

ACTION OF SOME AMIDES OF SUBSTITUTED ETHYLENE-DIAMINES ON CENTRAL NERVOUS SYSTEM

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(Received on March 11, 1986)

Summary : Five of the substituted ethylenediamine amides (LMG I to V) were tested for various CNS attributes and for acute toxicity (24 hr mortality). All compounds were potent analgesics in various animal tests, LMG V being most potent. All reduced spontaneous activity of mice and potentiated ether anaesthesia. However, CAR was not altered and anti-MES were not pronounced in rats. Compounds appear to have a wide safety margin considering ED₅₀ and LD₅₀ in mice.

Key words : ethylenediamine amides
ether narcosis

analgesic
Anti-MES

locomotor activity
CAR LD₅₀

INTRODUCTION

Actions of five substituted ethylenediamine-amides (LMG I to V) on blood pressure of dog, on skeletal and smooth muscles were reported earlier (4). Present paper describes some of their actions on central nervous system, alongwith their acute toxicity in mice.

MATERIAL AND METHODS

Male or female albino mice (18-25 g) and rats (150-200 g) were used in groups of 5. All drugs were dissolved in distilled water prior to use.

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(A) Analgesic activity :

Acetic acid-induced writhing : Acetic acid (300 mg/kg; a 3% solution, ip) was used to induce writhings (6), 20 min after administration of the test compounds (5 to 100 mg/kg, ip) or acetylsalicylic acid (5 to 60 mg/kg) or vehicle (control). ED₅₀ values or protection from writhing were calculated according to Miller and Tainter (3).

Rat tail flick method : Time to the flick of the tail placed on hot wire was recorded in control rats and in rats treated with LMG (30 to 150 mg/kg, ip) at 20 min and at various times upto 75 min. 'Analgesic index' (the square root of the ratio of the average reaction time after and before drug injection) was calculated. Potency rating was compared from dose required to produce analgesic index of 1.4.

Pododolarimetry : The metal grid floor of the cage delivered shocks of known voltage to a mouse. The 'critical voltage' that made the animal either to emit a cry or jump was recorded before (control values) and after the administration of LMG I to V (10 to 100 mg/kg, ip) given 20 min before, and at 10 min interval upto 90 min. Potency rating was calculated from doses causing 11 to 12 V rise in the critical voltage. Saline treated mice (control) showed only a maximum rise of 2 ± 0.32 V (n=5).

(B) Other central effects :

Spontaneous locomotor activity : 'Techno 6-beam photoactometer' was used to record the spontaneous locomotor activity (10 min-record) before and 1, 2 and 4 hr after the administration of the test compounds (5 to 100 mg/kg, ip) or methaqualon (40 mg/kg, ip).

Ether-induced narcosis : Mice were kept under the jars containing a cotton swab soaked with 10 ml ether. Time to the loss of righting reflex (onset time) and regaining of the righting reflex (duration) was recorded in animals treated with vehicle or test drugs (25 mg/kg, ip) 20 min before.

MES test : Prevention of hind limb extension was considered as an evidence for anticonvulsant activity in MES test (5). LMG I to V (5 to 50 mg/kg, ip) or diphenylhydantoin sodium (2.5 to 15 mg/kg, ip) were given 30 min before the test compounds. ED₅₀ values for anticonvulsant activity were calculated by the method of Miller and Tainter (3).

Condition avoidance response (CAR) : Albino rats trained to climb the pole of Cook's pole climbing apparatus on auditory signal within 30 sec were used. CAR was observed after giving LMG I to V (100 mg/kg, ip) or chlorpromazine (8 mg/kg, ip) at 30, 60 and 120 min.

(C) Acute toxicity :

LD₅₀ estimation : Mice were given graded doses of LMG I to V (ip) and observed for 24 hr for mortality for computing LD₅₀.

RESULTS AND DISCUSSION

Analgesic activity (Table I) :

All these 5 test compounds (LMG I to V) exhibited dose related analgesic activity when tested by writhing method, rat tail flick method or pododolarimetry, compound LMG V being the most potent. In writhing test, LMG V, I and IV were almost equipotent to acetylsalicylic acid. By the rat tail flick method, analgesic indices obtained at 30 mg/kg, were 1.39 (LMG V), followed by 1.16 (LMG I and IV) and 1.0 (LMG II and III). LMG I (150 mg/kg, ip) LMG II (100 mg/kg, ip) LMG III (100 mg/kg, ip), LMG IV (100 mg/kg, ip) and LMG V (150 mg/kg, ip) exhibited maximum analgesic index of 1.75, 1.40, 1.41, 1.80 and 1.88, respectively. In pododolarimetry, the analgesic effect was obvious in 10 to 20 min, was at the peak at 30 to 40 min and subsided by 90 min, as judged from 11-12 V rise in critical voltage. Table I shows the potency of the test compounds obtained in this test from a 10 to 11 V rise in critical (threshold) voltage; LMG I (100 mg/kg) and LMG V (30 mg/kg) produced even a higher rise in critical threshold voltage (19 V at 20 min and 15 V at 30 min, respectively).

