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Some pharmacological properties of piperazine

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Summary

1. The action of piperazine on mammalian smooth, cardiac and skeletal muscles has been studied.

2. Piperazine increased tone and produced a dose dependent contraction of isolated smooth muscle, which was antagonized by atropine.

3. With cardiac muscle, piperazine depressed both the rate and force of contraction and was antagonized by atropine. At higher concentrations, a non-specific depression of cardiac muscle was found. The intravenous injection of piperazine produced a transient decrease in both heart rate and blood pressure and this was followed by an increase.

4. In about half of the mammalian skeletal muscle preparations, piperazine potentiated the twitch of the muscle evoked by electrical stimulation.

5. On the frog neuromuscular preparation, piperazine produced potentiation of the twitch but this was followed first by blockade of the effects of nerve stimulation and then by depression of the effects of direct muscle stimulation.

Introduction

Piperazine is a drug widely used in human and veterinary medicine against the intestinal roundworms, *Ascaris lumbricoides* and *Enterobius* (*Oxyuris*) *vermicularis*. It was first administered clinically at the turn of the century as a uriscosuric agent for the treatment of gout but without much success. It was noted, however, that piperazine had a very low toxicity in mammals, and its anthelmintic properties were reported by Fayard in 1949.

Norton & de Beer (1957), investigating the mechanism by which piperazine paralyses *Ascaris* muscle, showed that low doses of acetylcholine induced contractions of the parasite and that piperazine competitively antagonized these contractions. They concluded that piperazine acts as a myoneural blocking agent in this species, so enabling the paralysed worms to be expelled from the hosts' gut by normal peristalsis. The action was compared with that of (+)-tubocurarine on mammalian skeletal muscle. Recently Aubry, Cowell, Davey & Shevde (1970) showed that piperazine produced a depression of mammalian isolated neuromuscular preparations by a direct action on the muscle, and Hanahoe & Sturman (1971) confirmed this, reporting that potentiation of both *in vivo* and *in vitro* mammalian skeletal muscle twitches occurred with piperazine.

We have attempted to elucidate the mechanism of action of piperazine on muscle cells using several skeletal, smooth, and cardiac muscle preparations.

Methods

In vitro *preparations*

Guinea-pig ileum was used in Tyrode solution at 30° C, using a 90 s cycle with a 30 s drug contact time. Responses were recorded on a kymograph using an isotonic frontal writing lever (magnification × 8). Rabbit duodenum was set up in Tyrode solution at 37° C, using a 45 s drug contact time and 150 s time cycle. Innervated rabbit duodenum preparation, as first described by Finkleman (1930), was used in Krebs solution at 37° C; the nerve was stimulated supramaximally for 30 s duration with impulses of 0·5 ms duration and 0·5 Hz. Rat duodenum at 33° C in Krebs solution was set up using 120 s time cycle with a 30 s contact time.

The isolated phrenic nerve-diaphragm preparation of the rat (Bülbring, 1946) was used in Krebs solution at 37° C. The phrenic nerve was stimulated supramaximally using bipolar platinum electrodes at 0·1 Hz, and at 0·5 ms duration. The diaphragm was stimulated directly with supramaximal impulses of 1·5 ms duration and 0·2 Hz.

The frog isolated sciatic nerve-gastrocnemius muscle was suspended in a 40 ml organ bath containing frog Ringer at 22° C and gassed with air. The sciatic nerve was stimulated supramaximally at 0·1 Hz and 0·5 ms duration; the muscle was stimulated directly at 0·1 Hz and 1·5 ms duration. Contractions were recorded on a kymograph with a spring-loaded lever.

Guinea-pig isolated atria were set up in Locke solution at 39° C and 100% oxygen was used to aerate the preparation. Force and rate of contractions of the atria were measured using a Devices isometric force displacement transducer (type 2ST.02, 0–1 kg) and Devices heart rate meter respectively and displayed on a Devices pen recorder (M2).

The method for perfusing isolated rabbit and guinea-pig hearts as first reported by Langendorff (1895), was used. The hearts were perfused with Locke solution at 34° C which had previously been aerated with 100% oxygen. Contractions and rate of the heart beat were displayed on a Devices pen recorder (M2) with a force displacement transducer and heart rate meter respectively.

