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SOME PHARMACOLOGICAL EFFECTS OF GRIFFONIN

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ABSTRACT

Griffonin stimulated intestinal smooth muscle preparations from the guinea-pig and the rabbit. It also stimulated pregnant and non-pregnant rat uterus preparations. The responses produced by griffonin in these preparations were dose-dependent and were antagonised by atropine. Low doses of griffonin produced a reduction in the force of contraction of the isolated rabbit perfused heart preparation. The reduction in force of contraction of the heart was antagonized by atropine. High doses of griffonin produced irreversible reduction in force and rate of contraction of the heart followed by death of the heart preparation. Griffonin also caused atropine-sensitive depressor effect on the systemic blood pressure of the anaesthetized cat. Griffonin (up to 6.4 mg/ml) had no effect on isolated skeletal muscle preparations and the isolated guinea-pig auricle.

Keywords: Griffonin, *Griffonia simplicifolia*, glycoside, antisickling, muscarinic, hypotensive.

INTRODUCTION

The plant *Griffonia simplicifolia*, (Baill), (Twi: Kagya; Ga: Kanya; Ewe: Gbogbouri) of the family Caesalpinaceae is used for various ailments. The leaves are fed to sheep and goats to stimulate reproduction. The juice of the leaves is used as an enema for the treatment of kidney troubles. A decoction of the leaves is used as an antiseptic to aid in the healing of suppurating wounds, to stop vomiting, diarrhoea and to treat congestion of the pelvis. The decoction is also used as an aphrodisiac [15]. A decoction of the roots is reported to be used to control sickle-cell crisis in Nigeria [9].

Griffonin, a nitrile glycoside, was first isolated by Dwuma-Badu, Watson, Copalakirshina, Okarter, Knapp, Schiff, Jr. and Slatkin in 1976 from the roots of *Griffonia simplicifolia* [10]. Preliminary pharmacological investigations have shown that griffonin has ganglion blocking activity and that it produces an initial increase in the force of the heart beat followed by a reduction in the force and rate of contraction of the isolated rabbit perfused heart preparation. Haematological studies have also shown that griffonin has antisickling effect on *in vitro* sickled red blood cells reverting the sickled cells to normal size and shape [1, 9].

The aim of the present work is to study further the pharmacological effects of griffonin.

MATERIALS AND METHODS

Extraction of Griffonin

One kilogram of dried, powdered root bark of *Griffonia simplicifolia* was refluxed with 8 litres of 95% alcohol for 2 hours. The alcoholic extract was concentrated by evaporation *in vacuo* at 65°C to about a tenth of its original volume. The concentrate was left to stand overnight and white flaky crystals of griffonin precipitated out. The crystals were filtered *in vacuo* and successive amounts of chloroform were used to wash the crystals until all the brown colour had been discharged. The crystals were left to dry on a filter paper and then they were refluxed for one hour in 200ml petroleum ether to remove all impurities. Griffonin was recrystallised from 90% methanol in water.

The melting point of the crystals obtained was determined and a comparative T.L.C. was run using a pure sample of griffonin provided by Professor Dwuma-Badu as standard and the Rf values were calculated. The Infra-red spectrum was also run for the crystals obtained using Philips Infra-red spectrophotometer (Model PU 9700). The peaks obtained were compared with the published results obtained by Dwuma-Badu and colleagues [10]. The results confirmed that the crystals obtained were griffonin.

Guinea-pig Ileum

Guinea-pigs weighing between 350g and 400g were stunned by a blow on the back of the head and bled. The ileum was removed and pieces of about 2cm length were cut from the distal end after discarding approximately 10m nearest to the ileo-caecal junction. The tissue was suspended in a 10ml organ bath containing Tyrode physiological solution (Composition: NaCl 137mmol/1, KCl

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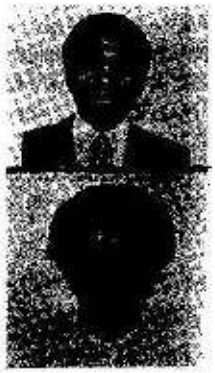

2.7mmol/l, $CaCl_2$ 2.2mmol/l, $MgCl_2$ 0.5mmol/l, NaH_2PO_4 0.4 mmol/l, $NaHCO_3$ 1.9mmol/l, glucose 5.5mmol/l), aerated with air and maintained at 32°C. Contractions were recorded isotonically on smoked paper by means of an isotonic frontal writing lever. The resting tension was 0.5g.

