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Effects of general depressant drugs on the electrical responses of isolated slabs of cat's cerebral cortex

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Summary

1. In the neuronally isolated cortex of the cat, local application of diphenhydramine, promethazine, gammahydroxybutyrate, gammabutyrolactone, gamma aminobutyric acid, hyoscine and pethidine, and the intravenous injection of diazepam and meprobamate depressed or abolished the surface negative and surface positive response to direct stimulation and raised the stimulus threshold of the positive burst response. These effects were the same as previously demonstrated for general and local anaesthetics on the same preparation.

2. Chlorpromazine produced a similar depression in small concentrations but caused spontaneous activity in higher concentrations.

3. In contrast to local application, pethidine when given by intravenous injection in a high dose produced convulsant activity in the isolated cortical slab. The possibility was suggested that the convulsant activity was produced by a metabolite of pethidine.

4. The results of this investigation suggest that the central depression produced by a number of structurally unrelated drugs is indicative of an anaesthetic-like property of these drugs.

Introduction

In tests carried out on intact mice a large number of central depressant drugs produced a depression of the central nervous system which is nonspecific in character and corresponds in some respects to general anaesthesia (Frank & Jhamandas, 1970). The mechanism of the depressant action of these drugs is not known. Since their gross actions, both depressant and stimulant, are very similar to those produced by local and general anaesthetics, it is possible that the mechanisms underlying the anaesthetic action of all these drugs are the same. In recent years it has been shown that volatile anaesthetics such as chloroform (Yamaguchi, 1961) and ether (Inoue & Frank, 1965); ethyl alcohol (Gage, 1965; Inoue & Frank, 1967); local anaesthetics such as procaine (Inoue & Frank, 1962); and a number of non-volatile anaesthetics (Thesleff, 1956) block the production of the action potentials in the skeletal muscle by suppressing the specific increase in the membrane sodium conductivity which follows an adequate stimulus and which is responsible for the rising phase of the action potential in the excitable tissue. Based on some of this evidence, Inoue & Frank (1962) proposed that all anaesthetics, local or general,

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acted by a common mechanism of action both at peripheral sites and in the central nervous system. Subsequently Frank & Sanders (1963) showed that local and general anaesthetics have fundamentally similar effects on the central nervous system both at the gross level in intact animals and at the cellular level in isolated neuronal tissue. When tetrodotoxin became available as a substance which specifically blocked the mechanism responsible for the increase in sodium conductance, Frank & Pinsky (1966) showed in similar tests that it also produced a central depression which was essentially similar to that produced by the local and general anaesthetics.

The object of the present investigation was to study the effects of central depressants on neurones in the cerebral cortex and to determine how closely their effects correspond to the effects of the local and general anaesthetics. For this purpose, experiments were carried out using neuronally isolated cortical slabs in unanaesthetized, decerebrate cats.

Methods

All experiments were carried out on cats of either sex weighing between 2 and 3 kg. The methods used have been described in detail elsewhere (Frank & Sanders, 1963).

Slabs of neuronally isolated cortex were prepared in decerebrate cats according to the technique of Burns (1950) and Burns and Grafstein (1952). The surface of the slab was stimulated every 15 s and evoked potentials recorded from the cortical surface. A stimulus strength was chosen to produce a surface negative response followed by a surface positive burst response.

Control responses were obtained during the 5–10 min before applying the drug. Next approximately 0·015 ml of the drug solution, prepared in the normal saline, was applied to a strip of filter paper 1 mm wide located on the surface of the slab between the stimulating and recording electrodes, and stimulation continued. The filter paper strip containing the drug was removed 5–10 min later. The stimulation and recording continued after the drug was removed until responses equivalent in size to control responses reappeared.

The responses obtained were measured to determine the amplitude of the surface negative response, and the amplitude and duration of the surface positive burst response. In addition stimulus thresholds were determined during the test and the recovery periods and these were compared to the control threshold levels.

All the drugs except meprobamate and diazepam (which were not water soluble) were applied topically to the cortical surface. The latter drugs were given by injection into the femoral vein. In addition, convulsant doses of pethidine were given intravenously. The blood pressure of the animal was monitored continuously on a Dynograph (Type RB) using Statham pressure gauge (P23AC).

Since most of the solutions were acidic in nature control experiments were carried out with sodium chloride solutions in the range of pH between 4 and 8. The cortical responses were not modified by these solutions.

Results

The effect of central depressants on the responses of the isolated cortex were investigated in cortical slabs undergoing direct electrical stimulation. Each drug

was applied in both low and high concentrations. The central depressants investigated in this manner were diphenhydramine, promethazine, chlorpromazine, gamma-hydroxybutyrate (GHB), gammabutyrolactone (GBL), hyoscine, pethidine, and gamma aminobutyric acid (GABA).

