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Comparison of sleeping time, immobility time and arousal time in mice

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The sodium salts of the five barbiturate hypnotics hexobarbitone, pentobarbitone amylobarbitone, secobarbitone and phenobarbitone, were studied in mice for their effect on duration of sleeping time, immobility time and arousal time. The latter measurement is described herein for the first time and is a measure of the duration of sleep with a moderate shocking stimulus to differentiate sedation and hypnosis in the mouse. The comparative potency of the drugs by these measurements is evaluated.

A standard method of measuring the depressant action of barbiturates in small animals is by the determination of what is usually, if unphysiologically called "sleeping time". This may be defined as the time from which the animal cannot right itself to that time when a righting reflex has returned. With a drug such as ethanol, this is not a useful procedure because the end-point is indistinct. The animals exhibit excitatory movements such as twitching and rolling with alternate appearance and disappearance of the righting reflex (Forney, Hulpieu & Hughes, 1962).

The present report consists of a comparison of "sleeping time," "immobility time" and "arousal time" after treatment with barbiturates. We have described immobility time as the interval between the loss of righting reflex and the time when an animal regains normal exploratory movements (Forney, Hulpieu & Hughes, 1962).

Sleeping time, in mice, comprises central nervous depression ranging from anaesthesia to hypnosis with a lingering sedative effect. An electrical stimulus might hasten the arousal of an animal from drug-induced sleep if it were strong enough to antagonise sedation. If such a current were applied continuously, the true hypnotic action of the drug might then be measured in time. Arousal time would be determined similarly to sleeping time except that the animals would be subjected to a continuous stimulating electric current. The technique for the measurement of this arousal time is described herein for the first time.

Experimental

Male Swiss albino mice, 20–30 g, were used. The five representative barbiturates selected have actions varying in duration from very short, intermediate to long; they were hexobarbitone sodium, 100 mg/kg; pentobarbitone sodium, 45 mg/kg; secobarbitone sodium, 60 mg/kg; amylobarbitone sodium, 100 mg/kg and phenobarbitone sodium, 175 mg/kg. Preliminary experiments with multiple dosages showed that, at the dosages selected, all mice slept, even though shocking stimuli were

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applied. The drugs were all administered intraperitoneally in aqueous solutions. The concentrations of the solutions were prepared so that each animal received 0·5 ml/20 g weight. Sleeping time was calculated as the time from the loss of the righting reflex to the return of the righting reflex (Brodie, Shore, & Silver, 1955; Kopmann & Hughes, 1958). Immobility time was calculated from the time of non-movement after injection to the time when the mouse was able to move beyond the border of a 6-inch diameter circle (Forney & others, 1962). The third measurement was "arousal time." For this, the "sleeping" mice were placed on a gridded platform on absorbent paper saturated with 0·85% saline. The absorbent papers were kept moistened throughout the experiment to insure maximum conduction. An electric current, supplied by a variable transformer having an input of 125V at 60 cycles and an output of 10V rated at 1·25 A, was introduced into the grid system. The arousal time was calculated, like sleeping time, as time elapsing from the loss of righting reflex to its return. Preliminary testing indicated that the stimulus provided by this circuit was the optimum necessary to arouse a mouse from a state of light sleep or near wakefulness.

Results and discussion

Table 1 gives the comparative effects of the five barbiturates on the sleeping, arousal and immobility times of the mice. The order of increasing duration of sleeping time was hexobarbitone, pentobarbitone, amylobarbitone, secobarbitone and phenobarbitone. These mean durations were reduced with a low-voltage current after all drugs. The greatest decrease in duration of sleep brought about by electrical stimulus was noted after pentobarbitone>hexobarbitone>amylobarbitone>phenobarbitone>secobarbitone. This could be a measurement of depth of sleep in mice, an increase in depth causing a decrease in the percentage reduction of sleeping time brought about by continuous stimulation. Arousal time therefore may be a measure of the true duration of hypnosis in animals.

TABLE 1. MEASUREMENT OF THE DEPRESSANT ACTION OF BARBITURATES IN MICE AS AROUSAL TIME, SLEEPING TIME, AND IMMOBILITY TIME*

Drug	Dose mg/kg	Arousal time (min)	Sleeping time (min)	Immobility time (min)
Hexobarbitone sodium ..	100	22 ± 1·8	30 ± 3·9	57 ± 2·1
Pentobarbitone sodium ..	45	34 ± 2·2	57 ± 5·7	75 ± 4·7
Amylobarbitone sodium ..	100	54 ± 5·6	66 ± 11·9	71 ± 12·2
Secobarbitone sodium ..	60	92 ± 7·6	98 ± 13·7	101 ± 13·7
Phenobarbitone sodium ..	175	135 ± 16·3	149 ± 21·2	173 ± 20·5

* Mean values of 10 mice and s.e. of mean (drugs administered i.p.).

A post-hypnotic sedative action of the drugs extends the immobility time beyond the arousal time. The order of duration of immobility time (prolonged sedation) was phenobarbitone>secobarbitone>pentobarbitone>amylobarbitone>hexobarbitone. The duration of arousal

and sleeping time after amylobarbitone was greater than that of pentobarbitone. However, as measured by immobility time, pentobarbitone had the longer duration of action (more sedative?).

If the arousal time (true hypnotic drug action) is subtracted from the immobility time, the order of post-hypnotic depression is obtained. This was found to be pentobarbitone>phenobarbitone>hexobarbitone>amylobarbitone>secobarbitone.

The techniques described should be useful in the evaluation of the hypnotic and post-hypnotic properties of barbiturates and other depressants such as ethanol. Additionally, their potentiation by drugs of the same class or of different classes, e.g., tranquillising drugs, could be quantified.

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