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THE EFFECT OF PICROTOXIN ON MOTOR ACTIVITY AND THE ELECTROENCEPHALOGRAM OF MICE

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Picrotoxin and strychnine have been used to elucidate the mechanism of action of inhibitory and excitatory transmitters at vertebrate and invertebrate central synapses. While both drugs are effective in antagonizing the action of the inhibitory transmitter at invertebrate synapses (Florey, 1954, 1957; McGeer, McGeer & McLennan, 1961), they are known to have different sites of action within the vertebrate central nervous system; strychnine reduces postsynaptic inhibition (Eccles, Schmidt & Willis, 1963; Desmedt & Delwaide, 1963) whilst picrotoxin has recently been shown to antagonize presynaptic inhibition of cat spinal motoneurones (Eccles *et al.*, 1963). Before using subconvulsive doses of these drugs to elicit release of noncholinergic substances from the cerebral cortex of cats (Ramwell & Shaw, 1963a) it was considered necessary to re-investigate the physicochemical and pharmacological properties of picrotoxin, for although picrotoxin consists of a loose combination of two nearly identical molecules (picrotoxinin and picrotin) most of the analeptic activity is associated with picrotoxinin (Ramwell & Shaw, 1963b). Subconvulsive doses of picrotoxin and picrotoxinin in mice were found to produce an unusual effect which appears to be unique for analeptic drugs, and is described here.

METHODS

Animal behaviour. Activity of isolated mice was assessed by selecting groups of male albino mice, weighing 25 ± 1g; one mouse within each group served as a control. All drugs were injected into the lateral tail vein in 0.1 ml. of 0.9% saline. The mice were then placed in separate compartments, carefully screened from distraction, and their activity was observed every 15 sec for 10 min; thus for each mouse a total of forty observations was recorded. The animals normally assumed one of four positions, namely preening rearing, running or stationary. The effect of picrotoxin (1.0 mg/kg), dexamphetamine, (−)-amphetamine, methylamphetamine, bemigride (0.4 to 4.0 mg/kg), leptazol, nikethamide (0.4 to 4.0 mg/kg) and strychnine (0.4 mg/kg) was determined on the behaviour of conscious mice.

For characterization of the flaccid posture detected after injection of picrotoxin, groups of six to seven mice were used once only. To reduce variance, mice were transferred to the laboratory at least 24 hr before use, and the animals were kept in groups of two to six per cage (Macintosh, 1962). Picrotoxin (0.4 to 2.0 mg/kg) was injected in a volume of 0.1 ml. of 0.9% saline, the mouse was placed in a confined space, and motor activity was observed. The flaccid posture time was recorded as the time elapsing between injection of the analeptic and first appearance of the flaccid posture. With convulsive doses of picrotoxin (4 to 8 mg/kg) both flaccid posture and convulsion times could be recorded.

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Blood pressure. Mice (weighing 30 g) were lightly anaesthetized with 0.25 ml. of a 15% urethane solution, intraperitoneally. The trachea was cannulated, and a fine polyethylene catheter was inserted into the left carotid artery. Heparin (50 U) was injected through a hypodermic needle in the lateral tail vein. The needle was left *in situ* for subsequent saline and drug injections, which were administered in a volume of 0.1 ml. from a micrometer syringe over a period of 20 sec. Blood pressure was recorded using an inductance transducer and an ultraviolet photokymograph.

Electroencephalogram. Mice (weighing 25 g) were anaesthetized with 150 mg/kg of hexobarbitone sodium, intraperitoneally. A fold of skin was removed from the head, the tissues were scraped away to expose the skull, and three indentations ranging from the occipital to the frontal region were made on one side using a dental drill and fine burr. The skull was not penetrated, for this was found to result in masking of the electroencephalogram by the electrocardiogram and respiratory artefacts. A little electrode jelly was placed in each of the indentations, followed by the silver-plated end of a No. 40-gauge enamel-covered copper-wire recording lead; this was held in position by Simplex acrylic cement. The three recording leads were then taken to the back of the head and, after a skull cap of cement had been formed, the leads were connected to an Ediswan pen recorder. To ensure that the recording leads were secure and correctly placed, the electroencephalogram was recorded during recovery from anaesthesia. No chronic preparations were made; mice were prepared immediately before use, after which they were allowed to move freely within a confined space. All injections were given in a volume of 0.1 ml. while the mouse was manually restrained.

