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The Role Of Mast Cell Depletion On The Acute Toxicity Of Morphine In Mice

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ÖZET :

Morfin Sülfat'ın LD_{50} değeri büyük değişkenlik göstermektedir. Farelerde yapılan deneylerde ayrı elevajlardan alınan sonuçlarda farklılık bulunmuştur. LD_1 değeri 40 — 200 mg/Kg arasında sabit kalırken, doza bağlı ölüm yüzdeleri 200 mg/Kg dan yukarı dozlarla elde edilebilmektedir. Morfin sülfat akut toksik etkisini hisamin açığa çıkararak yapmaktadır. Güçlü bir histamin salıcı madde olan 48/80 akut olarak verildikten sonra morfin sülfatın LD_{50} değeri 30 mg/Kg'a düşmektedir. Kronik olarak 48/80 maddesi verilerek mast hücre deplesyonu yapılmış farelerde morfin sülfatın LD_{50} değeri 795 mg/Kg çıkmaktadır. 48/80 maddesi ile morfin sülfat arasındaki bu etkileşme, morfin sülfatın akut toksik etkisinde histamin salınımının önemli rolü olduğunu ortaya koymaktadır. Santral etkili analjezik bir madde olan fakat histamin salınımına neden olmayan aminopyrine ile 48/80 maddesinin endiferans etkileşmesi bu bulgumuzu doğrulamaktadır.

INTRODUCTION

In 1933, Schmitt and Livingston[11] suggested that in the decerebrated and anesthetised cats and in the unanesthetised dogs, the depressing effect of morphine on the arterial blood pressure may be related to the release of histamine. But he later dismissed this possibility. Later, McIntosh and Paton[6] showed that the opium alcaloids also have properties of releasing histamine. Subsequently, Nasmith and Stewart[8] and Feldberg and Paton[4] demonstrated more clearly that morphine causes release of morphine in the cat's skin and the perfused gastrocnemius muscle. Furthermore they stated that the release of histamine was reduced if the injection of morphine was repeated.

It is known that morphine exibits its analgesic effects through its own receptor located in the central nervous system. Furthermore. if it is administered in subtoxic doses, it also produces some non-selective effects,[1, 2]. In 1952, Evans et al. demonstrated that the effects of morphine on the circulation, when injected i. v. to the cat, dog, and, rat, were related to the released histamine.

In the literature, the lethal dose of morphine varies greatly from one investigator to the other, departing from the hypothesis in which different factors may play a role in the acute toxicity. Therefore, with the aim of determining whether or not these differences were related to the histamine releasing effect of morphine, we decided to investigate the influence of compound 48/80, a potent histamine releaser, on the acute toxicity of morphine. Although the mechanism of histamine releasing activity of compound 48/80 is different from that of other histamine releasers, as exposed by the work of Rotchild[10] and Lamaski and Ende[14], their effects on the target organ during the histamine release must be similar.

MATERİAL AND METHOD

Swiss albino mice of both sexes weighing 24±7 grams were used throughout the experiments. All injections were made subcutaneously to animals kept fasting 24 hours prior to experiments. The deaths seen within 48 hours were evaluated for determination of LD_{50}. The calculation of LD_{50} was made according to the graphic method described by Miller and Taintes[7]. Percentage of death was indicated as probit on the ordinate, and log dose recorded on the absissa of the graph. The LD_{50} of morphine was found on the absissa corresponding to the value of probit 5 on the ordinate. LD_{50} of morphine determinations were carried out on 350 mice divided into seven groups of 50. Each group received five different doses of morphine, so that each point on the graph. consisted of seven different death percentage values which enabled us to evaluate the doses statistically.

The second series of LD_{50} estimations was made on 200 mice, and deaths with the influence of compound 48/80 on LD_{50} of morphine was determined in the first group. 200 mice were divided into four groups: in the first group of 50, 10 mg/kg of 48/80 was injected together with the 5 different doses of morphine, each dose given to 10 mice: in the second groups of 50, 10 mg/kg of 48/80 was injected 30 minutes before the administration of the 5 doses of morphine: in the third and fourth groups of 50, the mice recieved 10 mg/kg of 48/80, 60 and 120 minutes before morphine, respectively.

In the third series of experiments, LD_{50} of morphine was estimated in 50 previously mast cell depleted mice by injecting compound 48/80 in doses of 10 mg/kg three consecutive days, twice a day. In the last group of fifty mice, the influence of compound 48/80 on the LD_{50} of aminopyrine, which is not a mast cell depletor and which causes convultions before death, was tested. This group served as a control for showing that compound 48/80 has no effect on LD_{50} of aminopyrine.

