot be interpreted as indicating that the carbamate is converted to mephenesin in order to become active since it has been shown by several observers(5,6) that there is no significant excretion of beta-toloxy lactic acid, the main metabolic product of mephenesin, after the administration of large doses of mephenesin carbamate. Richardson(4,8) found the ester to be excreted mainly in the free and in a conjugated form (probably with glucuronic acid). We are dealing here, therefore, with a derivative which in itself has the action of mephenesin, with equal potency according to this technic. It has been shown(4) that it requires the infusion of less carbamate than of mephenesin after a large priming dose to maintain a constant plasma level.

We must conclude, therefore, that any difference in action on multineuron reflexes, *i.e.*, duration after a constant molar dose, is due to differences in physiologic disposition of the compounds. The rapid metabolism of mephenesin must be considered one of the most important factors in any such difference.

Changes in the rate of infusion in these experiments unfortunately tell very little about the comparative rates of distribution and disposition of the 2 drugs. A very great variability was encountered, with both compounds, in the time course of buildup of plasma level. This variability was perhaps somewhat greater with mephenesin itself. The analytical method detected only non-conjugated, unmetabolized mephenesin and mephenesin carbamate. The possibility of a difference in potency at the receptor site is not completely ruled out. It has been shown in dogs, however, that the tissue plasma ratio in brain at high plasma levels is approximately the same for both compounds(4,5).

Summary. Mephenesin and its ester with carbamic acid have been shown to be of equal potency when their effect on a multineuron reflex was correlated with their plasma levels.

We would like to thank Dr. I. H. Slater for a most helpful discussion of the problem with one of us (PED).

1. Riley, R. F., *J. Pharmacol. Exp. Therap.*, 1950, v99, 329.

2. Hassert, G. L., Jr., and Burke, J. C., *ibid.*, 1954, v110, 25.

3. Thomas, B. G. H., Poutsiaka, J. W., Kulesza, J. S., and Linegar, C. R., *ibid.*, 1954, v110, 48.

4. Richardson, A. P., unpublished observations.

5. Morrison, J. L., Saperstein, P., Walker, H. A., and Richardson, A. P., PROC. SOC. EXP. BIOL. AND MED., 1948, v68, 339.

6. Dresel, P. E., and Slater, I. H., *ibid.*, 1952, v79, 286.

7. Titus, E., Ulick, S., Richardson, A. P., *J. Pharmacol. Exp. Therap.*, 1948, v93, 129.

8. Richardson, A. P., Jones, P. S., and Walker, H. A., *Fed. Proc.*, 1953, v12, 361.

Received June 28, 1954. P.S.E.B.M., 1954, v86.