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Full Length Research Paper

Pharmacodynamic effect of piperazine citrate on the blood pressure of anaesthetized cat

Samuel Ghasi*, Chioli P. Chijioke and Raphael Anakwue

Department of Pharmacology and Therapeutics, College of Medicine, University of Nigeria, Enugu, Nigeria.

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Piperazine citrate produced a dose-dependent decrease in the blood pressure of the anaesthetized cat. Both 15 mg and 30 mg/kg piperazine showed average significant reduction in blood pressure of 29.8 ± 1.65 and 78.3 ± 3.18 mm Hg respectively. The effect produced in each case was transient and returned to baseline value within 2 min. The reduction in blood pressure caused by piperazine 30 mg/kg was statistically significant compared to the average due to piperazine 15 mg/kg (P < 0.0001). The maximum falls over the baseline blood pressure were also determined to be 34 and 82 mm Hg respectively. Adrenaline 5 µg increased the blood pressure of the cat by 68 mmHg and this effect was antagonized by equipotent doses of piperazine, nifedipine and propranolol to varying degrees. The antagonism produced by equipotent doses of piperazine (15 mg/kg) or nifedipine (200 µg/kg) to blood pressure elevation by adrenaline was quite small (8.8%), compared to propranolol (10µg) which antagonized the vasopressive action by as much as 26.5% (68 - 50 mmHg). Piperazine although severely reducing the blood pressure when given intravenously to the anaesthetized cat as shown in this study, may not be an effective antihypertensive agent as its hypotensive effect is always very transient. This would rather be seen as an untoward effect and in any event that may demand that piperazine be given intravenously, the patients should be warned of hypotension and dizziness as possible adverse effects.

Key words: Piperazine citrate, anaesthetized cat, blood pressure.

INTRODUCTION

Piperazine is a cheap and readily available anthelmintic agent with very wide therapeutic index. It has been shown to have non-specific non-vascular smooth muscle relaxant activity brought about by its ability to directly depress the smooth muscle (Onuaguluchi, 1966). In the mammalia, piperazine has been shown to have a direct non-specific, non-vascular smooth muscle relaxant action as it inhibits barium chloride, histamine, 5HT and acetylcholine- induced contractions in the guinea-pig ileum and rabbit duodenum by a direct smooth muscle depressant action (Onuaguluchi, 1966, 1981, 1984). It also antagonized effect of adrenaline on the guinea-pig vas deferens and oxytocin induced contractions in the rat uterus. Also, piperazine was shown to decrease the rate and force of contraction of the isolated frog heart and the rabbit heart Langendorff preparation (Onuaguluchi, 1966). It was therefore suggested that piperazine could have a quinidine-like action, and therefore could possess antiarrhythmic properties. Consequently, ECG patterns of piperazine citrate in both human volunteers and laboratory animals, and the antiarrhythmic potentials of the drug in anaesthetized rats were investigated in our laboratory (Onuaguluchi and Ghasi, 2006; Ghasi and Onuaguluchi, 2007; Ghasi, 2008). In these studies piperazine citrate was shown to exhibit antiarrhythmic activity by prolonging repolarization as it decreased the heart rate of the rat but significantly prolonged the duration of the P-R, Q-Tc, and J-T intervals. It, however, had no effect on the QRS complex. These ECG changes were identical to the characteristics of class three anti-arrhythmic drugs according to Vaughan Williams' classification of the antiarrhythmic agents (Vaughan Williams, 1984). Many antiarrhythmic agents are known to affect the blood pressure (Roden, 2001). Therefore, the effect of piperazine on the blood pressure of the anaesthetized cat was undertaken in this series.

*Corresponding author. E-mail: samuelghasi@yahoo.com.

MATERIALS AND METHODS

Four cats weighing between 1.7 and 2.1 kg were used for the study. Anaesthesia was induced through intraperitoneal administration of thiopentone 50 mg/kg body weight and the animal placed in supine position on the Brown-Schuster myographic table (C.F. Palmer, London). The limbs were fastened to the table by means of strings and the neck shoved with a sharp razor blade. The longitudinal muscle in front of the trachea was exposed via a skin incision with a pair of scissors.

