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 uricosuric 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 paralysis 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 paralyzed 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 (Bülbstring, 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-1, 4-phenylpiperazinium iodide; diphenhydramine hydrochloride; eserine sulphate; guanethidine sulphate; hexamethonium bromide; histamine acid phosphate; 5-hydroxytryptamine creatine sulphate complex; isoprenaline sulphate; phenotolamine methane sulphonate; piperazine hexahydrate; 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
In 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.

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).

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 phenolamine, 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, dimethylylpiperazinium 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

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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
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

![Graph](image_url)

**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.

![Graph](image_url)

**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 brady-
cardia 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 quaternary 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 guanethi-
dine and bethanidine antagonized the rise in blood pressure. However, ganglion
blocking drugs were found to have very little effect in modifying these cardio-
vascular 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 antimuscarinic properties. On isolated sympathetically innervated tissues, the action of piperazine hexahydrate was prevented by \( \alpha \) - and \( \beta \)-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 'spasmylic' 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).

REFERENCES


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