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Pharmacodynamic effects of dehydroemetine

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The pharmacodynamics of dehydroemetine were studied in isolated preparations and in whole animals. Dehydroemetine had no effect on the guinea-pig isolated ileum but non-specifically antagonized the spasmogenic effect of histamine, acetylcholine and nicotine on this tissue. Dehydroemetine in small concentrations inhibited relaxations of the guinea-pig and rabbit ileum produced by stimulating the sympathetic nerve without inhibiting the effect of noradrenaline. Twitches of the guinea-pig ileum elicited by transmural stimulation were blocked by dehydroemetine while higher doses of the drug also inhibited the indirectly elicited contractions of the isolated rat diaphragm. *In vivo*, neuromuscular blocking action could not be demonstrated for dehydroemetine while toxic doses were required to demonstrate the presence of an adrenergic neuron blocking action. Dehydroemetine had no anaesthetic action when applied locally to the rabbit cornea. Concentrations of the drug which caused local anaesthesia when injected intradermally into guinea-pigs also caused skin necrosis. It is concluded that dehydroemetine lacks any specific adrenergic neuron blocking action or neuromuscular blocking action or local anaesthetic action. It is suggested that the effects demonstrated are due to non-specific action at the nerve endings.

Little is known of the pharmacological actions of the synthetic alkaloid dehydroemetine. Herrero, Brossi & others (1961) studied the effects of the drug on the cardiovascular system and on smooth muscle. Dehydroemetine reduced the blood pressure, pulse rate and femoral and carotid blood flow in the cat and antagonized the smooth muscle contraction produced by barium chloride and acetylcholine. Schwartz & Herrero (1965) in a comparative pharmacokinetic study of emetine and dehydroemetine on guinea-pigs found that the two compounds were bound to the same tissues, but that dehydroemetine was bound to a less extent and was eliminated from the tissues more rapidly than emetine. Recently, Salako (1970) and Ng & Ng (1970) have suggested that dehydroemetine might possess an adrenergic neuron blocking action. In this study, the pharmacological actions of dehydroemetine have been considered in more detail.

METHODS

Guinea-pig isolated ileum

Short lengths of guinea-pig terminal ileum were set up in the usual manner in aerated Tyrode solution at 34° in a 45 ml organ bath. Isotonic contractions of the tissue in response to drugs added to the bath were recorded using a frontal writing lever.

Rabbit ileum with periarterial nerves

Pieces of rabbit ileum with their attached mesentery were mounted in 70 ml aerated Tyrode solution at 37° by the method of Finkleman (1930). The periarterial nerves

were stimulated at known frequencies through a pair of bipolar platinum electrodes using supramaximal square pulses of 20–50 V and 0·5 ms duration.

Guinea-pig ileum with attached mesentery

Segments of guinea-pig proximal ileum, prepared according to Finkleman (1930) were mounted in 70 ml oxygenated Tyrode solution at 37° for transmural and periarterial sympathetic stimulation (Paton, 1955; Wilson, 1962). The parasympathetic cholinergic nerves in the intestinal wall were stimulated transmurally (10 V; 1·0 ms) with a pair of coaxial platinum electrodes placed on either side of the ileum, and twitches of the ileum were obtained at the rate of 0·125 Hz. The mesenteric nerves were stimulated with square pulses of 20 V and 0·5 ms at the rate of 40 Hz for 15 s every 5 min. For simultaneous sympathetic and parasympathetic stimulation a baseline of transmural twitches was first obtained with supramaximal stimuli and these were then inhibited by the simultaneous maximal stimulation of the periarterial sympathetic nerves.

Rat isolated phrenic nerve-diaphragm

Hemidiaphragms were prepared according to the method of Bülbring (1946) and mounted at room temperature (26–28°) in a 50 ml bath filled with aerated Tyrode solution containing double the normal amount of glucose. Isotonic contractions of the diaphragm in response to supramaximal nerve or muscle stimulation were recorded. Nerve stimulation was with square pulses of 2–5 V and 0·5 ms duration and direct muscle stimulation was with pulses of 20–50 V and 5·0 ms.

Nerve-muscle preparation in the intact animal

The effect of dehydroemetine on neuromuscular transmission in the intact animal was studied using the sciatic nerve-gastrocnemius muscle preparation in the rat and the sciatic nerve-tibialis anterior muscle preparation in the cat (Brown, 1938). Twitches (0·125 Hz) and tetani (40 Hz) of the rat gastrocnemius or cat tibialis elicited by stimulating the nerve with supramaximal square pulses of 0·2 ms duration were recorded.