RESULTS

The activity of mice treated with picrotoxin and other analeptics. The most characteristic feature of the behaviour of mice after treatment with picrotoxin (1 mg/kg) was a considerable increase in the number of stationary positions assumed by the animals in the 10 min after injection, compared with the behaviour of mice given saline only. When other analeptic drugs were tested similarly to determine whether this response was unique to picrotoxin, only two compounds, (−)-amphetamine (0.4 to 4.0 mg/kg) and bemigride (1 to 4 mg/kg), considerably increased the number of stationary positions observed. The closely related drugs, dexamphetamine and methylamphetamine (0.4 to 4.0 mg/kg), as well as leptazol, nikethamide (0.4 to 4 mg/kg) and strychnine (0.4 mg/kg), either had little effect or decreased the number of stationary positions when compared with the controls (Table 1).

TABLE 1

INCIDENCE OF STATIONARY POSTURES IN MICE TREATED WITH ANALEPTIC DRUGS

Mice were injected in the tail vein with the compound dissolved in 0·1 ml. of saline, and were observed every 15 sec for 10 min. Figures refer to the mean number of stationary postures recorded during this period; those in brackets refer to the number of mice observed

Compound	Dose (mg/kg)	Stationary postures
Picrotoxin	1·0	35 (6)
Bemigride	4·0	30 (10)
	1·0	18 (9)
	0·4	6 (6)
(−)-Amphetamine	4·0	30 (13)
	1·0	22 (6)
	0·4	15 (12)
Dexamphetamine	4·0	13 (12)
	1·0	7 (11)
	0·4	4 (6)
Methylamphetamine	4·0	6 (10)
	1·0	4 (8)
	0·4	1 (8)
Saline	—	7 (15)

On close observation a difference was detected between the stationary positions assumed by mice treated with picrotoxin and those of mice treated with (−)-amphetamine or bemigride; the former mice adopted a well-defined flaccid posture described later. Dexamphetamine and racemic amphetamine have previously been reported to decrease the voluntary activity of rats (Randrup, Munkvad & Udsen, 1963) and monkeys (Davis, 1957) and in consequence their effect on motor activity was not studied. However, the flaccid posture in mice after subconvulsive doses of picrotoxin was investigated further.

Effect of subconvulsive doses of picrotoxin. When mice were injected intravenously with 0.4 to 2.0 mg/kg of picrotoxin, there was a period of 1 to 3 min after injection during which the mouse exhibited a pattern of behaviour similar to that shown by mice given saline, namely locomotion, rearing and general exploration of its new environment. The first sign of picrotoxin administration was a decrease in general motor activity associated with ataxia of the back legs and general relaxation of the trunk musculature. Finally the mouse assumed a posture in which the extended head was laid flat on the ground between the outstretched front paws. The righting reflex was present, but slightly impaired, and any distraction sufficed either to disturb or to delay the assumption of the posture. If the mouse were not distracted, the log of the dose administered was inversely proportional to the time interval between injection and first assumption of this flaccid posture (Fig. 1). The duration of the posture was unrelated to the dose of picrotoxin. The mouse frequently re-assumed this posture after periods of locomotor activity.

