Bradykinin Writhing: A Method for Measuring Analgesia. (28768)

JANE FRANCES EMELE AND JOHN SHANAMAN (Introduced by Martin M. Winbury)
Department of Pharmacology, Warner-Lambert Research Institute, Morris Plains, N. J.

Bradykinin is the name given by its discoverers, Rocha e Silva *et al*(1), to the polypeptide formed by the action of trypsin upon the pseudoglobulin fraction of plasma protein. Since the elucidation of the structure by Elliott(2) and its synthesis by Boissonnas (3), bradykinin has been intensively studied. It has been suggested that bradykinin may be involved in the mechanism of visceral pain (6). Armstrong(4) has demonstrated pain production by application of bradykinin to the raw surface of a blister in human skin. Braun(5) reported that intraarterial injection of bradykinin evoked manifestations of pain in cats and dogs; these manifestations consisted of vocalization and signs of sympathetic stimulation. Bradykinin has been implicated in inflammation because upon injection it produced the 4 classic components of inflammation: vasodilation, increased capillary permeability, accumulation of leucocytes and pain.

The purpose of this study was to devise a simple method in which the algesic activity of bradykinin could be utilized to study drug-induced analgesia.

Materials and methods. Manor Farms, M-1 strain, female mice weighing between 20 and 26 g were used. Each mouse was housed separately in a glass beaker during the study. The drugs were administered orally, by stomach tube, as solutions or suspensions in 1% gum tragacanth. The drugs studied were acetylsalicylic acid, ethoxybenzamide, aminopyrine, salicylamide, phenylbutazone, *d*-propoxyphene hydrochloride, codeine sulfate, chlorpromazine hydrochloride, imipramine hydrochloride, phenelzine sulfate, tripelennamine hydrochloride and mephenesin. The synthetic bradykinin, supplied by Sandoz Pharmaceutical Co. in aqueous solution in a concentration of 100 μg/ml, was diluted with water to a concentration of 10 μg/ml for intraperitoneal administration.

Control studies. One hundred eighty mice were used as controls. Each mouse was injected intraperitoneally with 2.5 μg of synthetic bradykinin in a total volume of 0.25 ml. The bradykinin-induced writhing syndrome observed was similar to the phenylquinone-induced writhing syndrome previously described by us(7) and other investigators (8). The characteristic pattern consisted of abdominal torsion, drawing up of the hind legs to the body, marked contraction of the abdominal area and arching of the back so that the caudad ventral surface of the mouse touched the floor of the beaker. After injection of bradykinin 5-10% of the mice began to writhe within 10 minutes. The percentage of animals writhing in the sample increased to 85% at 20 minutes, plateaued at this percentage for the next 20 minutes, then the incidence of writhing gradually decreased. Therefore, the peak of the writhing response induced in mice by intraperitoneal administration of 2.5 μg of bradykinin occurred during the period of 20 to 40 minutes after its injection; during this time the average number of writhes per mouse was six.

Drug studies. Groups of 10 mice per dose of drug were used. The specific dose of a drug was given orally to the animals, followed 10 minutes later by challenge with 2.5 μg of bradykinin injected intraperitoneally. Twenty minutes after bradykinin injection the percentage of writhing in the group of mice was noted. Percentage of writhing was noted for 10 minutes. Drug activity was studied 30 minutes after drug administration. One writhe per mouse was considered a positive response. The ED50 value of each drug was calculated according to probit analysis from a minimum of 2 dose levels by plotting the dose against the percentage of mice which did not writhe. Confidence limits, 95% probability, were calculated according to the method of Litchfield and Wilcoxon(9). The activity of the drugs against bradykinin-induced writhing was compared to that ob-

TABLE I. Comparative Analgesic Activity.

Drug	Analgesic ED_{50}, mg/kg p.o. Bradykinin method	Phenylquinone method
Acetylsalicylic acid	21 (11.9 – 37.2)*	150 (129 –174)*
Ethoxybenzamide	98 (55 –173)*	100 (50 –200)*
Aminopyrine	71 (53.4 – 94)*	70 (40 –123)*
Salicylamide	225 (145 –348)*	240 (146 –396)*
Phenylbutazone Na	62 (47 – 82)*	150 (97 –232)*
d-Propoxyphene HCl	>40	5.5 (4.2 – 7.2)*
Codeine SO_4	3 (.91– 9.9)*	10 (7.2 – 13.8)*
Phenelzine SO_4	22 (10.7 – 45)*	16 (9.4 – 27)*
Chlorpromazine HCl	> 5	.5 (.36– .70)*
Imipramine HCl	69 (43 –112)*	120 (87 –165)*
Tripelennamine HCl	>40	8.5 (5.7 – 12.6)*
Mephenesin	>200	140 (114 –172)*

* Confidence limits: 95% probability.

tained by a modification of phenylquinone-induced writhing(8). Drug activity in the phenylquinone method was also studied 30 minutes after drug administration.

Results. The results are presented in Table I. Acetylsalicylic acid was more potent against bradykinin writhing than against phenylquinone writhing. There was no significant difference between the activity of salicylamide or aminopyrine as measured by either method. Phenylbutazone was twice as potent in the bradykinin method as in the phenylquinone method. No significant difference was noted with ethoxybenzamide. It was of interest that propoxyphene was relatively inactive and there was no difference between the activity of codeine and phenelzine by the two methods.

Chlorpromazine was relatively inactive against bradykinin writhing, compared to its marked degree of potency in the phenylquinone method. The activity of imipramine against bradykinin writhing did not differ significantly from that against phenylquinone-induced writhing. Tripelennamine and mephenesin were without effect against bradykinin, although tripelennamine was very potent in the phenylquinone method.

