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THE EFFECT OF CONVULSANT DRUGS ON COAXIALLY STIMULATED GUINEA-PIG ILEUM

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Parenteral injection of the convulsant drugs strychnine or leptazol increases the electrical activity of the brain and the amount of acetylcholine (ACh) released from the surface of the cerebral cortex (Mitchell, 1963; Beleslin, Polak & Sproull, 1965; Neal, 1967), but little is known of the mechanism of action involved.

In the hope of obtaining a clue to the mechanism of action the effects of convulsants on a peripheral cholinergic synapse have been investigated.

Morphine and other narcotic analgesics reduce the amount of Ach released from the coaxially stimulated guinea-pig ileum (Paton, 1957), as they do from the brain (Beleslin & Polak, 1965). The cholinoceptive receptors on cells of the cerebral cortex exhibit the properties of muscarinic receptors with respect to blocking drugs (Krnjević & Phillis, 1963; Curtis, 1966), as do those of the smooth muscle of the guinea-pig ileum. These facts suggest that the guinea-pig ileum shows some of the properties of central cholinergic synapses and for this reason this tissue was chosen for the present investigation.

METHODS

Coaxially stimulated guinea-pig ileum

Male guinea-pigs which had been starved overnight were stunned by a blow on the head and bled out. The ileum was removed and placed in oxygenated Krebs solution. A piece of tissue 6–7 cm in length was removed from the mid-region of the ileum.

The gut was mounted in an organ bath (volume 15 ml.) containing Krebs solution at 37° C which was bubbled with 95% oxygen and 5% carbon dioxide. The electrodes were arranged essentially as described by Paton (1957). The lower end of ileum was tied on to a tubular glass support, which allowed the contents of the gut to be gradually extruded during the course of the experiment.

The ileum was stimulated with rectangular shocks of 0.5 msec duration delivered from a Grass SD5 stimulator at a frequency of 6/min. Under these conditions each shock produces a twitch-like response which is due to stimulation of the post-ganglionic parasympathetic nerves (Paton, 1957; Harry, 1962). The twitch responses were recorded on smoked paper using a frontal writing lever. Drugs were dissolved in Krebs solution and warmed to 37° C before addition to the bath. A contact time of 3 min was always sufficient to obtain maximum responses. Doses of drugs could normally be repeated after 10–15 min.

A.C. stimulated ileum

In some experiments guinea-pig ileum was stimulated using A.C. field stimulation in the presence of a high concentration of atropine (0.2×10^{-6} g/ml.). Under these conditions the response is due to direct stimulation of the smooth muscle, the effect of nerve stimulation having been abolished.

The shocks were administered via two electrodes, one situated at the top and one at the bottom of the bath. The A.C. stimulus was 50 c/s obtained from a mains transformer with multiple secondary tappings. The voltages available by suitable switching ranged from 5 to 25 V. The voltage delivered was monitored with an oscilloscope. The preparation was stimulated 2/min for periods of 15 sec.

Drugs and solutions

Krebs solution of the following composition was used: (g/l.) NaCl 6.9; KCl 0.35; $CaCl_2$ 0.28; $MgCl_2$ 0.11; $NaHCO_3$ 2.1; NaH_2PO_4 0.14; glucose 2.0.

The following drugs were used: strychnine hydrochloride (British Drug Houses), picrotoxin (British Drug Houses), brucine hydrochloride (British Drug Houses), pentamethylene tetrazole (leptazol (L. Light & Co.), bemegride (Megimide, Nicholas), nikethamide (Anacardone, British Drug Houses). Doses are expressed in terms of the base.

RESULTS

Strychnine, brucine, picrotoxin, bemegride, leptazol and nikethamide all produced an inhibitory effect on the twitch response of guinea-pig ileum to electrical stimulation. This is illustrated for strychnine, leptazol and bemegride in Fig. 1.