The results obtained using promethazine are illustrated as an example of the general pattern of effects produced by the central depressant drugs. Some of the responses obtained during an experiment in which promethazine was directly applied to an isolated slab of cerebral cortex are shown in Fig. 1. Measurements made on all the responses obtained in a different but similar experiment are plotted in Fig. 2. Although the details differed in the plots obtained from experiments with different animals, the general pattern obtained was the same in all animals tested with promethazine and in fact with all the depressant drugs tested in this study. This general pattern consisted of a depression in the amplitude of the surface negative response and in the amplitude and duration of the surface positive burst response. In general when higher doses of a drug were used, they tended to depress the cortical responses to a greater extent and to prolong the time required for full recovery of the responses after removing the filter paper strip containing the drug from the surface of the cortex. During the depressed phase of activity the surface positive responses could be elicited by increasing the intensity of the stimulus indicating an increase in the stimulus threshold.

The results obtained with the other drugs used are listed in Table 1. Meprobamate (Fig. 3) and diazepam had to be given intravenously and probably as a consequence recovery times were prolonged.

Some interesting differences were obtained when using chlorpromazine. As will be noted in Table 1, when chlorpromazine was applied to the surface of the cortical slab, recovery of control size responses following removal of the paper strip containing the drug required considerably longer than for any other drug applied in this manner. In addition, chlorpromazine was the only central depressant which induced spontaneous electrical activity in the slab when directly applied to the

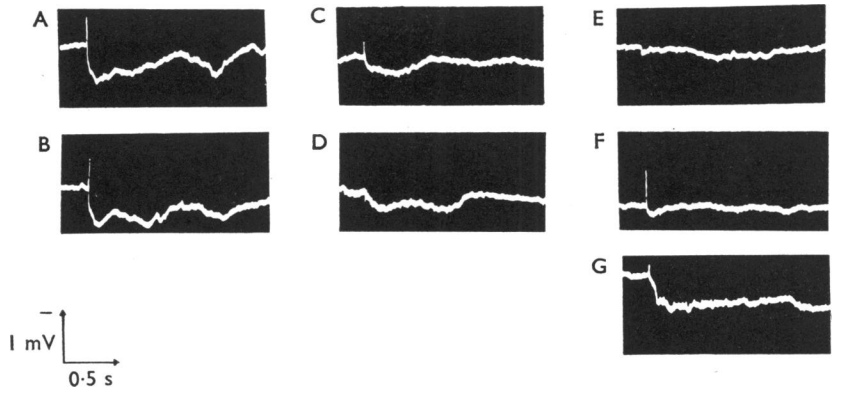

FIG. 1. Responses to direct electrical stimulation recorded from the surface of cat's isolated cerebral cortex before and after the local application of promethazine (2% w/v). Control responses, A and B. Responses after drug application, C, 75 s; D, 4 min. The filter paper strip containing the drug was removed after D. Responses after removal of the drug, E, 5 min; F, 30 min; G, 60 min.

surface of the cortex. This activity consisted of positive bursts (Fig. 4) following application in higher concentrations (5% and 10%).

In three decerebrate cats maintained on artificial respiration, pethidine was administered intravenously in a dose of 20 mg/kg. Administered in this manner the drug caused obvious convulsions in the animals as indicated by arching of the back, extension of the limbs, tachycardia and twitching. Convulsant activity also was recorded simultaneously from the isolated cortical slab. The convulsant activity recorded 5 min after the injection of pethidine consisted of spike discharges (Fig. 5) which were unrelated to the applied stimulus. The discharge lasted for 10–12 min, following which the slab became unresponsive to control stimuli. Recovery from the effects of pethidine took place 90–100 min after the injection and normal responses could be elicited by direct stimulation after this period.

