Pharmacodynamics and Biophasic Drug Levels of Methionine Enkephalin

Randall J. Erb,[1,3] Lih-Min Her,[2] Anna Abdallah,[2] and Ashim K. Mitra[2]

Received August 22, 1990; accepted January 17, 1991

Methionine enkephalin (Met-E) is a naturally occurring pentapeptide. It appears to mediate pain perception by blocking CNS pathways. Using rabbits, a log dose relationship was obtained between Met-E-induced dilation of the pupil (mydriasis) and constant intravenous infusion dose rates (14, 20, 28, 37, 56, 73, and 129 μg/min/kg). Steady-state dilation is reached within 9 min. Dose–effect curves (DEC) were fitted by a linear regression analysis of the log dose versus percentage dilation plots. Fitted DEC were used to determine temporal profiles for the relative biophasic drug level.

KEY WORDS: biophase; mydriasis; dose–effect curve; methionine enkephalin; pharmacodynamics.

INTRODUCTION

Although radioimmunoassays and high-pressure liquid chromatographic analyses of enkephalins (1–5) are available for measuring physiological concentrations of enkephalins, most of the work has concentrated on detecting the presence of these neuropeptides in various tissues and examining their neural actions. To date little information is available on relative biophasic drug concentrations of enkephalin dosage forms.

Methionine enkephalin (Met-E) is a naturally occurring pentapeptide (6). Opiates have been shown to cause changes in pupil size in humans and laboratory animals (7–10). Although endogenous enkephalins do not appear to exert tonic control of pupil size, pupil size can be affected by exogenous enkephalins through local, CNS, and peripheral effects. The presence of opioid receptors in the rabbit iris has been demonstrated by evoking naloxone antagonized miotic responses from intravitreally injected morphine and 2-met-enkephalinamide (11). In the rabbit, miosis is produced by low plasma levels of enkephalins. At higher plasma levels, enkephalin-induced mydriasis is a secondary phenomenon due to adrenergic mobilization from the adrenal medulla, which obscures the primary centrally mediated miosis (12).

The purpose of this investigation is to determine whether a pharmacodynamic response parameter, i.e., Met-E induced changes in pupil size, can be used to quantitate the relative biophasic level of Met-E.

[1] Drug Delivery Research Center, School of Pharmacy and Pharmaceutical Sciences, Purdue University, West Lafayette, Indiana 47907.

[2] Industrial and Physical Pharmacy Department, School of Pharmacy and Pharmaceutical Sciences, Purdue University, West Lafayette, Indiana 47907.

[3] To whom correspondence should be addressed at Quincy Research Center, 5100 East 24th Street, Kansas City, Missouri 64127.

MATERIALS AND METHODS

Three New Zealand white rabbits, approximately 3 kg (Indian Creek Rabbit Farm, Lafayette, Indiana), were given iv infusions of Met-E (Sigma Chemical Company, St. Louis, Mo.) in normal saline. Standardized fluorescent lighting conditions were used. Infusions were administered into the left marginal ear vein using an infusion pump (Sage Instruments, Boston, Mass.). The dosing rates of 14, 20, 28, 37, 56, 73, and 129 μg/min/kg of the Met-E acetate salt were administered in sequence for 9 min in a stepwise gradient manner. To establish baseline pupil size, rabbits were equilibrated under the standardized lighting conditions for at least 30 min prior to treatment. The pupil response of the left eye was visually recorded on a magnetic tape at a distance of 0.6 m with an 8MM video camera (Sony CCD-F70) with 8:1 zoom lens to generate a pupil size of about 40 mm on a 19-in. color television monitor (RCA Colortrak). Size of the pupil was measured directly from the paused image upon playback after the data was collected. The playback image incorporated a time overlay output on the screen to allow pupil size to be determined on a second-by-second basis. Each rabbit was replicated three times with the same dosing routine to provide information on the reproducibility of the pupillary data. The mydriasis plateau was previously determined to occur within 9 min after the start of infusion.

Thirty-two data points were collected for each 9-min run. Data artifacts caused by movement or disturbance of the rabbit were discarded. Statistical outliers (greater than 2 standard deviations of pupil response with no drug treatment) were interpolated. Fitted data for 30-min control runs without drug treatment produced an average pupil change of $-1.2 \pm 0.8\%$. Steady-state pupil sizes were determined by the average of the last three data points (8.50, 8.75, and 9.00 min) after the data had been fitted, using 20 iterations of a computer routine. The routine weighted each data point by 50% for the current point and the remaining 50% divided between the preceding and the succeeding point. Each point was weighted by the inverse of its temporal distance from the current point. Percentage dilation was calculated as the change in pupil size from the average pupil size with no drug treatment.