Cannulation of the trachea

The trachea was freed from the surrounding connective tissue using a curved iris forceps, and two ligatures passed round the trachea but not tied. A cut was made on the trachea to allow the insertion of a trachea cannula. The proximal ligature was tied and pulled towards the head. This facilitated the insertion of the cannula and the distal ligature was then tied around the cannula to keep it properly aligned. The insertion of a trachea cannula was done as a precautionary measure so that if severe drug-induced respiratory depression or failure occurred the cat could be alive by means of artificial respiration and for recording of the animals' respiration on the rotating drum. This was made possible by connecting the cannula through rubber tubing to a tambour. The amount of air entering and leaving the cannula was controlled by means of an adjustable valve.

Cannulation of the femoral vein

Fur was cleared along the inguinal region close to the abdominal wall. The skin in this region was dissected, and the femoral vein was then exposed at about the junction where the saphenous vein empties into it. By means of blunt dissection, the inguinal ligament was freed of connective tissue and all feeding venules to the femoral vein tied off. Femoral vein of a suitable length was exposed and placing a bulldog clip at the proximal end occluded the blood flow. Two threads (3/0) were carefully passed around the vein and half tied. The vein was then made to distend fully by using a finger to push blood towards the bulldog clip. The distal threads was next firmly tied and held taut as to stretch the vein slightly.

A small cut was made with a sharp pointed scissors not more than half way through the vein as near to the tied thread (that is, the distal thread) and as far from the bulldog clip as possible. The cut was made at an angle of about 30° so that there was a small flap, which can be lifted up with a seeker while a fine plastic cannula (1.5 mm external diameter) was inserted and connected to a three way stop-clock which allowed the injection of the drugs. The proximal thread was tied around the cannula to keep it aligned. The cannula and the tubing connected to it were filled with heparinized saline (50 i.u. /ml) before insertion. The essence of this procedure was to prevent blood clotting within the vessels. Air bubbles were carefully excluded completely.

Cannulation of the carotid artery

The procedure for the cannulation of the femoral vein was also employed for the cannulation of the carotid artery.

Two ligatures (3/0) were passed round the exposed and cleared length of the carotid artery. The proximal thread (close to the head and away from the heart) was thereafter tied and when the artery became engorged with blood the bulldog clip was placed at the end close to the heart. An arterial cannula (3.5 mm external diameter) was now prepared. It was connected to a mercury manometer through a three-way stop clock with a syringe filled with heparinized saline. All air bubbles in this system were flushed out with the heparinized saline and arterial cannula shut off by means of the tap off the three-way stop-clock.

A slanting cut was made on the artery between the proximal thread and the bulldog clip. The cannula was then inserted and tied into place. The pressure in the manometer was raised by means of the syringe to the expected blood pressure of the animal. The stop clock was set so that the syringe was closed off and the arterial cannula was connected to the recording device. The bulldog clip was then removed. The animal was given heparin (1000 i.u. /kg) intravenously.

Recording device used

Changes in blood pressure of the animal were recorded with mercury manometer; a simple 'U' tube of about 5 mm bore. It was a simple device that gave direct readings via the pulsation of the mercury column. This was connected via a writing lever to a smoked paper on a Brodie-Startling Kymographic drum rotating at a speed of 16 mm per minute.

Drug administration

The animal was left for at least 15 min after the cannulation before any drug was injected. Drugs were administered through the cannulated femoral vein in volumes not exceeding 0.2 ml/kg. Heparin (0.2 - 0.4 ml of 5000 i.u. /ml) was given and the animal left for about 15 min before any drugs were injected. After each dose of drug, the drug was flushed in with normal saline and the blood pressure due to the drug was allowed to return to the equilibration value before the injection of the next dose. The drugs studied were, piperazine citrate (15 and 30 mg/kg), propranolol 10 µg/kg, and nifedipine 200 µg/kg body weight. Effects of the listed drugs on the adrenaline-induced increases in the blood pressure of the cat were also investigated. Piperazine citrate powder was weighed and dissolved in normal saline to get the two different doses of 15 and 30 mg/kg body weight. On the other hand, injections were used in the case of propranolol and nifedipine and the final concentrations were obtained by dilution.

Statistical analysis was performed using computer-assisted Graph Pad Prism. Averages were expressed as arithmetic means ± standard error of the mean (SEM). Student's t-tests were performed comparing the results obtained after piperazine was administered with pre-treatment baseline values for each dose, as well as the two different doses of piperazine, and a P value of <0.05 was taken as indicating a statistically significant difference.