Cat blood pressure and nictitating membrane

12 cats were anaesthetized with intravenous chloralose (80 mg/kg) to which sodium pentobarbitone (5 mg/kg) was added. The preganglionic or postganglionic trunk of the cervical sympathetic nerve was stimulated through a pair of bipolar platinum electrodes with square pulses of 20 V and 0·5 ms, and the resulting contractions of the nictitating membrane were recorded using an isotonic frontal-writing lever. Blood pressure was measured from a femoral artery with a Statham P23 transducer and recorded on a Schwarzer physiograph. Drugs were injected into a femoral vein through a polythene cannula.

Observations in unanaesthetized animals

12 white Wistar rats, 150 to 200 g, were given subcutaneous injections of dehydroemetine (1 mg/kg as a 1 mg/ml solution) daily for 10 days and the effect of the treatment on the animals observed.

Local anaesthesia, after intradermal injections of dehydroemetine (0·6, 6·0 and 30

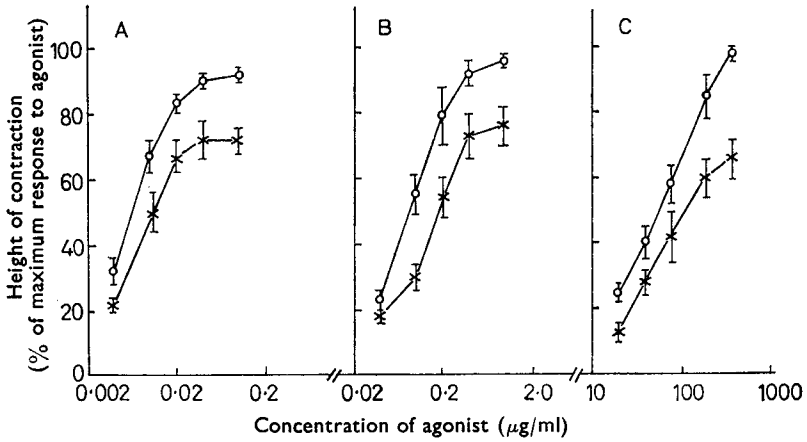

FIG. 1. Dose-response curves for A, histamine; B, acetylcholine; and C, nicotine; in the absence ○———○ and presence X———X of dehydroemetine (7 μg/ml). Each point represents the mean ±s.d. of A, eight; B, six; and C, six, experiments.

mg/ml solutions) into the shaved skin of the flanks of six guinea-pigs, was tested for by pricking the skin with a fine needle (Bülbring & Wajda, 1945).

Observations for surface anaesthetic activity were made after exposure of rabbit cornea to known concentrations of dehydroemetine (0·6, 6·0 and 30·0 mg/ml). Each solution was instilled into the corneal sac of 6 rabbits and the cornea was tested for anaesthesia by touching with the blunt end of a fine glass rod. Observations were made at 5-min intervals for 3 h and at 24, 48 and 72 h after administering the drug.

Drugs

The drugs used were acetylcholine chloride, atropine sulphate, cocaine hydrochloride, dehydroemetine dihydrochloride, hexamethonium bromide, histamine acid phosphate, mepyramine maleate, nicotine hydrogen tartrate, noradrenaline acid tartrate, phenoxybenzamine hydrochloride, propranolol hydrochloride and neostigmine methylsulphate. Drug solutions were made in isotonic sodium chloride solution and concentrations are expressed in terms of their salt.

The composition of standard Tyrode solution used in the *in vitro* studies was (%) NaCl 0·8, KCl 0·02, $CaCl_2$ 0·02, $NaHCO_3$ 0·1, $MgCl_2$ 0·01, NaH_2PO_4 0·005, glucose 0·1, and the pH was 7·3–7·4.

Results are expressed as mean ± standard deviation where appropriate.

RESULTS

Guinea-pig isolated terminal ileum

Dehydroemetine (2·0–100 μg/ml) had no spasmogenic effect on the guinea-pig terminal ileum, but reversibly antagonized contractions of the ileum produced by acetylcholine, histamine and nicotine. With all three compounds, dehydroemetine (7 μg/ml) caused a shift to the right of the dose-response curve and a reduction of the maximal response (Fig. 1). The inhibitory effects of dehydroemetine against acetylcholine, histamine and nicotine contractions of the guinea-pig ileum were quantitatively compared in terms of the dose ratio (Day & Vane, 1963), using 50% of the maximal response of the ileum to the agonist in each experiment to calculate the dose