Discussion. Bradykinin, a naturally occuring substance both in animals and man, has been implicated in the physiological and pathological response of inflammation and pain. Synthetic bradykinin, when administered intraperitoneally to mice, induced writhing which was inhibited by prophylactically administered analgesic drugs. Collier(10) has reported the specificity of acetylsalicylic acid, amidopyrine and sodium phenylbutazone in suppressing bradykinin-induced broncho-constriction in guinea pigs, and our study has demonstrated that these drugs were effective against bradykinin-induced writhing in mice. It is suggested that drug antagonism to bradykinin-induced writhing may be indicative of potential analgesic anti-inflammatory activity. The activity of imipramine against bradykinin writhing indicates the non-specificity of the method; however, in comparison to the phenylquinone method it would seem to be more specific if consideration is given to the relative inactivity of chlorpromazine, tripelennamine and mephenesin in the bradykinin method.

Summary. Intraperitoneally administered bradykinin to mice induced writhing which was antagonized by selected analgesic and anti-inflammatory drugs.

1. Rocha e Silva, M., Beraldo, W. T., Rosenfeld, G., *Am. J. Physiol.*, 1949, v156, 261.

2. Elliott, D. F., Lewis, G. P., Harton, E. W., *Biochem. J.*, 1960, v76, 16P.

3. Boissonnas, R. A., St. Guttmann, Jaquenoud, P. A., *Helv. Chim. Acta*, 1960, v43, 1349.

4. Armstrong, D., Jepson, J. B., Keele, C. A., Stewart, J. W., *J. Physiol.*, 1957, v135, 350.

5. Braun, C., Guzman, F., Harton, E. W., Lim, R. K. S., Potter, G. D., *ibid.*, 1961, v126, 508.

6. Guzman, F., Braun, C., Lim, R. K. S., *Arch. int. pharmacodyn.*, 1962, vCXXXVI, 353.

7. Emele, J. F., Shanaman, J., Warren, M., *J. Pharmacol. Exp. Ther.*, 1961, v134, 206.

8. Siegmund, E., Cadmus, R., Lu, G., PROC. SOC.

EXP. BIOL. AND MED., 1957, v95, 729.

9. Litchfield, J. T., Wilcoxon, F., *J. Pharmacol. Exp. Ther.*, 1949, v96, 99.

10. Collier, H. O. J., *Biochem. Pharmacol.*, 1961, v10, 47.

Received September 3, 1963. P.S.E.B.M., 1963, v114.

Metabolic Inertness of Orotidine in Man and the Rat. (28769)

SHERMAN WEISSMAN, VINCENT H. BONO, JR. AND MARVIN LEWIS
(Introduced by N. I. Berlin)
Metabolism Service and Medicine Branch, National Cancer Institute, National Institutes of Health, Bethesda, Md.

Orotidine is the pyrimidine nucleoside moiety of orotidylic acid, an intermediate in the biosynthesis of uridylic acid(1). It is found in elevated amounts in the urine of patients during 6-azauridine administration (2) and in the culture medium of a mutant of Neurospora crassa(3) but otherwise has not been described as occurring at more than trace levels in natural sources.

The only method presently available for quantitating pyrimidine production involves the assumption that the sole route for catabolism of orotic acid is by means of its initial conversion to uridylic acid(4). While conversion of orotic acid to orotidine and subsequent catabolism of orotidine would not change the formal validity of this method measuring pyrimidine ring synthesis, it would mean that the rate of pyrimidine ring biosynthesis would be an overestimate of utilizable pyrimidine synthesis, that is of synthesis of uridylic acid and its derivatives. Conversely, since no more than traces of orotidine normally occur in human urine(5), the conversion of orotic acid to orotidine could account for very little of the disposal of isotope from administered radioactive orotic acid, unless there are enzyme systems capable of catabolizing orotidine. Since it has been suggested that the capacity to metabolize orotidine might be significant in a pyrimidine biosynthetic pathway of lower organisms(6), it was considered necessary to exclude the possibility of such a pathway in humans. Finally, recent efforts to evaluate the effect of the metabolic antagonist 6-azauridine on pyrimidine metabolism in man(7) have been based on the assumption that orotidine excretion, following administration of this drug, is equal to orotidine production. Orotidine has not been found to be a substrate for phosphorylases and has been reported not to dilute the flow of isotope into pyrimidines in liver slices(8). However, because of the above considerations it was felt desirable to test the capacity of mammals to metabolize this substance using isotopic techniques and administering only tracer amounts of the compound.

Methods. C^{14} orotidine labeled in the 7 position was prepared from commercial C^{14} orotic acid as follows: A yeast enzyme preparation containing orotidylic acid pyrophosphorylase and decarboxylase was prepared according to the method of Lieberman *et al* (9). Orotidylic acid was synthesized by placing into a reaction vessel 10 ml of the yeast enzyme containing 14 units of orotidylic acid pyrophosphorylase per ml, 88 μc of orotic acid-7-C^{14} (specific activity 4.4 μc/μmole),* 20 μmoles 5 phosphoribosyl 1-pyrophosphate,† 20 μmoles 6-azauridine-5′ phosphate,‡ 400 μmoles $MgCl_2$, 1,800 μmoles NaF and 8,000 μmoles of Tris pH 8.0 in a final volume of 200 ml. This was allowed to react at 24°C for 10 minutes and the reaction stopped with perchloric acid. The acid soluble supernate was neutralized with KOH, brought to pH 8-9 with NH_4OH and placed on a 3 cm diameter, 40 ml bed volume Dowex-1-formate column. The column was eluted with a linear gradient of increasing concen-

* New England Nuclear Corp., Boston, Mass.

† Pabst Laboratories, Milwaukee, Wisc.

‡ Calbiochemical Corp., Los Angeles, Calif.