RESULT

Four cats weighing between 1.7 and 2.1 kg were used in this study. Evaluations using Student's't' test showed that piperazine caused a concentration dependent reduction in blood pressure of the anaesthetized cats ($P < 0.0001$). At 15 mg/kg, piperazine produced a transient but significant fall of between 29 and 34 mm Hg over the baseline values, with an average reduction in blood pressure of 29.8 ± 1.65 mm Hg. The percent blood pressure reductions caused by this dose of piperazine over the baseline values were found to be in the range of 20.8 - 25.9%. In every case, the effect produced did not last for more than 2 min before returning to the baseline value. When the dose of piperazine was increased to 30 mg/kg,

Table 1. Effect of piperazine on blood pressure of anaesthetized cat. Results are expressed as fall in blood pressure (BP) over baseline values. N = 4.

	Decrease in BP from the baseline value (mmHg)					
	Number of Cats				Mean ± SEM	p Value
Dose (mg/kg)	1	2	3	4		<0.0001
15	29	34	30	26	29.8 ± 1.65	
30	72	82	81	-	78.3 ± 3.18	

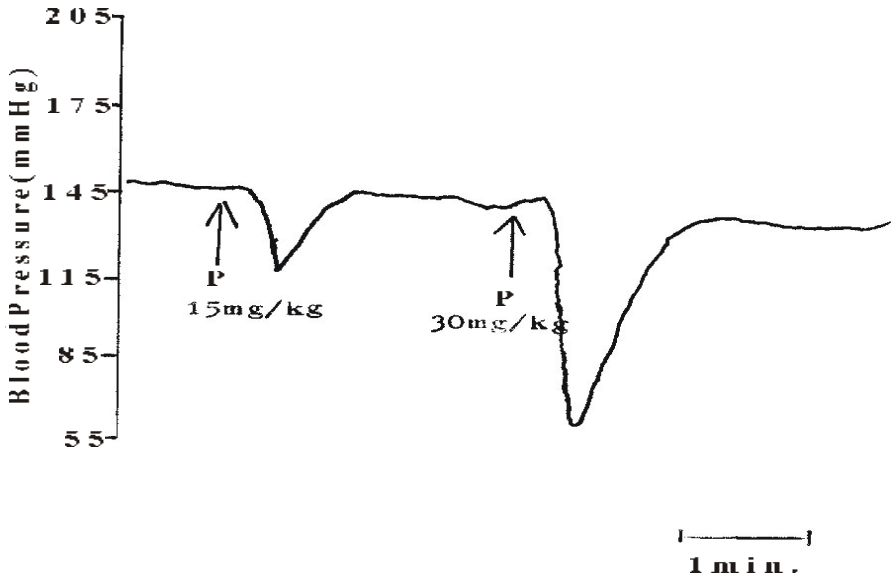

Figure 1. Manometer tracing showing the effect of two different doses of piperazine on the blood pressure of anaesthetized cat. Lowering of blood pressure was dose-dependent and statistically significant for the two doses of piperazine 15 and 30 mg/kg body weight.

it produced marked fall in the blood pressure of between 72 and 82 mm Hg over the baseline values with an average fall of 78.3 ± 3.18 mm Hg which was statistically significant compared to the average due to piperazine 15 mg/kg ($P < 0.0001$). The maximum fall over the baseline blood pressure was 82 mm Hg, from 140 to 58 mm Hg. Similarly, a minimum fall of 72 mm Hg (134 - 62 mmHg) was produced by piperazine, 30 mg/kg. The range in terms of percentage reduction was determined to be between 51.2 and 58.6% of the corresponding baseline values. The fall in blood pressure was again transient lasting not more than 2 min before returning to the baseline.

Table 1 shows the effects of increasing doses of piperazine on the blood pressure of the anaesthetized cat, while Figure 1 is a tracing showing reduction of blood pressure by piperazine 15 and 30 mg/kg.

Equipotent doses of piperazine, nifedipine and propranolol were determined to be 15 mg/kg, 200 µg/kg and 10 µg/kg respectively. Piperazine 15mg/kg caused a fall in the blood pressure of 28 mmHg (104 to 76 mmHg); a percent reduction of 26.9. With respect to nifedipine and propranolol, the cat's blood pressure showed decreases of 36 mmHg (108 – 72 mmHg) and 24 mmHg (108 - 84mmHg); percent reductions of 33.3 and 22.2 respectively. Figure 2 is a tracing showing reduction in blood pressure elicited by equipotent doses of piperazine, nifedipine and propranolol. It was observed that whereas the hypotensive action of piperazine was always transient, not lasting more than two minutes before returning to the baseline, reductions due to nifedipine and propranolol took much longer time before assuming the baseline values.

