Preliminary observations regarding the cataleptogenic activity of ethosuximide in the mouse

J. H. JADHAV*, J. J. BALSARA, A. G. CHANDORKAR, *Department of Pharmacology, V. M. Medical College, Solapur, India*

Ethosuximide has been used for many years in the treatment of petit mal epilepsy (Vossen 1958; Zimmerman & Burgemeister 1958). Goldensohn et al (1962) have reported the occurrence of parkinsonian symptoms in association with its use. As a lack of dopamine at striatal dopamine receptor sites can result in parkinsonian symptoms in man and catalepsy in the animals (Marsden 1975) we have investigated whether ethosuximide induces catalepsy in mice and the mechanism involved therein.

Male albino mice, 20–30 g, with free access to a standard diet and tap water were used once only. All observations were made between 10 and 16 h at 27–30 °C in a noiseless, diffusely illuminated room.

Catalepsy was scored according to Ahtee & Buncombe (1974). Mice were individually placed in Perspex cages 30 min before drug treatment to allow adaptation to the new environment. They were tested for the presence of catalepsy by placing both front paws on a 4 cm high wooden block. Those animals maintaining the cataleptic posture from 0 to 10 s scored 0, 10 to 30 s = 1, 30 s to 1 min = 2, 1 to 2 min = 3, 2 min to ∞ = 4. Mice were tested for catalepsy 0·5, 1·0, 2·0, 3·0, and 4·0 h after ethosuximide treatment. Further, the influence of apomorphine and atropine on ethosuximide-induced catalepsy was investigated.

The effect of ethosuximide pretreatment on apomorphine-induced stereotyped cage-climbing behaviour was also studied by the method of Costall et al (1978). Mice exhibited climbing behaviour following apomorphine (0·5–1·5 mg kg^{-1} s.c.). To quantify this behaviour, mice were placed in individual Perspex cages, 27 × 20 × 15 cm, lined with 1 cm^2 wire mesh, made of wire 2 mm in diameter. 30 min were allowed for acclimatization. Mice were individually tested for climbing behaviour taking 'the percentage of time spent climbing during the 30 min after the first climb' as the 'climbing index' (see Costall et al 1978). Further, the maximum time (in min) spent in a single climb throughout the duration of the apomorphine effect was also determined.

Ethosuximide (Parke-Davis) and atropine sulphate (BDH) were dissolved in distilled water, while apomorphine hydrochloride (Burroughs Wellcome) was dissolved in distilled water containing 0·2 mg ml^{-1} ascorbic acid. All agents were prepared immediately before use and were administered in a volume of 10 ml kg^{-1} weight. Except for ethosuximide doses refer to the salt. For each dose 10 animals were used. Atropine was injected 30 min before and apomorphine 60 min after ethosuximide treatment. Control groups received vehicles (10 ml kg^{-1}) by the same routes. Statistical differences were analysed by Student's *t*-test.

Ethosuximide at 12·5–50 mg kg^{-1}, i.p., induced no catalepsy while higher doses (100–400 mg kg^{-1}) induced a state of sedation and dose-dependent degree of catalepsy, without loss of righting reflex or apparent change in muscle tone. The cataleptic effect was present at 30 min and reached maximum at 1 h (Fig. 1). Doses beyond 400 mg kg^{-1} tended to produce motor incoordination and ataxia.

Apomorphine (1, 2, 4 mg kg^{-1}, s.c.) antagonized ethosuximide-induced catalepsy in a dose-dependent manner (Fig. 2). The anticataleptic effect of apomorphine was short-lasting (20–40 min) and well correlated in time with the locomotor stimulation, sniffing and gnawing seen in the groups receiving apomorphine only.

Atropine (20 mg kg^{-1}, i.p.) did not produce any detectable changes in the gross behaviour of the animals nor did it induce catalepsy. Pretreatment with atropine (20 mg kg^{-1}) was found to reduce significantly ($P < 0.05$ or less) the cataleptic effect induced by 200–400 mg kg^{-1} dose of ethosuximide, at all the testing time intervals, while the cataleptic effect induced by 100 mg kg^{-1} dose of ethosuximide was completely antagonized (Fig. 3).

Apomorphine caused dose-dependent climbing behaviour in doses of 0·5–1·5 mg kg^{-1}. A dose of 1·0 mg kg^{-1} was selected as a submaximal dose for subsequent

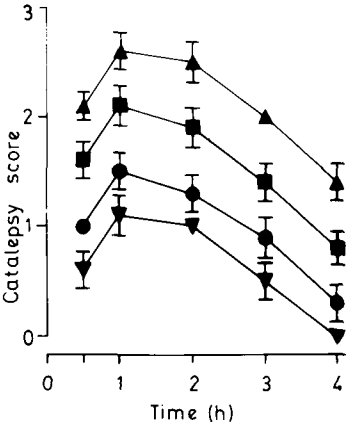

FIG. 1. Dose-dependency of the cataleptic effect induced by 100 (▼——▼), 200 (●——●), 300 (■——■) and 400 (▲——▲) mg kg^{-1} i.p. ethosuximide in the mouse. Each value represents the mean score of 10 mice. Vertical bars represent s.e. Times given are counted from the injection of ethosuximide.