Dose–effect curves (DEC) were constructed by linear regression analysis of the log dose versus steady-state percentage miotic response ($R_{p,ss}$) for each run. Variance analyses were conducted using Statgraphics software [Statistical Graphics Corporation, Rockville, Md. (13)]. Steady-state percentage dilation statistical outliers and missing data were estimated using the regression fit of the DEC for each run.

RESULTS

Table I shows the percentage dilation response at steady state ($R_{p,ss}$) for each rabbit at each infusion rate for three replicates. Missing data were estimated from the fitted dose–effect curve for the rabbit. In general, as the infusion rate increased, so did the $R_{p,ss}$ for each rabbit. The dilation effect attained a plateau between 73 and 129 μg/min/kg. From analysis of variance over all rabbits and replicates, the $R_{p,ss}$ response levels were observed to be different from each

0724-8741/91/0700-0930$06.50/0

Table I. Steady-State Response ($R_{p,ss}$) of Percentage Change in Rabbit Pupil Dilation for Constant iv Infusion Rates (R_0) for Met-E (Each Rabbit Was Run Three Times)

R_0 (μg/min/kg)	$R_{p,ss}$ (% dilation)						
	14	20	28	37	56	73	129
Rabbit 1	8.1	4.6	~10.2[a]	9.3	15.6	21.6	21.8
	2.7	~3.9	~5.9	5.8	8.0	~8.7	10.8
	7.2	4.5	6.7	12.3	13.2	13.2	16.2
Mean	6.0	4.3	7.4	9.1	12.3	14.5	16.3
SE	1.4	0.2	1.2	1.5	1.8	3.1	2.6
Rabbit 2	0.7	3.6	7.7	8.4	7.7	16.9	Plateau
	2.6	3.3	5.5	6.5	9.0	12.5	Plateau
	1.5	7.6	6.0	8.5	10.0	9.8	Plateau
Mean	1.6	4.8	6.4	7.8	8.9	13.1	N/A
SE	0.4	1.1	0.5	0.5	0.5	1.7	N/A
Rabbit 3	6.3	8.3	10.0	14.3	16.2	Plateau	Plateau
	9.1	9.5	13.1	14.7	~14.2	Plateau	Plateau
	8.5	10.6	13.8	22.5	~22.0	23.7	29.5
Mean	8.0	9.5	12.3	17.2	17.5	N/A	N/A
SE	0.7	0.5	1.0	2.2	1.9	N/A	N/A
Overall mean	5.2	6.2	8.7	11.4	12.9	N/A	N/A
Overall SE	1.5	1.3	1.5	2.4	2.0	N/A	N/A

[a] ~, indicates estimate of missing data from DEC fit.

other at $P < 0.001$. Individual DEC for the rabbits are different ($P < 0.0025$).

Figure 1 shows the linear regression fit for each rabbit's average DEC. Slopes are 0.129 ± 0.024, 0.155 ± 0.028, and 0.164 ± 0.033 for rabbits 1, 2, and 3, respectively. Intercepts are 0.281 ± 0.049, 0.300 ± 0.048, and 0.378 ± 0.062 for rabbits 1, 2, and 3, respectively. R-squared values for the fits are 0.93, 0.95, and 0.92 for rabbits 1, 2, and 3, respectively.

DISCUSSION

Although many pharmacological activities of enkephalins originate from action on the CNS (14), effects on the peripheral nervous system can complicate modeling of the pharmacological endpoints. In order for a pharmacological response to be useful as a biophasic drug level determinate, the response must be graded and monotonically increasing within the specific dosage range (15). These criteria appear to have been satisfied for the Met-E-induced pupil dilation.