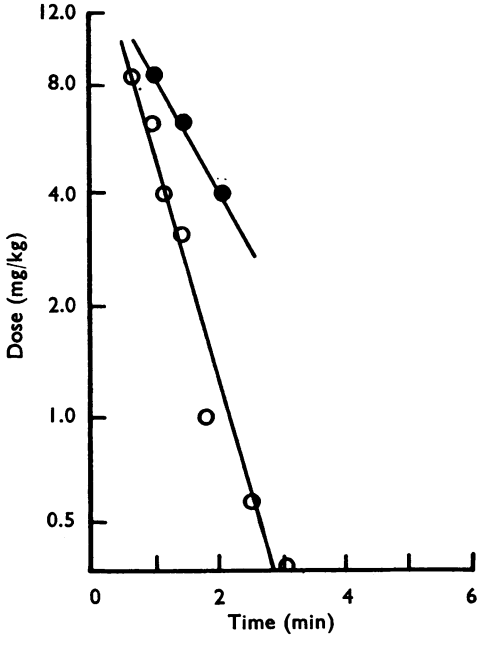

Fig. 1. Relationship between the log dose of picrotoxin and the time of the first appearance of the flaccid posture (○) and clonic convulsions (●). Ordinate: intravenous dose (mg/kg) of picrotoxin (log scale); abscissa: time (min) after injection.

Effect of convulsant doses of picrotoxin. Injection of convulsant doses of picrotoxin (4 to 8 mg/kg) was followed by a latent period before any effect of the drug became evident. The mouse then transiently assumed the flaccid posture; a period of restlessness followed which led to the appearance of a stance in which the animal stood with legs extended and held rigid. This posture was accompanied by trembling and progressively stronger twitching until finally a general clonic convulsion occurred, accompanied by apnoea, asphyxia, exhaustion and, depending on the dose, death.

The log of the dose administered was inversely proportional to the time interval between injection and the first clonic convulsion. The log dose/response curves for flaccid posture and convulsion time were not parallel, but intersected as the dose administered increased (Fig. 1). After administration of high doses of picrotoxin (>8 mg/kg) the flaccid posture, although detectable, was transient, being quickly followed by a convulsion, while determination of convulsion time with doses less than 4 mg/kg was unreliable.

Effect of picrotoxin on blood pressure. Because of the known ability of picrotoxin to increase blood pressure, the possibility arose that the flaccid posture might be the result of a peripheral rather than a central action of picrotoxin. Since the posture might conceivably have resulted from sudden and considerable hypertension, the blood pressure of the lightly anaesthetized mouse was determined. The small diameter of the carotid cannula prevented accurate recording of systolic and diastolic pressures and only the " mean pressure " was