Adrenaline 5 µg increased the blood pressure of the cat by 68 mmHg and this effect was antagonized by the equipotent doses of piperazine, nifedipine and propranolol to varying degrees (Figure 3). The antagonism produced by both piperazine 15 mg/kg and nifedipine 200

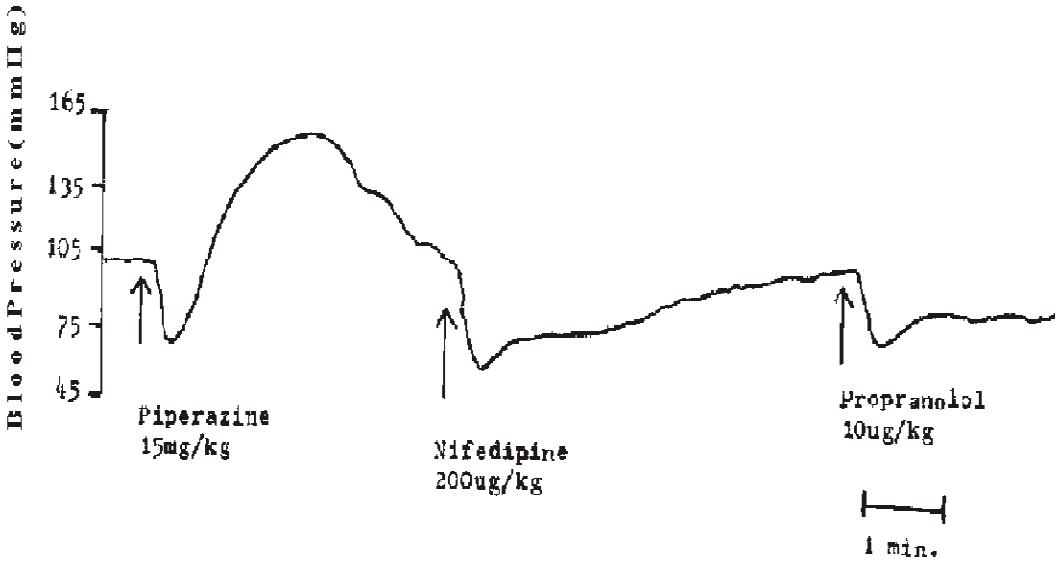

Figure 2. Effects of piperazine 15 mg/kg, nifedipine 200 µg /kg and propranolol 10 /µg kg on the blood pressure of anaesthetized cat. The three doses were found to be equipotent as they decreased the blood pressure by about the same magnitude.