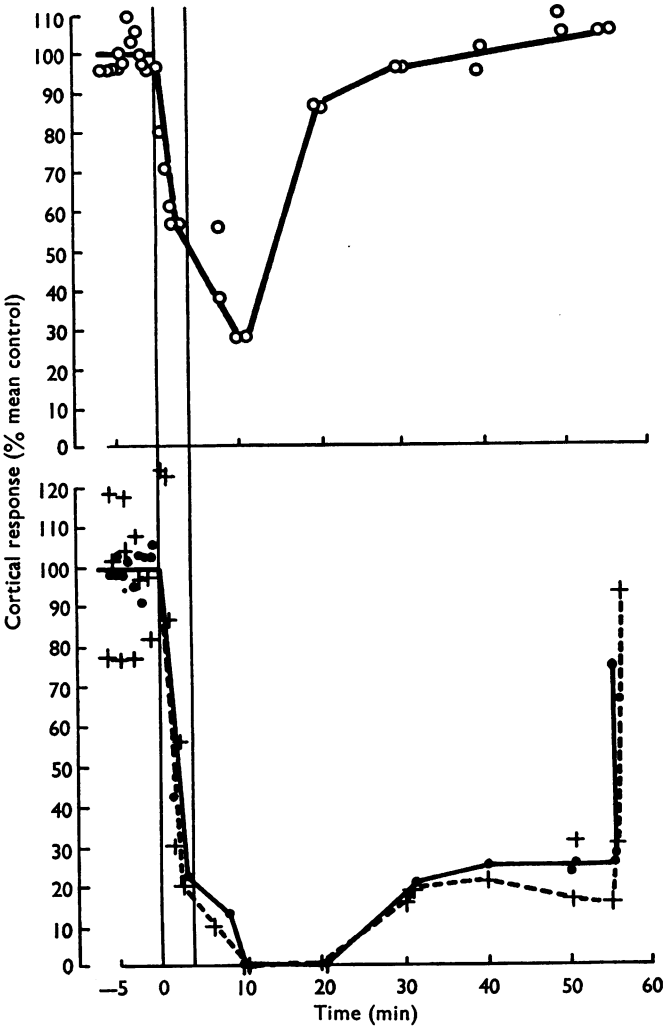

FIG. 2. Effect of topical application of promethazine (2% w/v) on the responses of a cat's isolated cerebral cortex stimulated directly. The cortex was exposed to the drug during the time between vertical lines. ○, Amplitude of surface negative response; ●, amplitude of surface positive response; +, duration of surface positive response.

TABLE 1. *Effect of central depressants on electrical responses of isolated cerebral cortex*

Drug	No. of experiments	Concentration (%)	Surface −ve response (amplitude)	Surface +ve response (amplitude)	Surface +ve duration	Recovery (min)
Diphenhydramine	8	1, 2, 4	Reduced	Reduced	Reduced	60–90
		8	Abolished	Abolished	Abolished	90
Promethazine	10	1	Reduced	Abolished	Abolished	90–120
		2	Reduced or Abolished	Abolished	Abolished	90–120
		5, 8	Reduced or Abolished	Abolished	Abolished	120
Chlorpromazine	12	1, 2	Reduced or Abolished	Reduced or Abolished	Reduced or Abolished	180–240
		5, 10	Spontaneous activity positive fluctuations in potentials			180–240
Gammahydroxybutyrate	7	2, 4	Reduced	Reduced	Reduced	60–90
		8	Reduced	Reduced	Reduced	>90
Gammabutyrolactone	8	2, 4	Reduced	Reduced	Reduced	60–90
		8	Reduced	Reduced	Reduced	90
Gamma aminobutyric acid	7	2, 4	Reduced or Abolished	Reduced	Reduced	60–90
Hyoscine	6	1, 2	No change	No change	No change	—
		4	Reduced	Reduced	Reduced	60–90
		8	Abolished	Abolished	Abolished	>90
Pethidine	11	0·5, 1·0	Reduced	Reduced	Reduced	30–60
		2·0, 4·0	Abolished	Abolished	Abolished	60–90
		20 mg/kg (i.v.)	Convulsant spike activity in isolated slabs			90–100
Meprobamate	5	20 mg/kg	Reduced	Reduced	Reduced	120
		40 mg/kg	Reduced	Reduced	Reduced	240–300
Diazepam	5	1 mg/kg	Reduced	Reduced	Reduced	90–120
		2 mg/kg	Reduced	Reduced	Reduced	>120