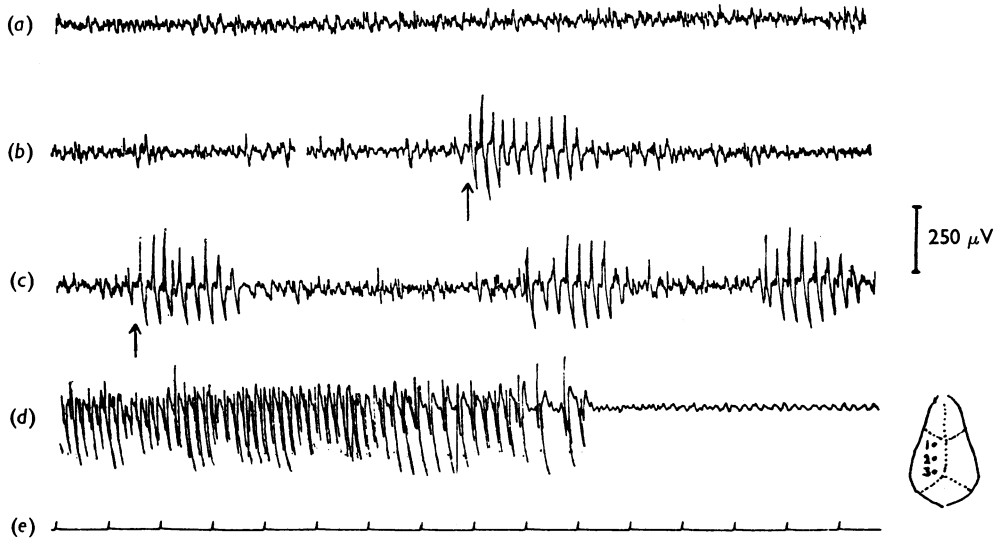

Fig. 2. Electroencephalogram of a conscious unrestrained 25 g mouse. Electrodes were inserted during hexobarbitone anaesthesia and the activity was recorded from leads 1 and 3. All injections were intravenous. (*a*) Normal electroencephalogram; (*b*) 110 sec after 1 mg/kg of picrotoxin; (*c*) 130 sec after 1 mg/kg of picrotoxin; (*d*) recording during and after a clonic convulsion following injection of 3 mg/kg of picrotoxin; (*e*) time scale (1.5 cm/sec). The arrows indicate the times of assumption of flaccid posture. Movement of the animal caused an artefact consisting of high-frequency (more than 50 cycles/sec) low-amplitude (less than 20 μV) activity.

recorded. The resting value was 110 to 130 mm Hg, but was appreciably reduced by the slightest blood loss incurred during preparation of the animal. Administration of picrotoxin (1 mg/kg, intravenously) caused an immediate transient rise in pressure of approximately 15 mm Hg. However, as a similar effect occurred after injection of 0.1 ml. of saline, this was accredited to an injection artefact. After 0.5 min, a second sustained rise in pressure was recorded; this increase in pressure varied between 15 and 45 mm Hg depending on the resting blood pressure.

Other analeptics which also increased the blood pressure did not affect motor activity or increase the number of stationary postures as did picrotoxin. Thus a somewhat similar pressor response was recorded after the injection of 25 μg of racemic amphetamine. After the injection artefact the pressor response was biphasic, a rise of approximately 45 mm Hg being preceded by a fall of 15 mm Hg, and the increase in pressure was transient, returning to resting level after 2 min.

Effect of picrotoxin on the electroencephalogram. After implantation of the electrodes, mice were left for 3 hr to allow for complete recovery from the anaesthetic during which time the electroencephalogram consisted of high-voltage (100 to 300 μV) slow-wave activity (1 to 4 cycles/sec) with spindles of about 12 cycles/sec. At the end of this period the electroencephalogram showed normal low-voltage rhythmic activity (Fig. 2,*a*) and electrocortical-arousal could be elicited, associated with full behavioural alerting. No difference was detected between the immediate electrical and behavioural effects after injection of 0.1 ml. of saline, and the same volume of saline containing 25 μg of picrotoxin (1 mg/kg). However, as the flaccid posture time was approached a gradual decrease in amplitude and increase in frequency of the electroencephalogram was observed, which culminated in a short burst of high-voltage (at least 350 μV) slow-wave (4 cycles/sec) activity (Fig. 2,*b*). The appearance of the first burst of electrical activity coincided with the mouse assuming the characteristic flaccid posture. The presence of the electrodes and the acute operative procedure did not affect the flaccid posture time. After being disturbed the mouse would re-assume the posture coincident with the first appearance of the characteristic burst of electrocortical activity. The length of the burst was very constant, lasting 1.5 to 2.0 sec. There was no correlation between the duration of this high-voltage slow-wave activity and the length of time for which the posture was held. The bursts were abolished by inhalation of 3% halothane, and appeared again upon recovery from the anaesthetic without further administration of picrotoxin. After administration of 3 mg/kg of picrotoxin, only the first few characteristic bursts of electrical activity were coincident with assumption of the flaccid posture, the bursts then became more frequent, and finally merged as the mouse passed into the first convulsion (Fig. 2,*d*). Leptazol, at doses which had no effect on motor activity, did not produce the bursts of electrocortical activity characteristic of picrotoxin. Practically all recordable activity of the brain temporarily ceased after convulsions resulting from a non-lethal dose of picrotoxin (Fig. 2,*d*). Movement artefact was recorded as very fast (40 cycles/sec) low-amplitude activity which was readily discernible.

DISCUSSION

So little is known concerning the mode of action within the central nervous system of analeptic drugs that any information which permits discrimination between them is of

potential value; this is particularly true for picrotoxin owing to its use in investigations into neurohumoral transmission at vertebrate and invertebrate inhibitory synapses.