* Correspondence.

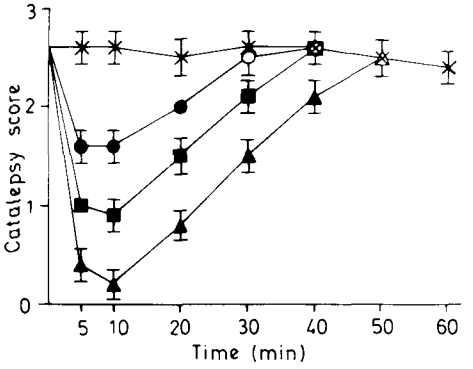

FIG. 2. Effect of apomorphine treatment at the doses of 1·0 (●——●), 2·0 (■——■) or 4·0 (▲——▲) mg kg^{-1} on ethosuximide (×——×) induced catalepsy in mice. Ethosuximide was injected at the dose of 400 mg kg^{-1}. Each value represents the mean score of 10 mice. Vertical bars represent s.e. Solid symbols indicate statistical significance ($P < 0.05$ or less). Times given are counted from the injection of apomorphine.

studies. Pretreatment with ethosuximide (25, 50, 100 mg kg^{-1}) significantly antagonized apomorphine-induced climbing behaviour in a dose-dependent manner (Table 1).

The present behavioural studies show that ethosuximide, in addition to inducing catalepsy in mice, also antagonizes apomorphine-induced cage climbing behaviour which is reported to occur as a result of direct stimulation of post-synaptic striatal dopamine receptors

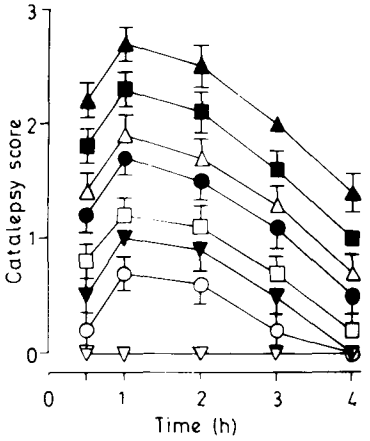

FIG. 3. Effect of atropine (20 mg kg^{-1}, i.p.) pretreatment on ethosuximide-induced catalepsy in mice. ▼——▼ ethosuximide 100 mg kg^{-1} i.p., ▽——▽ atropine + ethosuximide 100 mg kg^{-1} i.p., ●——● ethosuximide 200 mg kg^{-1}, ○——○ atropine + ethosuximide 200 mg kg^{-1}, ■——■ ethosuximide 300 mg kg^{-1}, □——□ atropine + ethosuximide 300 mg kg^{-1}, ▲——▲ ethosuximide 400 mg kg^{-1}, △——△ atropine + ethosuximide 400 mg kg^{-1}. Each value represents the mean score of 10 mice. Vertical bars represent s.e. Times given are counted from the injection of ethosuximide.

Table 1. Effect of pretreatment with ethosuximide on apomorphine-induced climbing behaviour. Ethosuximide was given i.p. 60 min before apomorphine (1 mg kg^{-1} s.c.). The climbing index represents the percentage of time spent climbing during the 30 min following the first climb. The second measure of climbing behaviour represents the maximum time spent in a single climb throughout the duration of the apomorphine effect. Both the climbing index and the maximum time are expressed as the mean ± s.e.m. 10 mice were used at each dose of drug. Animals with dose designated 0 received vehicle 60 min before apomorphine.

Drug	Dose mg kg^{-1}, i.p.	Climbing index (%)	Max. time (min)
Ethosuximide	0·0	72·7 ± 2·2	12·1 ± 0·8
	12·5	71·4 ± 2·5	11·8 ± 0·7
	25·0	42·6 ± 4·8*	5·7 ± 1·1*
	50·0	10·5 ± 2·4**	1·4 ± 1·0**
	100·0	0·0	0·0

* Differs from vehicle treated, $P < 0.01$.
** Differs from vehicle treated, $P < 0.001$.

by apomorphine and it is effectively antagonized by dopamine-receptor blocking drugs like haloperidol (Protais et al 1976; Baldessarini et al 1977; Costall et al 1978). Our finding that ethosuximide effectively antagonizes the climbing behaviour suggests that it possesses post-synaptic striatal dopamine-receptor blocking activity and this action may also be responsible for its cataleptogenic effect. Further apomorphine also antagonized ethosuximide-induced catalepsy thereby supporting our contention that the cataleptogenic effect of ethosuximide is most probably due to blockade of post-synaptic striatal dopamine-receptors by ethosuximide. The antimuscarinic agent atropine, which counteracts neuroleptic-induced parkinsonism and dystonias in man as well as the cataleptic effect of these drugs in rodents (Friedman & Everett 1964; Ezrin-Waters et al 1976) was also found to counteract the cataleptic effect of ethosuximide. Recently, Westerink et al (1977) have provided biochemical evidence for a functional blockade of striatal and mesolimbic dopamine receptors by ethosuximide in the rat brain. Our finding that ethosuximide induces catalepsy in mice, is in agreement with the clinical observation of Goldensohn et al (1962) regarding the occurrence of parkinsonian symptoms in association with ethosuximide therapy.