Table 1. *Dose ratios for histamine, acetylcholine and nicotine before and in the presence of dehydroemetine.*

Experiment No.	Dose in the presence of DHE (7 μg/ml) / Control dose		
	Histamine	Acetylcholine	Nicotine
1	3·0	2·6	2·7
2	2·4	1·9	1·8
3	2·3	1·6	1·7
4	2·0	1·5	2·5
5	2·5	1·4	1·6
6	1·9	1·6	1·8
7	3·9	—	—
8	2·7	—	—
Mean	2·6	1·8	2·0
±s.d.	±0·64	±0·45	±0·46

ratio. The results summarized in Table 1 show that in the presence of 7 μg/ml dehydroemetine a 2 to 3 fold increase in the concentrations of acetylcholine, histamine and nicotine was necessary to produce the same contraction as in the control period.

Rabbit ileum with sympathetic nerves

Salako (1970) showed that dehydroemetine (0·5–10 μg/ml) reversibly blocked the effect of sympathetic stimulation on the rabbit ileum without antagonizing the direct effects of noradrenaline. In this study, the relation of the block to the frequency of stimulation was examined. Four frequencies were chosen (10, 20, 40 and 80 Hz), and a total of 1000 shocks were given at each stimulation. The inhibition of intestinal movement caused by stimulation of sympathetic nerves was first diminished and finally abolished at all frequencies by dehydroemetine (0·5 μg/ml). The response at the highest frequency of stimulation was inhibited first and that at the lowest frequency last. A progressive reduction in the amplitude of the pendular movement was also observed regularly.

Guinea-pig ileum with autonomic nerves

Dehydroemetine (0·5–5 μg/ml) reversibly inhibited the relaxation of the ileum caused by sympathetic stimulation of the periarterial nerves (20 V; 0·5 ms; 40 Hz for 15 s every 5 min) as described by Munro (1953). Relaxation of this tissue produced by a low concentration of noradrenaline (0·3 μg/ml) was not antagonized by dehydroemetine.

Twitches of the guinea-pig ileum obtained by transmural stimulation of the cholinergic parasympathetic nerves were inhibited by dehydroemetine (0·5–20 μg/ml). The effect developed slowly at the low doses but was rapid at the higher doses. The effect was also reversible on washing. After the transmural twitches had been abolished by a relatively large dose (20 μg/ml) of dehydroemetine, the response to acetylcholine persisted though reduced.

The relative sensitivity of the sympathetic and parasympathetic nerves to the action of dehydroemetine was examined in the simultaneously stimulated preparations of the guinea-pig ileum. In a series of 18 experiments, dehydroemetine (0·5, 1 and 2 μg/ml)

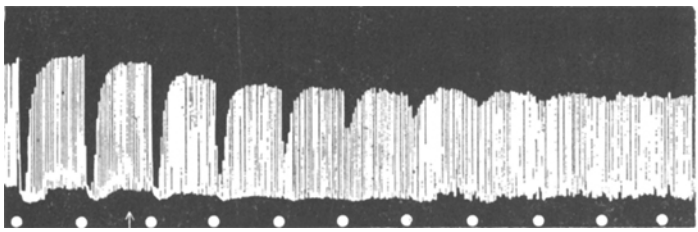

FIG. 2. Guinea-pig ileum. Continuous transmural stimulation (10 V; 1·0 ms, 0·125 Hz) interrupted at 5-min intervals by simultaneous periarterial nerve stimulation at the dots (20 V; 0·5 ms; 40 Hz for 15s). At ↑ dehydroemetine, 0·5 μg/ml, was added.

inhibited the sympathetic response completely before the parasympathetic response (Fig. 2).

Nerve-muscle preparations

Dehydroemetine in concentrations above 0·1 mg/ml inhibited the contractions of the rat diaphragm elicited by stimulating the phrenic nerve. On the other hand concentrations of up to 1 mg/ml acting for 30 min had no effect on the direct contractions of the fully curarized preparation. The effect of dehydroemetine on neuromuscular transmission developed slowly and was reversible after washing several times with fresh solution. The block was more on tetanus than on single twitches, was not antagonized or increased by neostigmine (1–10 μg/ml) and a tetanus elicited during a partial block did not antagonize the block (Fig. 3). The possible relation between dehydroemetine block and stimulus frequency was examined in 12 hemidiaphragms by stimulating the nerve successively at 0·125, 0·5, 1 and 2 Hz, a total of 20 shocks being given at each frequency of stimulation. The results (Fig. 4) showed that the effect of dehydroemetine was greater at faster stimulus frequencies.