The discovery of a flaccid posture preceding convulsions decreased, but did not abolish, the well-known delay between administration of picrotoxin and any observed drug effect. The delay may be due either to inability of the drug to penetrate rapidly into the brain or to formation of an active metabolite. There is no evidence to suggest that the activity accredited to picrotoxin is derived from a metabolite, but there is evidence for the existence of a permeability barrier between brain and cerebrospinal fluid (Richards, Grimes & Smith, 1941; Bircher, Kanai & Wang, 1962) and between brain and blood (Jolly & Steinhaus, 1956).

A considerable decrease in motor activity was recorded in mice treated with (−)-amphetamine, bemigride or picrotoxin. However, only the mice treated with picrotoxin assumed a posture described as flaccid owing to the completely relaxed manner in which it was held. That this posture resulted from acute hypertension was improbable for racemic amphetamine, in doses which were equipotent on the blood pressure of lightly anaesthetized mice, did not induce the flaccid posture or decrease motor activity in the conscious animal. Further, (−)-amphetamine, which produced both an increase in blood pressure and a decrease in motor activity, did not induce the flaccid posture. It is considered that the posture may result from a direct central action of the drug, for it appeared coincident with changes in the electroencephalogram of the conscious mouse. This suggestion is supported by the demonstration of Bircher, Kanai & Wang (1963) that, in the dog, the systemic and electroencephalographic changes after picrotoxin are independent and unrelated.

The mouse showed some slight loss of righting reflexes when in a flaccid posture, an effect unlikely to be due to impaired neuromuscular transmission for the animal immediately assumed normal activity when disturbed; furthermore, whatever the dose, mice only assumed the posture when care was taken to prevent distraction. The posture is also unlikely to result from a direct action of picrotoxin on the spinal cord, for antagonism of presynaptic inhibition of spinal motoneurones by picrotoxin as reported by Eccles *et al.* (1963) would of necessity lead to increased muscle tonus and not the detected flaccidity.

The changes in the electroencephalogram observed in picrotoxin-treated mice were similar to that observed by Swank & Foley (1948), who found that the picrotoxin seizure pattern in dogs originated in the motor areas before spreading over the cortex. Such a localized intense discharge from a focus on the cortex is characteristic of an epileptic seizure during which changes in posture may occur. Thus *petit mal* seizures in children are often " accompanied by a sudden dropping of the head or bending of the whole body to the floor " (Penfield & Erickson, 1941). Furthermore, the manner in which the posture suddenly appeared and interrupted preening and exploratory behaviour of the mouse was similar to the reported interruption of normal activity of patients by sudden inattention, stupor and vagueness associated with attacks of *petit mal*. Electrocortical seizure patterns may also be recorded without the development of generalized convulsion for such a situation obtains during photic stimulation of epileptics.

In conclusion, the sudden appearance of a flaccid posture in the mouse with concomitant changes in the electroencephalogram after picrotoxin may result from a mechanism similar to that causing the slight changes associated with the *petit mal* seizure. Other analeptics

did not produce a flaccid posture in mice and thus it is conceivable that picrotoxin antagonism may prove more useful than other procedures for testing drugs effective against *petit mal.*

SUMMARY

1. The responses of mice to intravenous injection of convulsive and subconvulsive doses of picrotoxin have been studied.

2. Subconvulsive doses of picrotoxin (less than 2 mg/kg) decreased motor activity in isolated mice, associated with a well-defined characteristic flaccid posture. The posture was often re-assumed after periods of locomotor activity.

3. The time of first occurrence of this posture was inversely proportional to the log of the dose of picrotoxin. The duration of the posture was unrelated to the dose.

4. After injection of convulsant doses of picrotoxin (4 to 8 mg/kg), mice transiently assumed this flaccid posture before a general clonic convulsion.

5. Since first assumption of the posture coincided with a burst of high-voltage slow-wave electroencephalographic activity, it is concluded that the posture is mediated by central mechanisms.

6. Of the other analeptics tested none produced such a flaccid posture, but (−)-amphetamine (0.4 to 4.0 mg/kg) and bemigride (1 to 4 mg/kg) produced characteristic abnormal behaviour associated with decreased motor activity.

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