In vivo *preparations*

The effects of drugs on the blood pressure (1 mmHg≡1·333 mbar), heart rate and electrocardiogram of urethane anaesthetized (1·25 g/kg, i.p.), decerebrate or pithed rats (Sprague-Dawley, 200–500 g) were recorded. Carotid arterial blood pressure was recorded by a Bell and Howell physiological pressure transducer (ref no. 4-327-L 221) connected to a Devices pen recorder (M4). Lead II of the electrocardiogram was recorded. The heart rate was recorded, via a Devices rate meter, from the electrocardiogram or blood pressure. Intravenous injections were made through a cannula in a jugular vein. All drug solutions were washed in with 0·2 ml of saline. In some experiments the muscles of the hind limb were dissected free at the Achilles tendon. The leg was clamped in a horizontal position. The sciatic nerve was decentralized and the peripheral stump stimulated supramaximally with square wave pulses of 0·5 ms duration and 0·2 Hz. The resulting muscle contractions were recorded isometrically on a polygraph. Rats treated this way were artificially respired.

Cats were anaesthetized with chloralose (80 mg/kg, i.p.). The trachea was cannulated and the animals were artificially respired. Injections were made into a cannulated jugular vein. The blood pressure was monitored from a common carotid artery using a pressure transducer. The anterior tibialis and gastrocnemius muscles were dissected free at the ankle. The leg was clamped in a horizontal position by means of metal rods inserted into the femur and tibia. The sciatic nerve was decentralized and the peripheral stump was stimulated with supramaximal square wave pulses of 0·5 ms duration and 0·1 Hz. The resulting muscle contractions were recorded on a polygraph.

Drugs

Acetylcholine chloride; angiotensin; atropine sulphate and methyl bromide; barium chloride; bethanidine sulphate; chloralose; 1·1-dimethyl-l, 4-phenylpiperazinium iodide; diphenhydramine hydrochloride; eserine sulphate; guanethidine sulphate; hexamethonium bromide; histamine acid phosphate; 5-hydroxytryptamine creatine sulphate complex; isoprenaline sulphate; phentolamine methane sulphonate; piperazine hexahydrate and citrate; propranolol hydrochloride; tetraethylammonium chloride; tetramethylammonium chloride; urethane (ethyl carbamate).

All drugs used were dissolved in 0·9% saline or the appropriate Ringer solution and all drug concentrations are expressed in terms of their salts. Piperazine solutions were neutralized with hydrochloric acid before use.

The physiological solutions used were prepared according to Bowman, Rand & West (1968).

Results

Intestinal smooth muscle

At concentrations of below 200 μg/ml, piperazine hexahydrate potentiated acetylcholine-induced contractions on the guinea-pig ileum. At higher concentrations

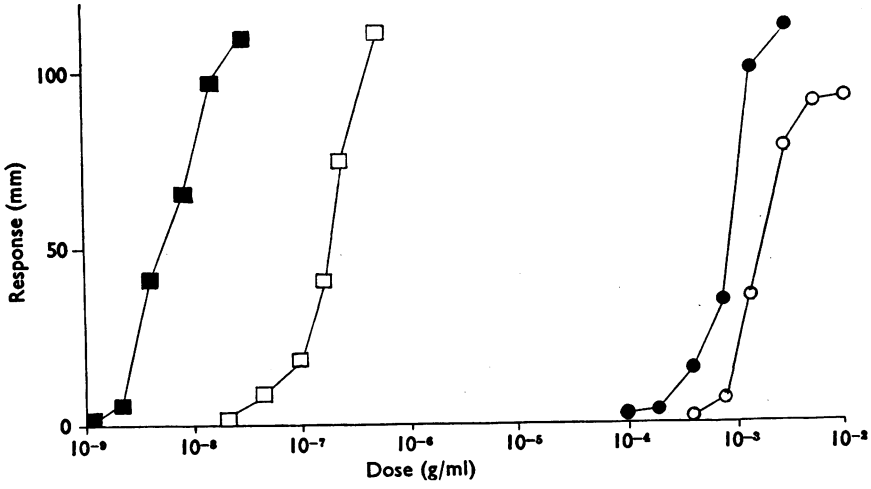

FIG. 1. Responses induced by piperazine hexahydrate alone (●——●) and in the presence of atropine (5 ng/ml) (○——○), and responses induced by acetylcholine alone (■——■) and in the presence of atropine (5 ng/ml) (□——□) on the guinea-pig ileum.