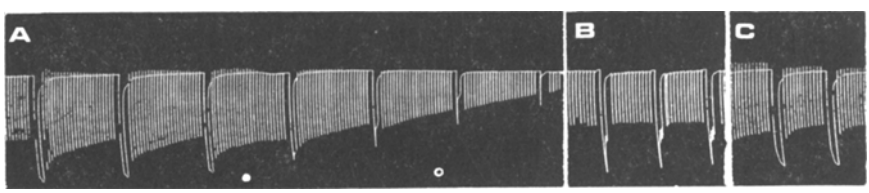

FIG. 3. Rat isolated phrenic nerve-diaphragm preparation. Twitches and tetani of the diaphragm elicited by stimulation of the phrenic nerve. At (○) dehydroemetine 0·15 mg/ml and at (○) neostigmine, 5 μg/ml. Between panels A and B, and B and C, the preparation was washed several times and rested for 15 min.

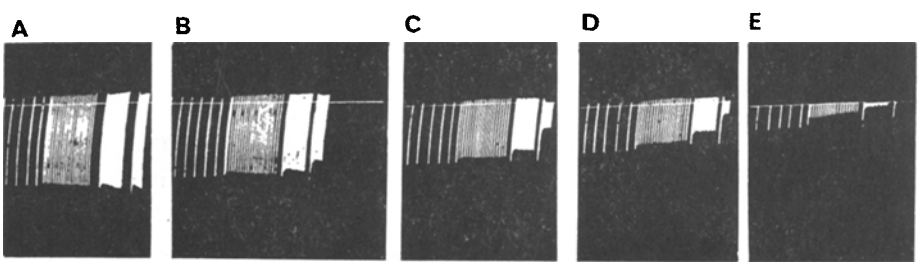

FIG. 4. Rat isolated phrenic nerve-diaphragm preparation. Indirect maximal twitches of the diaphragm due to stimulation of the phrenic nerve at 0·125, 0·5, 1 and 2 Hz successively. 20 shocks were given at each stimulus frequency but only the last 5 at 0·125 Hz are shown. A, control; B, C, D, E, 2, 5, 10 and 15 min respectively after adding 0·15 mg/ml dehydroemetine.

Dehydroemetine (1–5 mg/kg) had no effect on the indirect maximal twitches and tetani of the rat gastrocnemius and cat tibialis. The drug also had no effect on tubocurarine- or suxamethonium-induced neuromuscular block in the cat. Contractions of the tibialis muscle elicited by close-arterial injection of acetylcholine were not affected by dehydroemetine, and close-arterial injection of dehydroemetine had no effect on the maximal indirect twitches of the tibialis.

Cat blood pressure and nictitating membrane

Dehydroemetine inhibited the contractions of the cat nictitating membrane elicited by pre- or post-ganglionic stimulation of the cervical sympathetic (20 V; 0·5 ms), but had no effect on contractions due to injected noradrenaline (50 μg). The minimum effective dose of dehydroemetine was 3 mg/kg(i.v.). However, with doses of 3–5 mg/kg it was usually necessary to repeat the dose 2–3 times before obtaining an effect on the nictitating membrane but above 5 mg/kg, single doses caused a reduction. The doses of dehydroemetine which caused an inhibition of the contraction of the nictitating membrane also produced a prolonged fall in blood pressure associated with bradycardia and irregular heart beats, but whereas the animal usually recovered, at least partially, from the effect on blood pressure within 1 h, the effect on the nictitating membrane was not reversed after 3 h. The block was also not reversed by cocaine (0·5 mg/kg). The relation of the block to the frequency of stimulation was examined in 4 cats in which the preganglionic nerve was stimulated at 5 frequencies (5, 10, 20, 40 and 80 Hz), for 10 s at 2 min intervals, the cycle being repeated every 10 min. The heights of contraction of the nictitating membrane were measured before and 1 h after giving dehydroemetine. The results in each of the 4 cats showed that dehydroemetine (7 mg/kg) produced a parallel shift to the right of the curve relating the frequency of stimulation to the resulting height of contraction.

The blood pressure of cats anaesthetized with chloralose fell rapidly after intravenous doses of 0·5–3 mg/kg dehydroemetine. The effect was usually over in 2–10 min. Repetition of the dose at intervals of less than 30 min led to increasing effects. There was no change in heart rate. The fall in blood pressure was not influenced by vagotomy, atropine (1·0 mg/kg), hexamethonium (2 mg/kg), propranolol (2·5 mg/kg), phenoxybenzamine (2 mg/kg) and mepyramine (1 mg/kg). Doses of dehydroemetine of 5 mg/kg and above caused a more prolonged fall in blood pressure associated with bradycardia and irregular heart beats from which the animal sometimes failed to recover.