Statistical analises were made by Student T test, and all graphs were drawn on the basis of probit versus log. dose.

The estimation of LD_{50} of morphine in intact naive mice

LD_{50} of morphine was estimated on 350 mice divided into seven groups. Each group received morphine in doses starting from 40 mg/kg and reaching, in the last groups, 1600 mg/kg. The results are summarized in table 1/A and 1/B. Death percentages for doses of 40 mg/kg was 10: for 100 mg/kg was 10 and for 200 mg/kg was 10. It is obvious that the death percentages of these each tree doses were the same: this indicates that death percentages are not dose-dependent in these doses, whereas after 400 mg/kg, death rate increased according the dose administered to the mice. 1600 mg/kg was found as LD_{100} for morphine. The calculation of LD_{50} made by applying the method of Miller Tainter, taking LD_{100} as 1600 mg/kg, showed that LD_{50} for morphine is 502 mg/kg. If the LD_1 is taken as 200 mg/kg and LD_{100} 1600/kg the results showed that LD_{50} is 795 mg/kg. In our experiment, 795 mg/kg was accepted as LD_{50} of morphine, because between 40-200 mg/kg the death rate is not dose-dependent.

Table I/A : Morphine Sulphate LD_{50} values obtained in the first group.

Dose (mg/Kg)	Log. dose (x)	% Percentage of death (Y)	Probit	LD_{50} (mg/kg)
100	2.00	10	3.72	
200	2.30	20	4.16	
400	2.60	30	4.48	550
600	2.77	50	5	
800	2.90	90	6.28	
1000	3.00	100	+ ∞	

Table I/B : Morphine Sulphate LD_{50} values obtained in the first group.

Dose (mg/kg)	Log. dose (x)	% Percentage of death (Y)	Probit	LD_{50} (mg/kg)	
40	1.60	10	3.72		
100	2.00	10	3.72		
200	2.30	10	3.72		
400	2.60	20	4.16	795	502
800	2.90	40	4.75		
1200	3.07	60	4.25		
1600	3.20	100	+ ∞		

The Acute Influence of 48/80 On The LD_{50} Of Morphine

As indicated in table II, if morphine is administered with 10 mg/kg. of 48/80 which is not lethal, LD_{50} of morphine is diminished. In other words, the LD curve shifts to the left. The combination of morphine with the non-toxic dose shifts of 48/80 LD_{50} decreased from 795 mg/kg to 37 mg/kg. The shift to the left was even more when morphine doses were administered 30 minutes after the administration of 10 mg/kg of 48/80. Thus the LD_{50} became 22.5 mg/kg.

Table II. Morphine Sulphatte LD_{50} values 0, 30, 60, 120 minutes after compound 48/80 injection

Time lapse between 48/80 and morphine injection (min.)	Dose (mg/kg)	Log. dose (X)	% Percentage of death	Probit	LD_{50} (mg/kg)
0′	10	1	0	— ∞	37
	25	1.39	20	4.16	
	40	1.60	60	5.25	
	50	1.69	90	6.28	
	75	1.87	100	—	
30′	10	1	10	3.72	22.5
	20	1.30	40	4.75	
	30	1.47	60	5.25	
	40	1.60	80	5.84	
	50	1.69	100	+ ∞	
60′	25	1.39	10	3.72	64
	50	1.69	40	4.75	
	100	2.00	70	5.52	
	150	2.17	90	6.28	
	200	2.30	100	+ ∞	
120′	50	1.69	10	3.72	210
	100	2.00	30	4.48	
	250	2.39	60	5.25	
	300	2.47	80	5.84	
	400	2.60	100	+ ∞	

If the time between 48/80 and morphine is increased, the LD curve of morphine gradually shifts from the extreme left toward the LD curve estimated in control animals as illustrated in fig. 1.