Rabbit Duodenum

Pieces of duodenum obtained from rabbits weighing between 1.5 kg and 2.0kg were set up in a 10ml organ bath containing Locke solution (Composition: NaCl 154mmol/l, KCl 5.6mmol/l, $CaCl_2$ 2.2 mmol/l, $NaHCO_3$ 6.0mmol/l, glucose 11.1mmol/l) maintained at 37°C and bubbled with air. Contractions were recorded isotonically on smoked paper by means of an isotonic frontal writing lever. The resting tension was 1g.

Rat Uterus

Non-pregnant adult female albino rats (Wistar Strain) weighing between 120g and 150g, pretreated with 0.1µg/kg of stilboestrol 24 hours earlier were used for these experiments. Longitudinal pieces of uterus were set up in a 10ml organ bath containing De Jalon solution (Composition: NaCl 154 mmol/l, $NaHCO_3$ 6.0 mmol/l, KCl 5.6 mmol/l, $CaCl_2$ 0.54 mmol/l, glucose 2.75 mmol/l) maintained at 28°C and aerated with air. A resting tension of 0.5g was applied to the tissues and isotonic contractions were recorded on smoked paper by means of an isotonic frontal writing lever. The procedure was repeated using pregnant rats. The pregnant rats were not pretreated with stilboestrol.

Toad Rectus Abdominis Muscle

Rectus abdominis muscle isolated from toad was set up in a 10ml organ bath containing Ringer solution (Composition: NaCl 111 mmol/l, KCl 1.9 mmol/l, $CaCl_2$ 1.1 mmol/l, NaH_2PO_4 0.1 mmol/l, $NaHCO_3$ 2.4 mmol/l, glucose 11.1 mmol/l) maintained at room temperature and bubbled with air. Contractions were recorded isotonically on smoked paper by means of an isotonic frontal writing lever. The resting tension was 1g.

Chick Biventer-Cervicis Muscle [12].

Biventer-cervicis muscles obtaiend from 5 to 10-day old chicks were set up in a 10ml organ bath containing Krebs solution (Composition: NaCl 118 mmol/l, KCl 4.7 mmol/l, $CaCl_2$ 2.5 mmol/l, $MgSO_4$ 1.2mmol/l, NaH_2PO_4 1.2 mmol/l, $NaHCO_3$ 25 mmol/l, glucose 11.1 mmol/l) at 37°C. The solution was bubbled with air. A resting tension of 0.5g was applied to the tissue. Contractions were recorded isotonically on smoked paper by means of an isotonic frontal writing lever.

Rat Phrenic Nerve-hemidiaphragm [8]

Adult albino rats (Wistar strain) weighing between 150g and 200g were used. The left hemi-diaphragm together with its phrenic nerve was dissected out and mounted in a 50ml organ bath containing Krebs solution at 37°C and bubbled with oxygen. The preparation was stimulated electrically via the phrenic nerve using single square wave shocks of 1.0 msec duration and of supramaximal strength delivered from SRI stimulator, CAT. No. 6052 at a frequency of 0.2 Hz. Contractions were recorded on smoked paper by means of a light spring-loaded lever.

Guinea-pig Auricles (Clark's method)

Auricles obtained from guinea-pigs weighing between 350g and 400g were set up in a 20ml organ bath containing Locke solution bubbled with oxygen and maintained at 30°C. Contractions were recorded on smoked paper by means of a light spring-loaded lever.

Rabbit Heart Perfusion (Langendorff's Method).

Hearts obtained from rabbits weighing between 1.8kg and 2.0 kg were used. The heart was perfused with oxygenated Locke solution maintained at 37°C. The contractions were recorded on smoked paper by means of a light spring-loaded lever. The perfusate was collected and the rate of perfusion was measured. Readings were taken every two minutes. Further doses were not administered until the preparation had recovered or until new steady control levels were obtained.