(up to 20 mg/ml), it produced dose dependent contractions which were antagonized by atropine (Fig. 1), but not by hexamethonium. Diphenhydramine also prevented these contractions but only in doses which were effective against contractions produced by acetylcholine. On the rabbit and rat duodenum preparations, piperazine (4 mg/ml) initially increased the tone of the muscle, but this was followed by a relaxation with simultaneous increase in both rate and amplitude of spontaneous movements. The increase in tone was antagonized by atropine (100 ng/ml), and the relaxation was markedly reduced by a mixture of phentolamine and propranolol (20 μg/ml of each). The relaxation produced by nerve stimulation was also antagonized to the same degree using this mixture.

Cardiac muscle

On both the isolated atria and the perfused heart, piperazine hexahydrate produced a slowing of the rate and a reduction in the force of contraction. This effect was slower in onset than the action of acetylcholine on cardiac muscle but was antagonized by atropine. On increasing the concentration of piperazine, direct muscle depression occurred. Results from the perfused rabbit heart experiments are tabulated (Table 1).

On intravenous injection of piperazine hexahydrate (100–1,000 mg/kg) into the anaesthetized rat, there was an immediate transient change in the electrocardiogram pattern. Generally this alteration was a depression of the QRS complex and an inversion of the T wave.

Blood pressure and heart rate

In the anaesthetized cat and rat preparations, intravenous doses of piperazine hexahydrate lowered the blood pressure and reduced the heart rate, and this was followed by a transient increase in both (Fig. 2). The depressor component was reduced and the fall in heart rate was blocked by atropine methyl bromide (Fig. 2). Piperazine injected intravenously into decerebrate or pithed rats produced a similar pattern of changes as in the anaesthetized animals. Adrenalectomized anaesthetized animals still exhibited these responses. However, these actions of piperazine were completely antagonized by phentolamine, guanethidine and bethanidine (Fig. 2). Hexamethonium and mecamylamine were ineffective in modifying the responses when used in doses which completely antagonized the pressor responses to the ganglion stimulants, dimethylphenylpiperazinium and tetramethylammonium. These results indicate that the pressor component of the response produced by piperazine on the blood pressure is due to an action on postganglionic sympathetic nerves.

Skeletal muscle

In about half of the cat and rat *in vivo* experiments, piperazine (50–300 mg/kg i.v.) produced a potentiation (up to 50% increase) of the contraction of the muscles

TABLE 1. *Effect of different doses of piperazine hexahydrate alone, and in the presence of atropine (1 μg/ml), on the force of contraction of the isolated perfused rabbit heart preparation*

Piperazine hexahydrate (mg)	Force of contraction (% reduction) alone	with atropine
20	10	0
80	31	0
160	40	0
320	55	17
640	73	75

of the legs, with no subsequent depression. This potentiation lasted for several minutes (Fig. 3). When piperazine (200 mg/kg) was injected retrogradely via a