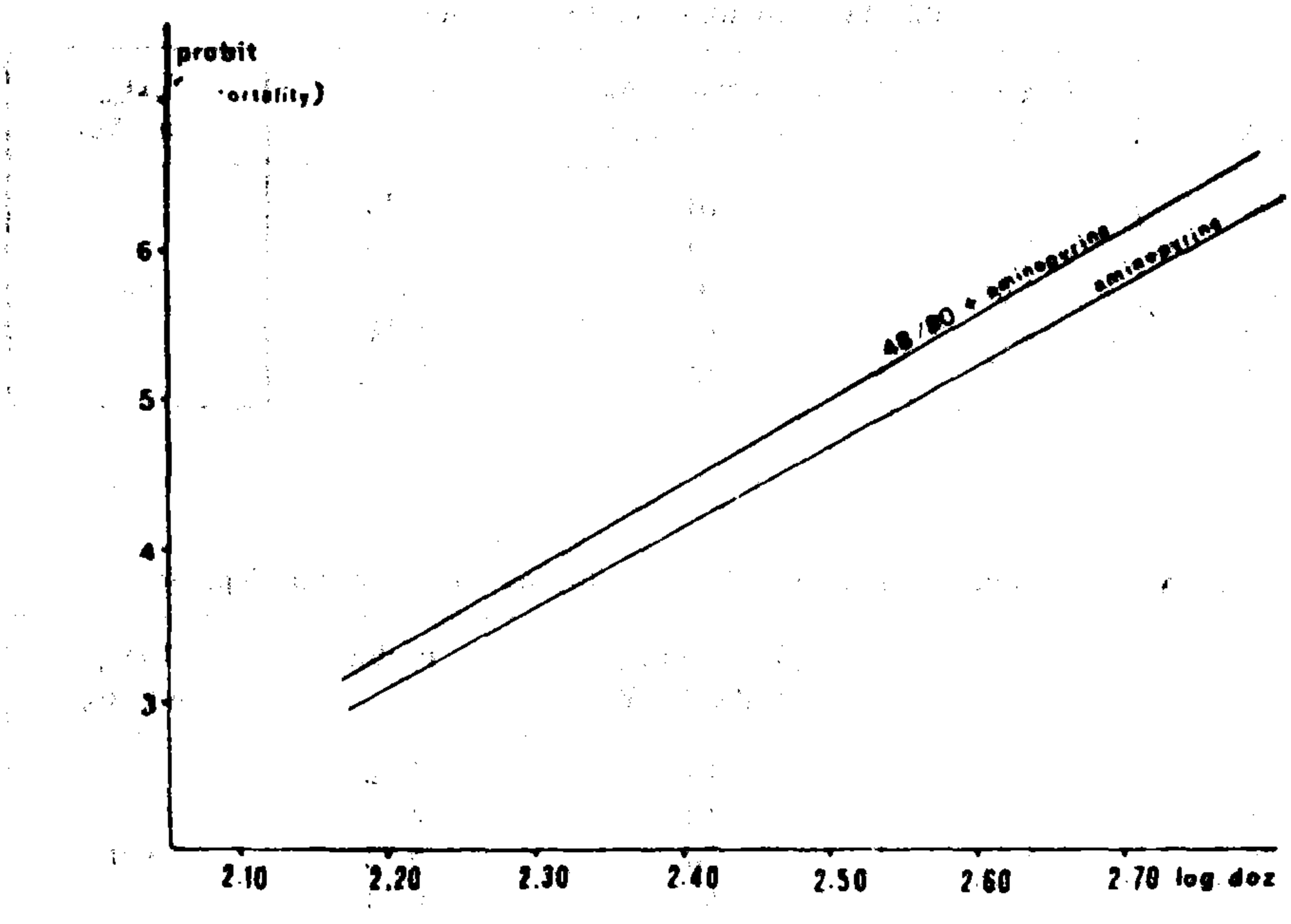

Figure 1.

LD_{50} of Morphine In Previously Mast Cell Depleted Animals

The mast cells of the mice were depleted prior to the toxicity test by injection of 10 mg/kg of compound 48/80 twice a day for three consecutive days. The depleted animals were subjected to the toxicity test 24 hours after the completion of mast cell depletion. The results of such experiments are tabulated in table III. In these groups of animals, LD_{50} of morphine was calculated as 880 mg/kg.

Table III. Morphine injection following chronic 48/80 depletion

Dose (mg/kg)	Log. dose (x)	% Percentage of death (Y)	Probit	LD_{50} (mg/kg)
525	2.72	0	— ∞	
700	2.84	20	4.16	
800	2.90	60	5.25	
1000	3.00	70	5.25	880
1200	3.07	100	+ ∞	

The difference between 790 and 880 mg/kg is significant statistically. The LD curve is also shifted to the right which can be seen in figure 1.