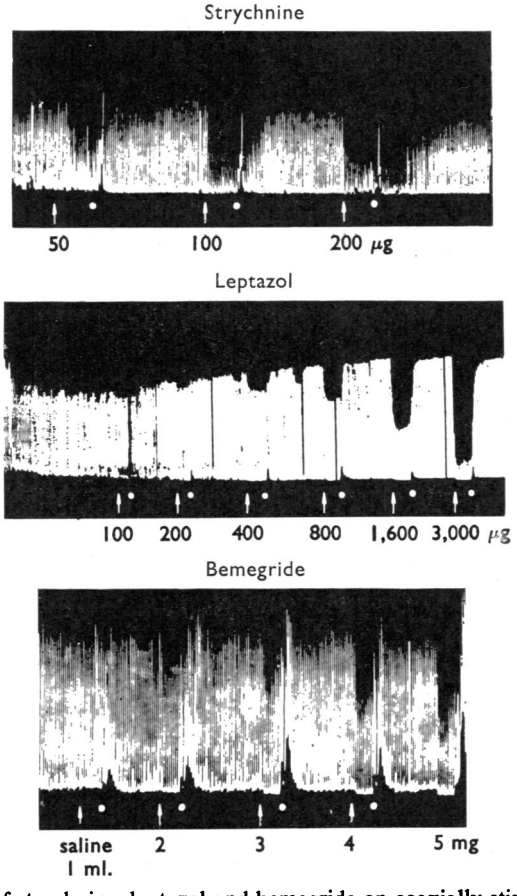

Fig. 1. Inhibitory effect of strychnine, leptazol and bemegride on coaxially stimulated guinea-pig ileum.

Initially, submaximal shocks were used in order to reveal any increased response which would be expected if convulsants caused an increase in the amount of transmitter released by a nerve impulse. In subsequent experiments supramaximal shocks were used.

In order to compare the relative potencies of convulsants on the guinea-pig ileum, log concentration-response lines were constructed for strychnine, leptazol, picrotoxin, brucine, bemegride and nikethamide. These are shown in Fig. 2. Each line was obtained

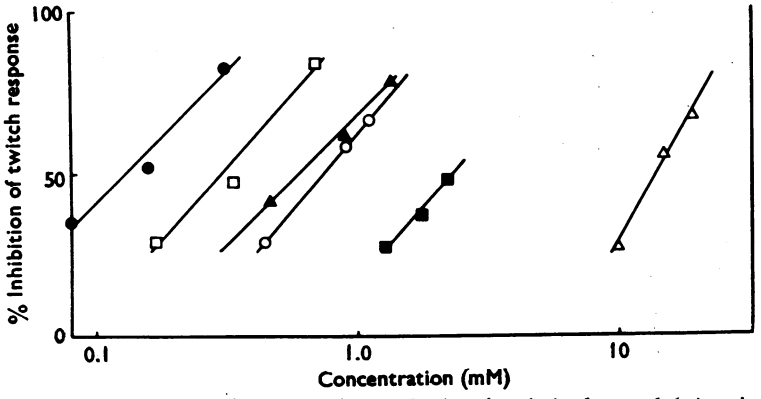

Fig. 2. Log dose response curves for strychnine (●); brucine (□); leptazol (▲); picrotoxin (○); bemegride (■); and nikethamide (Δ).

from four or five preparations. Table 1 shows the concentrations of convulsants required to inhibit the twitch response of guinea-pig ileum by 50% and also the doses of these drugs required to produce convulsions in 50% of mice (CD50). The CD50 values were taken from Setnikar, Murmann, Magistretti & Da Re (1960).

TABLE 1

CONCENTRATIONS OF CONVULSANT DRUGS WHICH INHIBIT COAXIALLY STIMULATED GUINEA-PIG ILEUM BY 50% (ED50), AND SUBCUTANEOUS DOSES OF CONVULSANTS WHICH PRODUCE CONVULSIONS IN 50% OF MICE (CD50)

Drug	ED50 (μM)	CD50 (μ-mole/kg)
Strychnine	130	1·2*
Picrotoxin	720	5·2*
Brucine	310	10
Bemegride	2,200	95*
Leptazol	580	310*
Nikethamide	13,500	1,200*

* From Setnikar *et al.* (1960).

It can be seen that the more potent convulsants such as strychnine and picrotoxin affect the guinea-pig ileum at lower concentrations than do the weaker convulsants such as nikethamide. This is illustrated in Fig. 3, which, with the exception of leptazol, shows a correlation between convulsant activity in mice and inhibitory activity in nerve stimulated ileum.

A preliminary investigation was made of the site of action of strychnine and leptazol. The response to electrical stimulation was matched with the response to a suitable dose of ACh added to the bath. During ACh administration electrical stimulation was

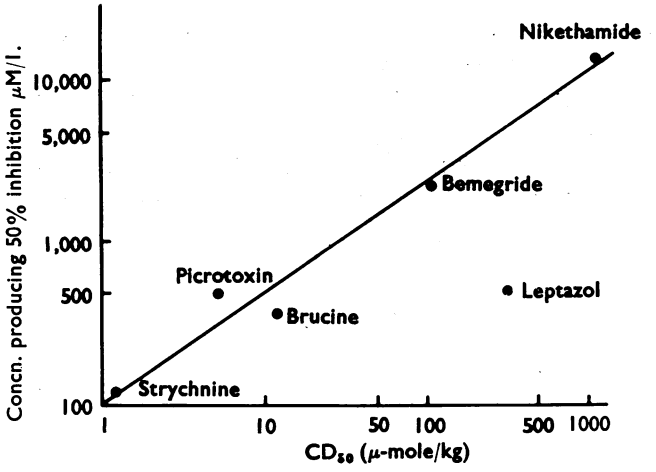

Fig. 3. Shows the correlation of the inhibitory action of convulsants on coaxially stimulated guinea-pig ileum with the dose of these drugs which produces convulsions in 50% of mice.