Anaesthetized Cat

Male and female cats weighing between 1.4 kg and 2.2 kg. were anaesthetized with a mixture of 1% w/v - chloralose and 0.6% w/v pentobarbitone sodium solutions administered intraperitoneally.

Drugs were administered through the femoral vein and the arterial blood pressure was recorded from the carotid artery by means of a Statham pressure transducer Type 21094-P23AC coupled to a Grass Polygraph, Model 7D. The cats were maintained on artificial respiration using the Harvard respirator Model 661.

Drugs

Drugs used were acetylcholine iodide, histamine acid phosphate, nicotine hydrogen tartrate, carbachol chloride, adrenaline acid tartrate, noradrenaline acid tartrate, stilboestrol, atropine sulphate, α-chloralose (BDH); propranolol hydrochloride, dibenamine, (Sigma); mepyramine maleate, hexamethonium bromide (May and Baker); (-)-isoprenaline bitartrate (Wyeth); d-tubocurarine (Borroughs Wellcome); pentobarbitone sodium (Halewood chemicals); griffonin.

RESULTS

Guinea-pig Ileum

Griffonin, in concentrations between 0.2mg/ml and 3.2mg/ml, caused dose-dependent contractions of the

guinea-pig ileum (five preparations). Atropine (0.001 µg/ml and 0.002 µg/ml) inhibited the responses produced by griffonin (0.4 mg/ml) by 86.2% and 100% respectively. The same concentrations of atropine (0.001 µg/ml and 0.002 µg/ml) inhibited the responses produced by acetylcholine (0.04 µg/ml) by 66.7% and 96.0% respectively. Mepyramine (0.01 µg/ml) inhibited the response produced by griffonin (0.4 mg/ml) by only 5.0% while the same concentration of mepyramine inhibited the response produced by histamine (0.08 µg/ml) by 55.0%. Hexamethonium (1.0µg/ml) had no effect on the response produced by griffonin (0.4 mg/ml) but inhibited the effect of nicotine (1.0 µg/ml) by 81.4%. These results indicate that the responses produced by griffonin are similar to those produced by acetylcholine.

The dose-response curves of griffonin and acetylcholine and of griffonin in the absence and in the presence of various concentrations of atropine are shown in Figs.1 and respectively. The antagonism between griffonin and atropine is non-competitive.

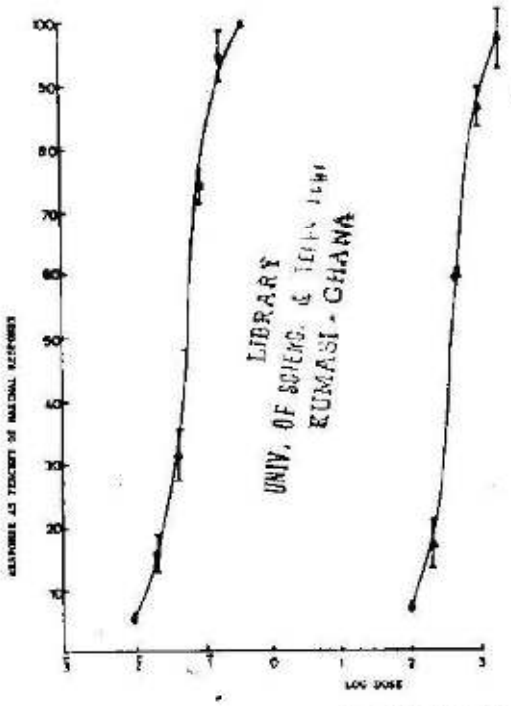

Fig. 1: Log dose/response curves to griffonin (Δ) and acetylcholine (●) on guinea-pig ileum. Each point is the mean ± s.e.m. of five observations.