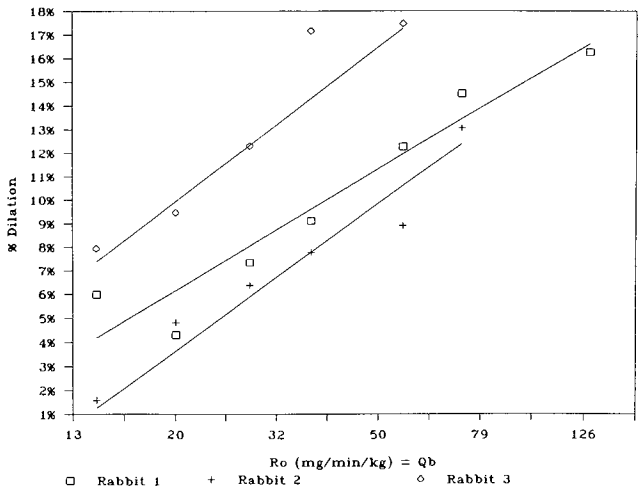

Fig. 1. Dose–effect curve fit, log infusion rate vs percentage dilation at steady state for three rabbits (average of three determinations per rabbit).

The dilation effect appears to be reproducible within a rabbit. Although statistical difference occurs among the rabbit parameters, the largest slope of 0.164 is only 27% higher than the lowest at 0.129. Similarly, the intercepts were only 34% different.

The relative biophasic drug level, Q_b, reflects the amount of drug at its site of action. The absolute amount of drug in the biophase is unknown. Q_b is related to the amount of drug in the plasma. To calculate Q_b (mg′/kg), one assumes that at steady state the amount of drug in the pupillary response biophase is related to the rate of infusion. Quick pupillary response to iv bolus dosing, producing a peak response in about 1 min, suggests that equilibration between the plasma and the pupillary biophasic compartments is rapid. This allows one to assume that the pupillary response is directly reflective of the amount of Met-E in the plasma. One can transduce experimentally derived percentage dilation data points into Q_b values by finding the Q_b which corresponds to a given percentage dilation using the DEC as a "calibration curve." For example, to calculate a relative biophasic Met-E level (Q_b) corresponding to a 10% pupil dilation, a perpendicular is dropped from the DEC curve at 10% dilation (Fig. 2). The resultant Q_b value is about 65 μg′/kg. Using the fitted curve equation for Q_b,

$$[Q_b = 1000 \times 10^{([\%\ \text{dilation} - 0.247]/0.123)}]$$

an estimate of the relative biophasic drug level (63.9 μg′/kg) can be calculated.

At the median dose of 37 μg/min/kg used in this study, a 9-min treatment delivers a total Met-E dose of 0.6 μmol/kg. This dose is of the same order of magnitude as the Met-E dose (1.3 μmol/kg) causing analgesia and bradycardia in rats (16). Thus, pupillary response appears to be sensitive within

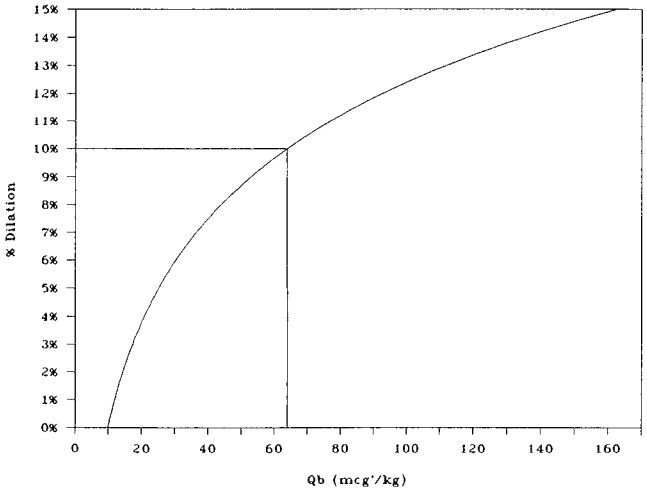

Fig. 2. Example dose–effect curve for Met-E. $Q_b = 1000 \times 10^{[(\%\ \text{dilation} - 0.247)/0.123]}$. Relative biophasic drug levels, Q_b, are determined from corresponding percentage dilation values.

a "therapeutic" dose range, demonstrating the relevancy of the method.

Since the relative amount of drug in the biophase can be determined using the pupillary response, one can use pupillometry to conduct evaluations of experimental enkephalin delivery systems by establishing individual iv infusion dose–effect relationships. Relative dosage form performance can then be evaluated from the transduction of pupil dilation data through dose–effect curves to find the relative biophasic drug level.

Finally, it should be noted that part of the attractiveness of using a pharmacodynamic response such as pupillometry) for evaluating enkephalin dosage form biophasic drug levels is that the biological system remains unperturbed by the noninvasive procedure; there is no physical contact with the rabbit during pupillometric measurements. This is especially important for the evaluation of drugs such as the enkephalins, since stress on a test animal can artifactually alter enkephalin levels (17).

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