discontinued. Strychnine or leptazol, in doses which produced a marked inhibitory response on coaxially stimulated preparations, was added to the bath and the dose of ACh was repeated. Neither drug reduced the response to ACh in concentrations which blocked the twitch response by up to 95%. Higher concentrations of strychnine or leptazol progressively decreased the response to added ACh. It seems, therefore, that these two convulsants produce their effect on guinea-pig ileum mainly by reducing the amount of ACh released from the nerve rather than by an effect on the smooth muscle.

Effect on smooth muscle

The direct effect of the convulsants on smooth muscle was further investigated using A.C. field stimulated ileum in the presence of atropine (0.2×10^{-6} g/l.). None of the convulsants had any consistent effect on the directly stimulated smooth muscle. However, in the case of strychnine it was sometimes possible to obtain a stimulant effect with low doses and an inhibitory effect with higher doses. Although these effects were reproducible on any given preparation, they were small in comparison with the inhibitory effect of strychnine on the coaxially stimulated ileum.

Noradrenaline

The possibility that convulsants produced their inhibitory effect by causing noradrenaline release was excluded by repeating the experiments using the ileum from a guinea-pig pre-treated with reserpine (5 mg/kg subcutaneous injection) 24 hr previously. The results did not differ significantly from those obtained using ileum from untreated animals.

Effect on ganglia

The addition of hexamethonium (10^{-6} g/l.) to the Krebs solution had little or no effect on the action of convulsants on guinea-pig ileum. This result was expected since electrical stimulation of this preparation excites post-ganglionic nerve fibres (Paton, 1957 ; Harry, 1962).

DISCUSSION

The present experiments were undertaken in the hope that they would help to explain why convulsant drugs increase the release of ACh from the central nervous system. However, all the convulsants investigated depressed the twitch response evoked by coaxial stimulation of the guinea-pig ileum and in the case of two of them (strychnine and leptazol) evidence was obtained which suggested that the effect was associated with a decrease in ACh output. Thus, the results obtained using a peripheral cholinergic synapse appear to be incompatible with those obtained from experiments on the central nervous system. Nevertheless, the fact that there appears to be a correlation between the inhibitory effect on a peripheral cholinergic junction and the ability of the drugs to cause convulsions in mice suggests that there may be similarities in the mechanisms involved in producing these effects at the different sites.

The ability of strychnine to block cholinergic synapses has been previously demonstrated at the neuromuscular junction (Lanari & Luco, 1939; Alving, 1961), at sympathetic ganglia (Brown & Quilliam, 1964; McKinstry & Koelle, 1967) and on coaxially stimulated guinea-pig ileum (Takagi & Takayanagi, 1966). As a result of experiments on the frog neuromuscular junction, Alving (1961) suggested that strychnine competed with ACh for the post-synaptic cholinoceptive receptor. However, McKinstry & Koelle (1967) and Takagi & Takayanagi (1966) concluded that strychnine acts presynaptically, reducing the amount of ACh released from nerve endings. The present results agree with the idea of a presynaptic site of action, although in the case of strychnine at least it is unlikely that the site involved is cholinergic, since the known cholinergic synapse which the lower motoneurone collateral forms with the Renshaw cell is unaffected by this drug. This is shown by the fact that strychnine does not depress the discharges of the interneurones (Eccles, Fatt & Koketsu, 1954; Eccles, 1957; Curtis, 1959).

It remains possible, however, that the convulsants which act higher in the neuraxis may increase the release of ACh from the brain by blocking the release of an inhibitory transmitter.

SUMMARY

1. The effect of six convulsant drugs was investigated on coaxially stimulated guinea-pig ileum.

2. Strychnine, brucine, picrotoxin, leptazol, bemegride and nikethamide depressed the twitch response.

3. A preliminary investigation of the site of action of strychnine and leptazol suggested that they act presynaptically by reducing acetylcholine release.

4. The convulsants had little effect on directly (A.C.) stimulated smooth muscle in the presence of atropine.

5. With the exception of leptazol there appeared to be a correlation between the inhibitory effect of convulsants on coaxially stimulated guinea-pig ileum with their ability to produce convulsions in mice.

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