Rabbit Duodenum

Griffonin (0.4 to 6.4 mg/ml) caused dose-dependent contractions of the rabbit duodenum (five preparations). In one out of the five preparations used, the contractions produced by griffonin were preceded by relaxations. The results were essentially similar to those obtained on the

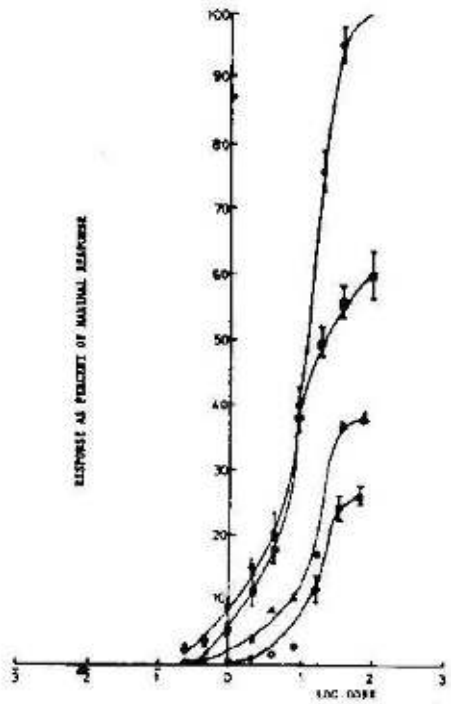

Fig. 2: Log dose/response curves to griffonin on guinea-pig ileum in the presence of atropine. The concentrations of atropine used were 0M (●, control); 10^{-9}M (■); 10^{-7}M (Δ); mean ± s.e.m. of five observations.

guinea-pig ileum in that the responses produced by griffonin (1.6 mg/ml) were inhibited by atropine (0.04 µg/ml, 30%) and mepyramine (0.04 µg/ml, 20%) but not by hexamethonium (2.0 µg/ml).

Rat uterus

Griffonin, in concentrations between 0.2mg/ml and 3.2 mg/ml, produced dose-dependent contractions of the non-pregnant and pregnant rat uterus preparations (five preparations each). On the non-pregnant uterus atropine (0.02 µg/ml) inhibited the responses produced by griffonin (0.2mg/ml) by 30%. On the pregnant uterus the contraction produced by griffonin (0.8 mg/ml) was inhibited by atropine (0.004 µg/ml) by 68%. Atropine inhibited the responses produced by acetylcholine on both the non-pregnant and pregnant uterus preparations. Dibenamine (0.04 µg/ml) did not inhibit the responses produced by griffonin (0.8 mg/ml) on the pregnant rat uterus. The dose-response curves of griffonin and acetylcholine on the non-pregnant and pregnant uterus preparations are shown in Figs. 3 and 4 respectively.

Effect of Griffonin on Isolated Skeletal Muscle

Griffonin, in concentrations up to 8mg/ml, had no effect on the toad rectus abdominis, chick biventer-cervicis and rat phrenic nerve-hemidiaphragm preparations (six preparations each). Griffonin (up to 2mg/ml) did not affect the responses produced by acetylcholine

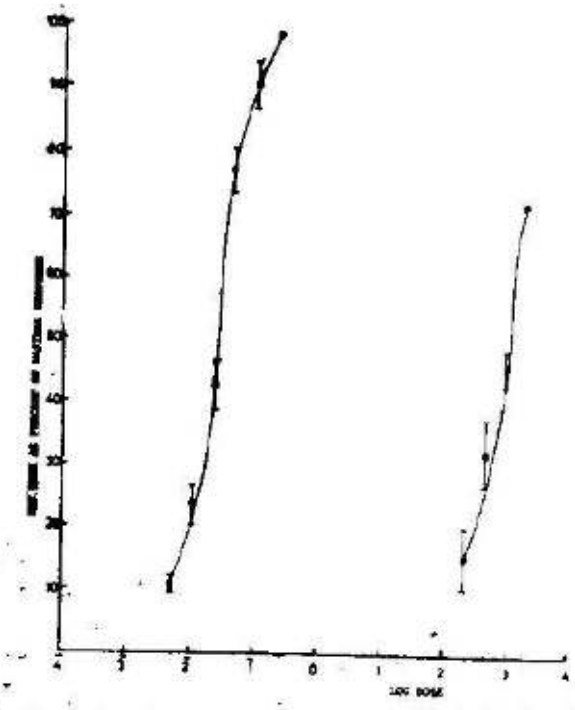

Fig. 3: Log-dose/response curves to griffonin (▲) and acetylcholine (O) on non-pregnant rat uterus. Each point is the mean ± s.e.m. of five observations.