Unanaesthetized animals

No untoward systemic effect was observed in 12 rats given 1 mg/kg dehydroemetine daily for 10 days (This dose is similar to the therapeutic dose in man). The mean weight of the rats was not significantly different from that of 12 control rats. Four of the 12 treated rats developed necrosis of the skin with ulceration at the site of subcutaneous injection.

The three solutions of dehydroemetine (0·6, 6 and 30 mg/ml) injected intradermally into guinea-pigs caused anaesthesia lasting between 24 and 48 h. Necrosis of the skin at the site of injection of the 30 mg/ml solution was observed after 72 h. The same three solutions instilled into the conjunctival sac of 6 rabbits did not produce corneal anaesthesia while the 30 mg/ml and 6 mg/ml solutions caused oedema of the conjunctiva and blepharospasm lasting between 24 and 48 h.

DISCUSSION

Like bretylium & guanethidine (Day, 1962) dehydroemetine in small concentrations, inhibited the response to stimulation of sympathetic "adrenergic" neurons while the response to noradrenaline was unaltered or potentiated. Dehydroemetine however differs from these drugs in that the sympathetic blockade was not reversed by cocaine or (+)-amphetamine (Salako, 1970). That dehydroemetine blocked the response to stimulation at high frequencies before the response to stimulation at low frequencies, suggests that the action of dehydroemetine was not that of a local anaesthetic (Burn & Seltzer, 1965). Although intradermal injection of dehydroemetine in guinea-pigs produced local anaesthesia lasting for several hours, effective concentrations caused skin ulceration. In both those characteristics the anaesthesia produced by dehydroemetine differs from that produced by conventional local anaesthetics. The observed local anaesthesia may therefore be related to local irritant action.

Twitches of the guinea-pig ileum elicited by transmural stimulation of the parasympathetic nerves were also inhibited by small concentrations of dehydroemetine. This suggests that dehydroemetine affects both sympathetic and parasympathetic fibres alike. It thus differs from emetine which affects only the sympathetic fibres (Ng, 1966). But, in the simultaneously stimulated preparation, the sympathetic fibres were blocked more rapidly than the parasympathetic fibres. Dehydroemetine also exerts direct actions on the smooth muscle of the intestine. It diminished the tone and pendular movement of the rabbit isolated proximal ileum and reduced the spasmogenic effect of acetylcholine, histamine and nicotine on guinea-pig distal ileum. The inhibition of histamine and acetylcholine contractions of the ileum by dehydroemetine might suggest that the drug has specific antihistamine and atropine-like actions but the characteristics of the curves shown in Fig. 2 would indicate that the antagonism is probably non-specific.

Dehydroemetine inhibited the indirect contractions of the rat isolated diaphragm but at concentrations several times higher than those required for adrenergic neuron blockade. As shown in Fig. 3, the characteristics of the block produced by dehydroemetine are unlike that produced by either tubocurarine or decamethonium. The neuromuscular block produced by dehydroemetine is therefore more likely to be due to a presynaptic action either at the motor nerve endings or on the nerve.

The failure to demonstrate any neuromuscular blocking action for dehydroemetine in the intact animal even at the toxic dose of 5 mg/kg is significant since it would suggest that the drug has no specific neuromuscular blocking action, and the observed effect *in vitro* is then more likely to be due to a non-specific effect due to local accumulation of the drug at or near the motor nerve endings.

Similarly the dose of dehydroemetine required to inhibit contractions of the cat nictitating membrane elicited by pre- or post-ganglionic stimulation of the cervical sympathetic was much higher than the usual doses (1–3 mg/kg) that produce a reversible fall in blood pressure. The fall in blood pressure produced by these doses of dehydroemetine can therefore not be explained on the basis of adrenergic neuron blockade. On the contrary the observation that adrenergic neuron block cannot be demonstrated with therapeutic doses of dehydroemetine, and is irreversible, further strengthen the suggestion that the inhibition of the effect of sympathetic stimulation is not due to a specific adrenergic neuron blockade of the type associated with bretylium, guanethidine and xylocaine. In conclusion it is suggested that the inhibition of

sympathetic and cholinergic neurons readily demonstrated for dehydroemetine *in vitro* is due to a non-specific action on the nerves at or near the neuro-effector junctions.

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