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The role of monoamine oxidase in catecholamine-stimulated prostaglandin biosynthesis of rat brain homogenates

MÁRTA KOMLÓS, ANDRÁS SEREGI*, ANDRÁS SCHAEFER, *Institute of Experimental Medicine, Hungarian Academy of Sciences, H-1450 Budapest 9. P.O. Box 67, Hungary*

Brain tissue is known to synthesize only small amounts of prostaglandin (PG) from exogenous precursors while it forms a considerable amount of $PGF_{2\alpha}$ and PGE_2 from an endogenous pool of arachidonic acid (Coceani & Wolfe 1965; Wolfe et al 1976 a,b). Endogenous PG-biosynthesis taking place during the incubation of cortex slices, brain homogenates and synaptosomes could be enhanced by catecholamines, 5-HT and tryptamine (Hillier et al 1976; Leslie 1976; Schaefer et al 1978). Our earlier investigations have shown that the stimulating effect of catecholamines and indolalkylamines on PG-biosynthesis in rat brain homogenates can be blocked by inhibitors of monoamine oxidase (MAO) (Schaefer et al 1978). On the other hand, PG-biosynthesis could be activated only by those catecholamines and indolalkylamines that were substrates of MAO. Thus, α-methyl- and α-carboxyl-derivatives, as well as isoprenaline, proved to be ineffective. It was suggested that some relationship might exist between the stimulation of PG-biosynthesis by catecholamines or indolalkylamines and the function of MAO. We have examined the effect of in vivo pretreatment with MAO inhibitors on the catecholamine-induced stimulation of endogenous PG-biosynthesis in rat brain homogenates.

CFY rats of either sex, 150–250 g, were treated intraperitoneally with various MAO-blocking agents or with 0·9% NaCl (saline). At a definite time after treatment the brains were rapidly removed and homogenized in three volumes of 0·1 M Tris-HCl buffer, pH 7·4. The soluble fraction was removed by centrifugation at 100 000 g for 1 h. The pellet was resuspended in the original volume of Tris buffer. This total particulate suspension obtained from the brain homogenate was tested for noradrenaline (NA)-stimulated PG-biosynthesis and for oxidative deamination of NA.

To measure the PG-biosynthesis, 1·4 ml of the particulate suspension was incubated at 37 °C for 20 min under constant shaking with or without 5×10^{-4} M NA. The final volume of the incubation mixture was 2 ml. The reaction was stopped by the addition of 6 ml ice cold isopropanol. PG-s were extracted, separated and measured by bioassay on rat stomach strips as described by Schaefer et al (1978). In preparations obtained from control animals, PGF-like activity measured against $PGF_{2\alpha}$ varied between 50–90, while PGE-like activity in terms of PGE_2 varied between 8–15 ng/100 mg brain tissue after incubation in the presence of 5×10^{-4} M NA. We have recently confirmed this value for $PGF_{2\alpha}$ by radioimmunoassay measurements. The changes in the total PG-like activity were determined.

For the determination of MAO activity, 200 μl of the incubation mixture contained 140 μl of particular suspension, 5×10^{-4} M NA and 0·1 μCi ^{3}H-NA ($8{\cdot}5 \times 10^{-8}$ M). Incubation was at 37 °C for 20 min and was stopped by the addition of 200 μl 0·02 M HCl saturated with NaCl. The metabolites were extracted from the acidified reaction mixture with 2 × 1 ml ethyl acetate by the method of Leeper et al (1958) and the organic phase was measured by liquid scintillation. Specific activity for MAO was: 10·8–13·5 nmol amine deaminated/100 mg brain tissue per 20 min.

Table 1. Effect of in vivo pretreatment with MAO inhibitors on oxidative deamination of NA, and on NA-stimulated PG-formation in rat brain homogenates. MAO inhibitors were given i.p. 1 h before decapitation, Concentration of NA: 5×10^{-4} M in both mixtures. Inhibitory effect of the drugs was determined at five different concentrations. For each concentrations 9 animals were used, ID50 values were determined graphically,

	ID50 values (mg/kg^{-1})	
Compounds	MAO-activity	PG-formation
Tranylcypromine	0·55	0·74
Clorgyline	0·77	2·3
Pargyline	13·9	24·0
Deprenyl	19·3	36·1

* Correspondence.