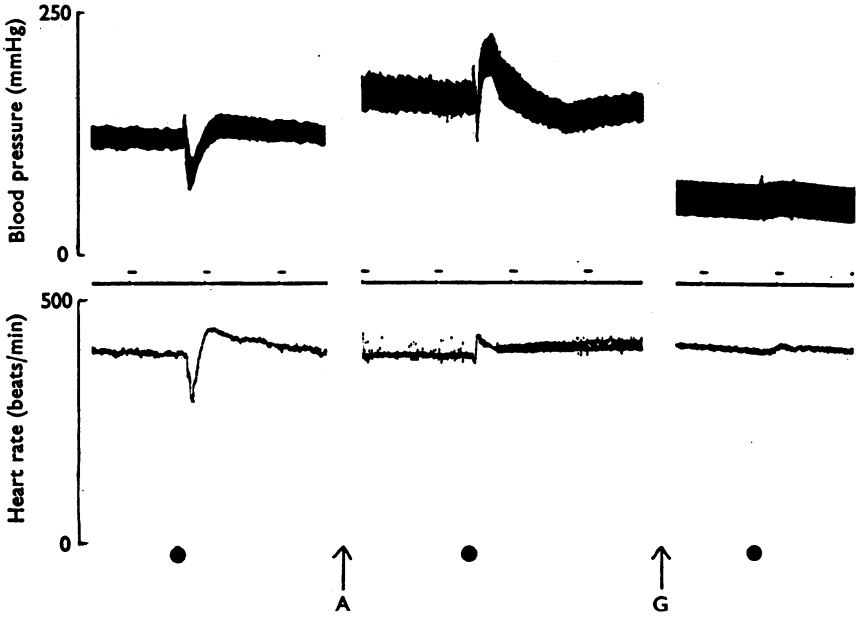

FIG. 2. Effect of atropine methylbromide (A) (4 mg/kg) and 1 h later guanethidine sulphate (G) (3 mg/kg) on response to piperazine hexahydrate (●) (15 mg/kg) on the blood pressure (upper tracing) and heart rate (lower tracing) of the anaesthetized rat.

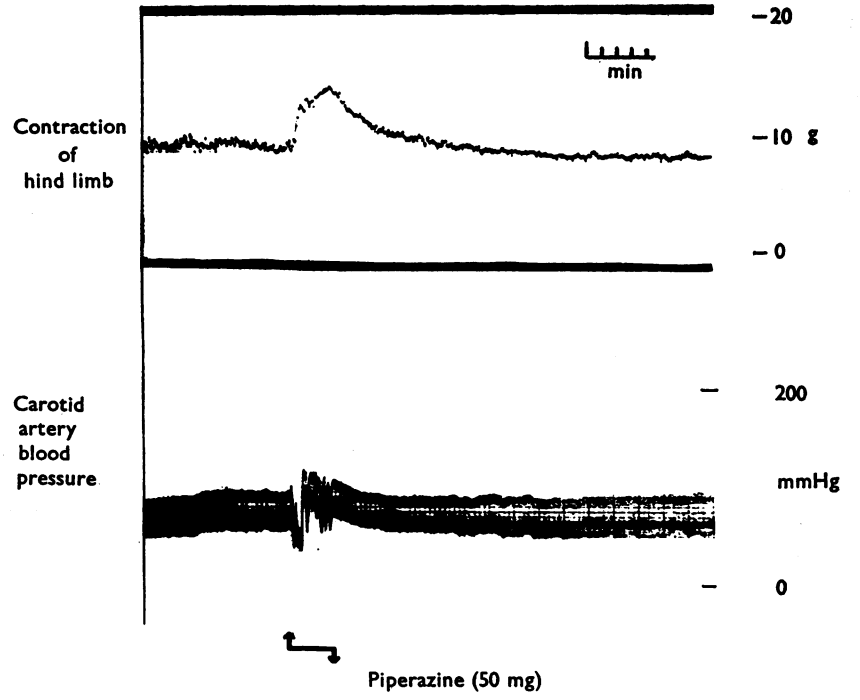

FIG. 3. Effect of piperazine (50 mg, i.v.) on the contraction of the muscles of the hind limb of the anaesthetized rat, induced by sciatic nerve stimulation. The sciatic nerve was stimulated by square wave pulses of 0·5 ms duration and supramaximal voltage at a rate of 0·2 Hz.

branch of the femoral artery to the muscles of the leg, a potentiation followed by a depression occurred. On the rat isolated phrenic nerve-diaphragm preparation and the frog isolated sciatic nerve-gastrocnemius muscle preparation piperazine (100 μg/ml) produced potentiation of the muscle twitches. This potentiation occurred both to nerve stimulation and to muscle stimulation and lasted up to 20 min, before gradually decreasing and finally producing depression of the contractions. In the frog preparation, however, piperazine inhibited the effect of nerve stimulation several minutes before it effected that of direct muscle stimulation. This two stage blocking action of piperazine in the frog is in direct contrast to the blocking action found in the rat.

An attempt was made to elucidate the mechanism of the potentiation of contraction of skeletal muscle by piperazine. The effect *in vivo* was accompanied by a transient alteration in the blood pressure and so catecholamines, acetylcholine, histamine and angiotensin were each injected intravenously to study whether or not this effect was due to an alteration in the blood flow to the muscles. However, when each of these drugs was used at doses which produced marked changes in the blood pressure, there was no alteration in contraction of skeletal muscle.