Table IV. Aminopyrine LD_{50} value

Dose (mg/kg)	Log. dose (x)	% Percentage of death (Y)	Probit	LD_{50} (mg/kg)
200	2.30	10	3.72	
300	2.47	30	4.48	
400	2.60	60	5.25	364
500	2.69	80	5.84	
600	2.77	100	+ ∞	

n = 5

Table V. The value aminopyrine LD_{50} following acute 48/80 depletion

Dose (mg/kg)	Log. dose (x)	% Percentage of death (Y)	Probit	LD_{50} (mg/kg)
200	2.30	10	3.72	
300	2.47	40	4.75	
400	2.60	70	5.52	330
500	2.69	90	6.28	
600	2.77	100	+ ∞	

n = 5

The control experiments were carried out with aminopyrine, which is not a histamin liberator, and its LD_{50} is fixed.

The first LD_{50} of aminopyrine was determined in our laboratory conditions. Its LD_{50} is 364 mg/kg. If is is administered together with 10 mg/kg of 48/80, the LD_{50} is found to be 330 mg/kg. The difference is not significant. The results of aminopyrine experiments can be seen in the table IV and V.

DISCUSSION

In literature, the LD_{50} values for morphine sulphate reported vary greatly from investigator to another in mice, it was determined as 200 mg/kg by Starkenstein (12); 200-400 mg/kg by Zunz (15); 500 mg/kg by Merck Index (13); and 600 mg/kg by Blozovski and Jacop[2]. In our experiments we also