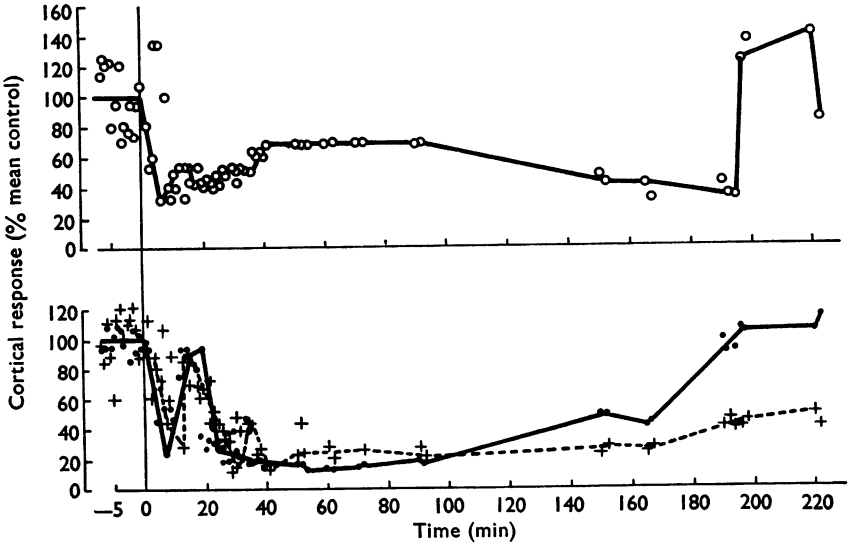

FIG. 3. Effect of meprobamate (40 mg/kg) given intravenously on the responses of a cat's isolated cerebral cortex stimulated directly. The drug was administered at the time shown by the vertical line. ○, Amplitude of surface negative response; ●, amplitude of surface positive response; +, duration of surface positive response.

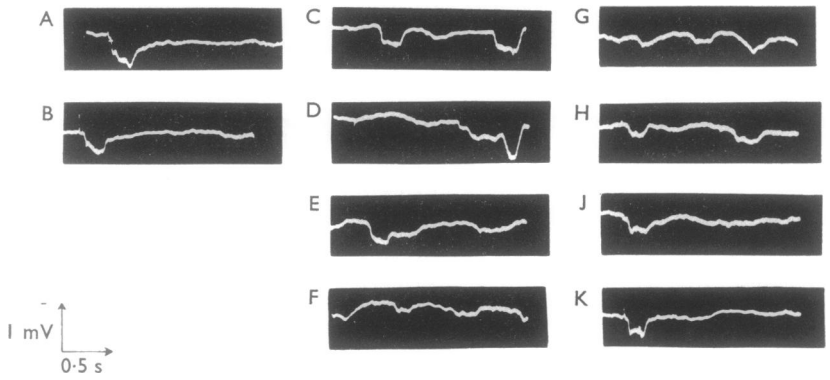

FIG. 4. Responses to direct electrical stimulation recorded from the surface of cat's isolated cerebral cortex before and after local application of chlorpromazine (5% w/v). Control responses, A and B. Responses after drug application, C, 2 min ; D, 3 min ; E, 6 min ; F, 10 min. The filter paper strip containing the drug was removed after F. Responses after removal of the drug. G, 15 min ; H, 30 min ; J, 120 min ; K, 220 min.

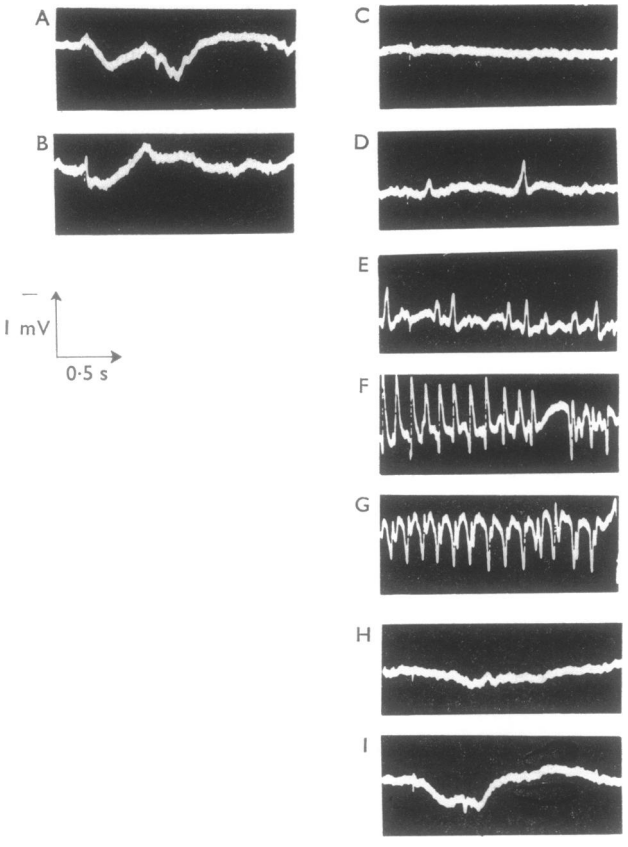

FIG. 5. Responses to direct electrical stimulation of cat's isolated cortex recorded before and after a convulsant dose (20 mg/kg) of pethidine given intravenously. Control responses, A and B. Responses after intravenous injection of pethidine, C, 1 min ; D, 5 min ; E, 6 min ; F, 8 min ; G, 11 min ; H, 30 min ; I, 90 min.