Discussion

The results from this study show that piperazine is inactive on mammalian tissues except at relatively high concentrations or doses. Norton & de Beer (1957) reported that the action of piperazine on *Ascaris* is at the neuromuscular junction. However, Aubry *et al.* (1970) and Hanahoe & Sturman (1971) showed that there is no neuromuscular blocking action in mammals and only on *in vitro* preparations was any direct muscle depression detected. In the present work, both *in vitro* and *in vivo* preparations showed a potentiation of the muscle twitches, and this action of piperazine may be analogous to the dose dependent contracture produced in the frog and leech (Hanahoe & Sturman, 1971). On investigation of the action of piperazine on the frog neuromuscular preparation, it was found that after slight potentiation of the muscle twitches there was a two-stage blockade, firstly on neuromuscular transmission and then directly on muscle. Norton & de Beer (1957) and Broome (1962) have reported pharmacological differences between mammalian and *Ascaris* neuromuscular systems and Bueding (1962) suggests that the frog occupies an intermediate position.

Intravenous injections of piperazine had a biphasic effect on rat and cat blood pressure and heart rate; there was initially a depression accompanied by bradycardia and this was followed by a pressor component accompanied by tachycardia. As these actions were observed in the anaesthetized, decerebrate and pithed animals, piperazine was not acting centrally. In addition, atropine sulphate which penetrates the brain, had a similar action to that of its quarternary salt (methyl bromide) which does not. Rollo (1966) reported that on intravenous injection, piperazine produced a transient fall in the blood pressure, and we found that the depressor action was antagonized by anticholinergic doses of atropine. Also it was found that guanethidine and bethanidine antagonized the rise in blood pressure. However, ganglion blocking drugs were found to have very little effect in modifying these cardiovascular responses. As there was no change in the characteristic piperazine response in the adrenalectomized animal, the blood pressure and heart rate changes produced by intravenous injections of piperazine were not the result of adrenal stimulation.

We conclude that piperazine has a stimulant action on the post-ganglionic neurones, either causing depolarization, facilitating normal impulse traffic, or increasing transmitter output, and not by an action on the central nervous system.

The results from the *in vitro* smooth muscle preparations suggest that piperazine may cause release of transmitter from para-sympathetic nerves also. Low concentrations of piperazine were found to potentiate acetylcholine contractions, while larger concentrations produce contractions of their own. The actions of piperazine on guinea-pig ileum preparations were only antagonized by compounds having anti-muscarinic properties. On isolated sympathetically innervated tissues, the action of piperazine hexahydrate was prevented by α- and β-adrenoceptor blocking agents but not by ganglion blocking agents, suggesting that stimulation of sympathetic postganglionic nerve fibres occurs. Our results with the rat and rabbit duodenum agree with those obtained by Krotov, Davydov & Abramova (1969). However, the use of selective blocking agents enabled us to examine the effects of piperazine on these tissues at a greater depth. We attribute the 'spasmolytic' effect described by Krotov *et al.* to piperazine causing stimulation of sympathetic neurones and the 'histaminic' effect to stimulation of parasympathetic nerves.

On investigating the action of piperazine on cardiac muscle, negative inotropic and chronotropic effects were produced. Low doses produced effects which were antagonized by atropine whereas higher doses produced a direct cardiac depression. These negative inotropic and chronotropic effects produced by piperazine were slow in onset when compared with those of acetylcholine.

In clinical studies on piperazine, only rarely have toxic symptoms been reported and these invariably have been in the form of central nervous system stimulation such as tremor, incoordination and psychic disturbances (Brown, Chan & Hussey, 1956; Savage, 1967; Jakubowska, Lebensztejn, Pedich, Rudzinski & Wollna, 1968). Savage (1967) has observed an abnormal electroencephalogram pattern with piperazine in toxic doses, but all authors agree that any neurological lesion is at risk during piperazine therapy and that epileptic attacks may be generated. Another side-effect of piperazine therapy which is less rarely seen is that of diarrhoea. The action of piperazine on the human alimentary canal is similar to that found on isolated mammalian intestinal preparations, namely potentiation of inherent movements, an action which probably aids the expulsion of roundworms from the host. Thus, the doses of piperazine used therapeutically paralyse the worms and may have an effect on the mammalian systems only at the sites where high concentrations are reached (that is the gastrointestinal tract).

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