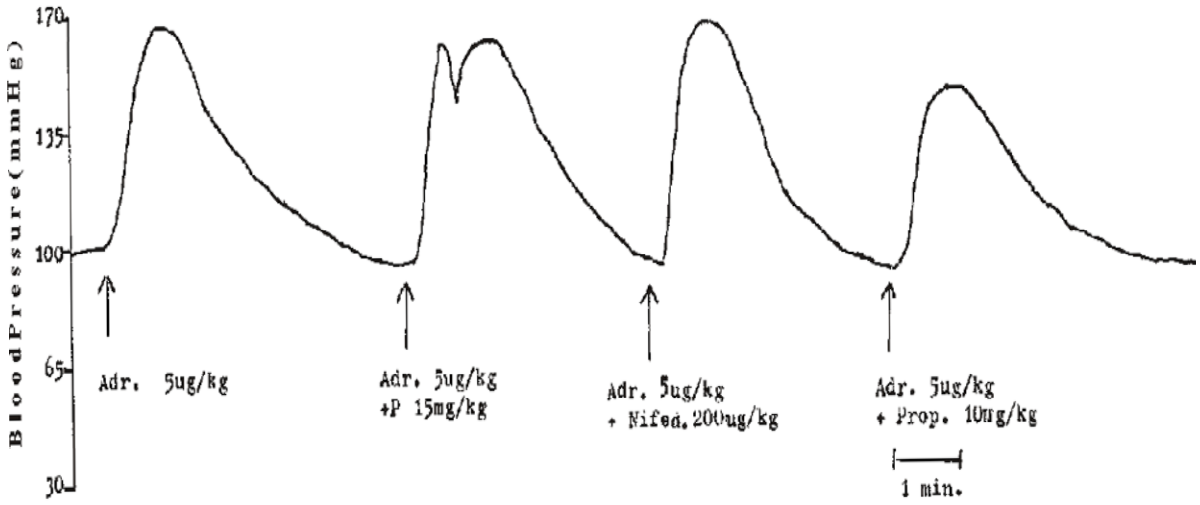

Figure 3. Manometer tracing showing the effects of equipotent doses of piperazine (P), nifedipine (Nifed.), and propranolol (Prop.) on adrenaline (Adr.)-induced increases in blood pressure of anaesthetized cat. In each case, adrenaline was administered first followed immediately afterwards by the antagonizing drugs. Only propranolol antagonized the pressor effect of adrenaline significantly.

µg/kg (68 – 62 mmHg in each case) was very small when administered concomitantly; a mere 8.8% reduction of the increase in blood pressure caused by adrenaline alone. Conversely, propranolol 10 µg antagonized the vasopressive action of adrenaline by as much as 26.5% (68 - 50 mmHg).

DISCUSSION

Piperazine citrate caused dose-dependent decreases in the blood pressure of anaesthetized cat ($P < 0.0001$). The increases were significant but transient in all cases as the fall always returned to its original position within two minutes. Piperazine 15 mg/kg, Nifedipine 200 µg/kg and propranolol 10 µg/kg produced hypotensive effects of equal magnitude. However, there was a basic difference in the duration of the hypotensive effect produced by nifedipine and propranolol on one hand and that due to piperazine on the other hand: whereas the fall in blood pressure caused by piperazine was very transient, nifedi-

pine and propranolol produced a more sustained fall in blood pressure that returned to the original level over a period of five minutes. Therefore, piperazine is not likely to be useful as an antihypertensive agent because the fall in blood pressure would not be sustained.

Piperazine and nifedipine failed to antagonise the adrenaline-induced increases in blood pressure unlike propranolol. It is therefore unlikely that piperazine produced its hypotensive action by blocking the β-adrenergic receptors. Nifedipine is a known calcium channel blocker with dominant effect on the smooth muscle. It blocks the Ca^{2+} channel, inhibiting the entry of extracellular Ca^{2+} and, therefore, binding of Ca^{2+} to the protein calmodulin (Kerins et al., 2001). The consequence of this is that myosin light chain kinase is not activated and the light chain of myosin is, therefore, not phosphorylated to promote interaction between actin and myosin which is essential for the contraction of the smooth muscle. Since equipotent doses of the drugs had the same minimal percent inhibition of the adrenaline-induced increase in blood pressure, it is suggested that similar mechanism of action involving inhibition of Ca^{2+} is central in the mode of action of piperazine in lowering of the blood pressure of the cat.

Because piperazine had negligible inhibitory effect on the blood pressure when given concomitantly with adrenaline, unlike the effect produced by equipotent dose of propranolol, it can be concluded that piperazine does not reduce blood pressure by predominantly blocking the β_1-adrenoceptors in the heart leading to direct depression of myocardium and lowering of heart rate or by stimulating the β_2-adrenoceptors. It is very likely that piperazine blocks the potassium channels, and since high external concentrations of K^+ promotes influx of Ca^{2+} through voltage-sensitive, or "potential-operated" Ca^{2+} channels (Bevan et al., 1982), its blockage would produce the opposite effect. Piperazine may also block the α-adrenoceptors within the vessels to some degree. Both of these physiological effects would result in vasodilatation which would account for the remarkable fall in blood pressure in the absence of adrenaline. The negative chronotropic and inotropic effects seen with piperazine citrate (Onuaguluchi, 1966) are likely due to prolongation of repolarization as a consequence of blocking of the myocardium potassium channels.

Smith (1961), in his analysis of effects of drugs on the cardiovascular system, suggested that any hypotensive drug which produced a fall in blood pressure that lasted for less than two minutes was likely to be producing the fall either through direct vascular smooth muscle relaxation, or direct depressant action on the myocardium, but certainly not through the autonomic nervous system.

Although piperazine was shown in this study to severely reduce the blood pressure when given intravenously to the anaesthetized cat, this may not likely be the case if the drug were administered orally. However, in any event that may demand that piperazine be given intravenously, the patients should be warned of hypotension and dizziness as possible adverse effects.

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