Discussion

In a previous study on intact mice (Frank & Jhamandas, 1970) it was found that a variety of centrally acting drugs produced a central depression which was similar in many respects to anaesthesia. The object of the present study was to investigate the action of these drugs at the neuronal level and to determine the extent to which the mechanism underlying their anaesthetic-like effects corresponds to the anaesthetic action of the local and general anaesthetics. Therefore the effects of these drugs were examined in a group of neurones which were neuronally isolated from the rest of the brain.

The central depressants which were investigated have diverse chemical structures, nevertheless they all produced a remarkably similar pattern of effects on the electrical responses of isolated cortex. As a group all these agents reduced the evoked potentials and increased the stimulus threshold. Even when applied in high concentrations, diphenhydramine, promethazine, gammahydroxybutyrate, gammabutyrolactone, hyoscine and pethidine diminished the amplitude of the surface negative and the surface positive response. No consistent facilitation of either response was ever observed in these tests. Diazepam and meprobamate, being insoluble in water, were given by injection. Given in this way, they reduced both responses. The effects of all these depressant drugs corresponds very closely to those produced by general anaesthetics such as phenobarbitone and ether and local anaesthetics such as procaine (Frank & Sanders, 1963). Although most of the depressant drugs studied here, like procaine, have a strong stimulatory action in intact animals, they failed to show such action on the isolated cortex.

The only deviations from this typically depressant action on isolated cortex were provided by chlorpromazine and pethidine. Chlorpromazine when topically applied to the cortical slab in concentrations of 1–2% produced only depression of the evoked responses which persisted for a prolonged period. In higher concentrations (5% and 10%) it produced spontaneous activity in the cortical slabs consisting mainly of positive fluctuations in the surface potential. This stimulatory effect of chlorpromazine seems to be peculiar to the drug itself, since promethazine, which is also a phenothiazine, failed to show a similar effect. The mechanism of action of this effect is not known but the observation is similar to other isolated reports of its facilitatory action in the central nervous system (Rinaldi & Himwich, 1955).

Pethidine when applied topically to the cortex in the concentration range 0·5–4·0% produced only a depression of the evoked responses. However, when injected intravenously in a large dose (20 mg/kg) it produced a convulsant response in the whole animal and in the isolated cortex undergoing normal electrical stimulation. One possible explanation for this difference in the response of isolated cortex to topically applied, as compared to systemically administered, pethidine would be that a metabolite of pethidine, rather than pethidine itself, is responsible for the central nervous system stimulant effects observed. Deneau & Nakai (1961) showed that in the monkey, the degree of the central excitation depends in a large part on the rate at which pethidine is converted to its metabolite, norpethidine. Such a process would explain our previous finding that lower doses of pethidine had a greater potentiating effect on subanaesthetic doses of phenobarbitone than did larger doses of pethidine. However, other explanations are possible, and further evidence would be required to prove that the convulsant action of pethidine is due to one or more of its metabolites.

In view of the marked similarity in neuronal depression produced by the central depressants to the depression produced by procaine, ether and phenobarbitone in previous tests (Franks & Sanders, 1963) it is attractive to postulate that the cellular mechanisms underlying this depression are the same for all these agents and are responsible for their anaesthetic property. Implicit in this hypothesis is the assumption that like the local and the general anaesthetics, which have already been shown to have a suppressive effect on the sodium conductance and hence the action potential (Frank, 1968), these central depressants also suppress the sodium conductance in a similar way. Their effects in the central nervous system also correspond very closely to those of tetrodotoxin (Frank & Pinsky, 1966), a substance which specifically blocks the sodium conductance in the excitable tissue (Narahashi, Moore & Scott, 1964). However, direct evidence for such an effect of the central nervous system depressants on excitability is not available at present.

It must be recognized that the central depressants discussed here not only produce a generalized depression of the central nervous system but they also produce other effects on central nervous system function—for example, pethidine produces analgesia, chlorpromazine produces a tranquillizing effect and diazepam causes muscle relaxation. All these effects are undoubtedly a result of central nervous system depression. However, it is probable that such depressions occur selectively in limited areas or at specific sites in the brain. They therefore may be regarded as relatively specific depressions, the degree and mechanism of which vary according to the drug and its chemical structure. On the other hand the common depressant effects of the central depressants studied here cannot be related to the chemical structure of these drugs which vary greatly, and we would suggest that these depressant effects are a generalized but weak form of anaesthetic depression.

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