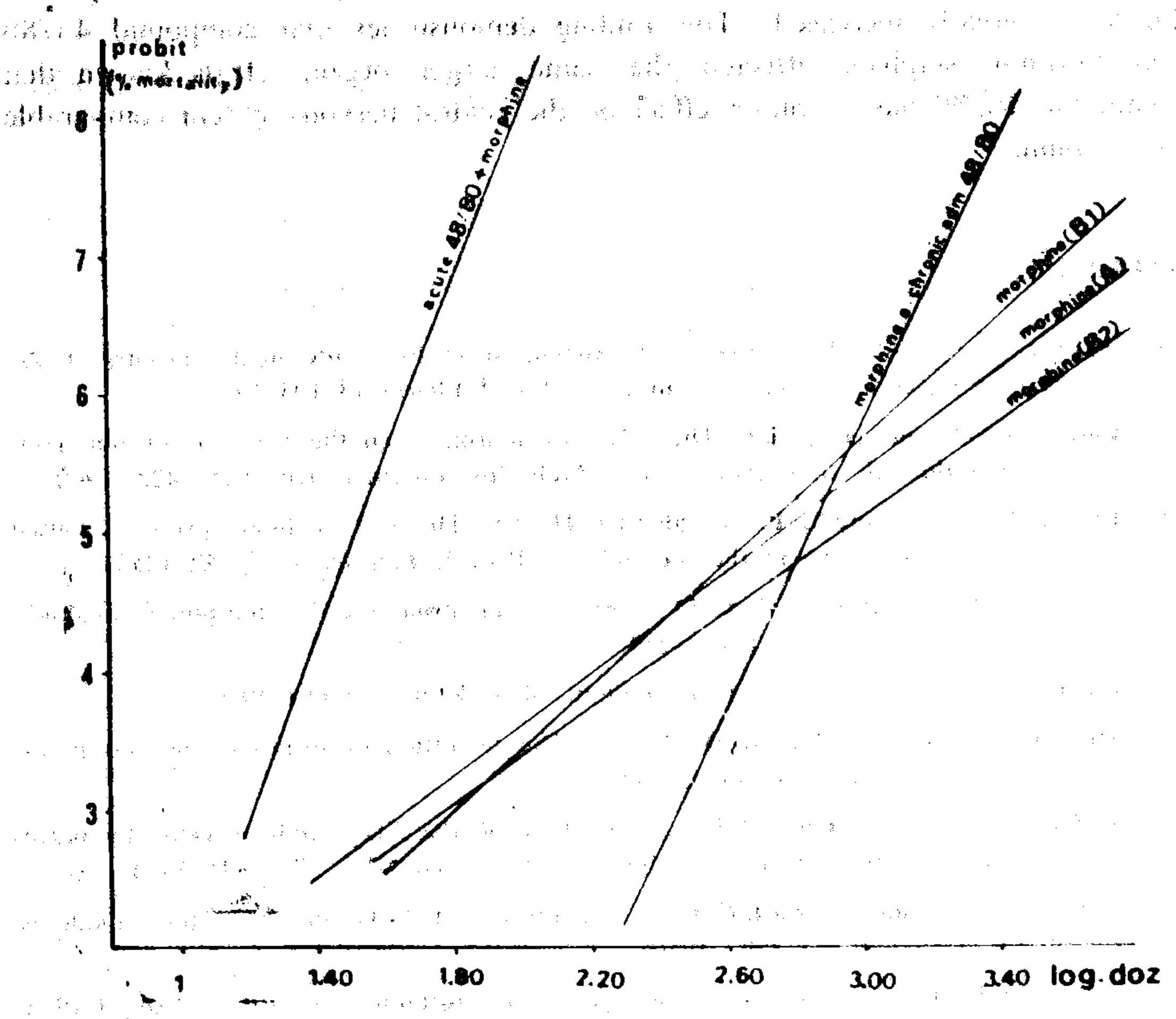

Figure 2.

found different results for the LD_{50} of morphine sulphate. A result of 550 mg/kg was obtained in breeding A, and values of 502 mg/kg and 795 mg/kg were found in breeding B, two rather different results.

As seen in Table I/B, the percentage of deaths at doses ranging from 40 to 200 mg/kg, remain at a constant value of 10 %. when a dose of 40 mg/kg is considered as the LD_1 value and that of 1600 mg/kg as the LD_{100} value, the LD_{50} value rises to 795 mg/kg. The fact that the percentage of deaths produced by doses ranging from 40 to 200 mg/kg is not dose-dependent, only becoming dose dependent when over 200 mg/kg, supports the hypothesis that the lethal dose of morphine sulphate in mice is caused by more than one factor. In order to find this out, a subtoxic dose of compound 48/80, a mast cell depletor and potent histamine-releasing substance, was administered with different doses of morphine sulphate. It was then observed that the LD_{50} value morphine

sulphate was lowered to a value as low as 35 mg/kg: that is, morphine's toxicity is greatly increased. This finding demonstrates that compound 48/80 and morphine sulphate affected the same target organe. It is known that compound 48/80 has no major effect on the central nervous system comparable to morphine.

REFERENCES

1. **Akcasu, A.:** The role of mast cell distruption in the acute manifestations of the intravenous injection of morphin in dogs. Eur. J. Pharm. 13, 103, 1970
2. **Blozovski, M. and jacop, j.:** The effect of morphine on the behavior of mice trained to run through an elevated maze Arch. Int. Pharmacodyn. 124 : 422, 1960
3. **Evans, A. G., Nasmyth, P. A., Stewart, H. C.:** The fall of blood pressure caused by intravenous morphine in the rat and cat. Brit. J. Pharmacol. 7, 542, 1952
4. **Feldberg, W. and Patton, W. D. M.:** Release of histamine by morphine alkaloids, J. Physiol. (London), 111, 19P, 1950
5. **Koch, R.:** On the toxicity of pyramidon. Med. Klin. 45 : 661, 1950
6. **Mc Intosh, F. C. and Patton, W. D. M.:** The liberation of histamine by certain organic bases, J. Physiol. (London) 109, 190, 1949
7. **Miller, L. C. and Tainter, M. L.:** Estimation of the ED_{50} and its error by means of log. probit graph. paper. Proc. Soc. Exp. Biol. Med. NY. 57 : 261, 1944
8. **Nasmyth, P. A. and Stewart, H. C.:** The release of histamine by opium alkaloids. J. Physiol. (London) 11, 19P, 1950
9. **Paton, W.D.M.:** Compound 48/80 : A potent histamine liberator. Brit. J. Pharmacol. 6, 499. 1951
10. **Rotschied, A. M.:** Mecanism of histamine release by compound 48/80. Brit. J. Pharmacol. 38, 253, 1970
11. **Schmidt C. F. and Livingston, A. E.:** The action of morphine on the mammalian circulation. J. Pharmacol. Exptl. Therap. 47, 416, 1933
12. **Starkenstein, E. :** Die papaveracenalkaloide. Handbuch der experimenttallen pharmacologie. Berlin verlag von julius Springer, 817, 1924.
13. **The Merck Index:** Merck Co. Inc. Rahway USA. 9. Ed. 1976
14. **Yamasak, H. and Endo, K. :** Co-existence of energy dependent and non dependent processes in histamin release mechanism of compound 48/80 and sinomenine : experiments on chopped skin from various animal species. Jap. J. Pharmac. 17, 228, 1967
15. **Zunz, E. :** Elements de pharmacodynamie speciale. Masson et cie. Edit. Paris, 169, 1932