Central nervous system depression :

Spontaneous locomotor activity : Calculated on the basis of mean % reduction in 4 hr period (mean values at 1, 2 and 4 hr), Table I shows the doses of LMG's I to V producing 80% reduction in mean motor activity, comparable to a single dose of methaqualon (40 mg/kg, ip). Higher doses of LMG II, III and IV (30 mg/kg, ip) and V (20 mg/kg, ip) showed maximum % reduction of 91.6, 94.47, 98.70 and 96.33 respectively. The results show that the test compounds were highly potent in reducing locomotor activity.

Ether-induced narcosis : In control (saline treated) group of mice, onset time of 6.2 ± 1.07 min and the duration of narcosis was 42.5 ± 2.5 min (n=5): LMG I, II, III, IV and V (25 mg/kg, ip) reduced the onset time by order of 6.25 ± 1.25 , 9.99 ± 2.7 ,

8.2±0.58, 2.2±1.53 and 5.8±1.12 % respectively and increase duration of narcosis by order of 52.4±3.5, 39.3±1.98, 26.72±1.32, 12.80±0.39 and 12.80±0.95 % respectively (n=5) over the control values.

MES test : LMG I to V exhibited dose graded anticonvulsant activity. ED₅₀ (mg) values of diphenylhydantoin sodium was 5.31±2.95 and ED₅₀ values for LMG I, II, III,

IV and V were 12.2±7.25, 13.65±2.95, 23.01±2.71, 71.88±4.79 and 12.09±5.68, respectively.

Condition avoidance response (CAR) : None of the test compounds showed any effect on CAR in rats.

TABLE 1 : Ethylenediamine effect on pain and on locomotor activity.

Compounds	Acetic acid-induced writhing in mice; ED ₅₀	Rat hot wire analgesiometry, Dose (mg/kg) leading to analgesic index of 1.4	Pododolarmetry in mice. Dose (mg/kg) leading to 10-11 V rise in threshold	Locomotor activity in mice. Dose (mg/kg) leading to 80% reduction in 4 hr motility
N-(β-diethyl amino ethyl) N-p-methylphenyl Benzamide. (LMG I)	14.57	75	50	100
α-dimethyl amino-N-(β-diethyl amino ethyl) acetamide. (LMG II)	26.96	100	30	20
α-dimethyl amino-N-(β-diethyl amino ethyl) -N-α-naphthyl acetamide. (LMG III)	19.95	100	60	20
α-dimethyl amino-N-(β-diethyl amino ethyl) N-β-naphthyl acetamide. (LMG IV)	16.79	50	60	20
α-Morpholino-N-(β-diethyl amino ethyl) -N-α-naphthyl acetamide. (LMG V)	13.89	30	10	10
Acetylsalicylic acid	12.59	—	—	—
Methaqualon	—	—	—	40

All compounds were injected ip, 20-30 min before testing.

Acute toxicity :

LD₅₀ estimation : LD₅₀ values (mean \pm S.D., mg/kg, ip) were found to be 162.9 \pm 21.80, 94.19 \pm 24.98, 131.8 \pm 27.19, 112.2 \pm 19.72 and 173.8 \pm 54.12 respectively. This indicates that the test compounds had a wide safety margin.

In the present study five ethylenediamines showed significant analgesic activity. LMG IV and V are almost equipotent to acetylsalicylic acid and remaining are less potent. By three different tests LMG V has been found most potent. Non narcotic analgesics namely letimide HCl, proxazole citrate, and a narcotic analgesic, methadone show a remarkable structural similarity with LMG's I to V (test compounds) (1, 2). In addition to a distinct analgesic activity, the test compounds potentiated ether-induced narcosis (an evidence for a distinct CNS depressant effect), showed a remarkable; reduction of locomotor activity (probably a curaremimetic action; 4) and a weak anti-MES activity. None of the LMG compounds affected CAR, which was taken to mean that these were devoid of any 'tranquillizer activity', though they exhibited sedative activity.

Considering the LD₅₀ values and ED₅₀ values reported above and from the observations that relatively small doses reduced locomotor activity. LMG compounds appear to have a favourable therapeutic index for certain pharmacological attributes and are worthy for further studies.

ACKNOWLEDGEMENTS

The authors wish to thank Dr. J. J. Trivedi, Principal, M.G. Science College, Navarangpura, Ahmedabad, for the supply of LMG I to V. Generous gift of methaqualon by Mr. K.C. Jain, Roussel Pharmaceuticals (India) Bombay, is highly appreciated.

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