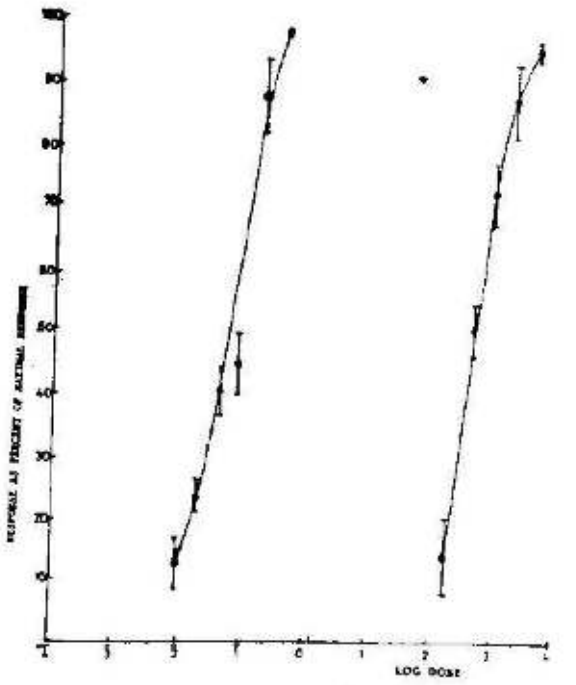

Fig. 4: Log dose/response curves of griffonin (O) and acetylcholine (●) on pregnant rat uterus. Each point is the mean ± s.e.m. of five observations.

on the rectus abdominis and biventer-cervicis muscle preparations.

Guinea-pig Auricles

Griffonin (up to 6.4 mg/ml) had no effect on the rate and force of contraction of the isolated guinea-pig auricles (six preparations).

Rabbit Heart Perfusion

Doses of griffonin below 8mg had no effect on the perfused heart. Griffonin (8mg) produced an initial slight increase followed by reduction in the force of contraction of the heart but had no effect on the rate of contraction. Griffonin (16mg) caused 53.8% reduction in the force of contraction of the heart without an initial increase and 29.7% reduction in the rate of contraction. This dose of griffonin also caused irreversible abnormal heart beats which occured about 20 minutes after administration of the drug. The abnormal heart beats and the reduction in the rate and force of contraction caused by griffonin (16 mg) were not affected by atropine (0.4 µg). Noradrenaline (up to 1.6 µg) had no effect on hearts that have been exposed to griffonin (16mg) though noradrenaline (0.4 µg) caused marked increase in the rate and force of contraction of untreated hearts. However adrenaline (3.2 µg) increased the rate but decreased the force of contraction of hearts that have been exposed to the high dose of griffonin (16 mg).

Griffonin (8mg and above) produced dose-dependent reduction in the rate of coronary perfusion. There was 92.5% reduction in the rate of coronary perfusion when griffonin (16 mg) was administered.

Anaesthetized Cat

Griffonin, in doses between 2.85mg/kg and 22.84mg/kg, produced dose-dependent lowering of the arterial blood pressure of the anaesthetized cat (Fig.5). Propranolol (up to 5.7 µg/kg) did not have any effect on the hypotensive effect of griffonin. Mepyramine (2.85 µg/kg) abolished the hypotensive effect of histamine (0.14 µg/kg) but had no effect on the response to griffonin (11.42 mg/kg) (Fig.6). Atropine (5.7 µg/kg) abolished the hypotensive effect of acetylcholine (0.14 µg/kg) and griffonin (11.42 mg/kg) (Fig. 7).

DISCUSSION

The contractions produced by griffonin on the guinea-pig ileum, rabbit duodenum and pregnant and non-pregnant rat uterus preparations were inhibited by atropine, suggesting that griffonin has muscarinic actions. On the guinea-pig ileum and the rat pregnant uterus preparations, griffonin behaved like a true agonist in that griffonin and acetylcholine produced similar maximum responses. However on all the smooth muscle preparations used, griffonin was found to be less potent than acetylcholine.

The antagonism between atropine and griffonin was of the non-competitive type. This interaction differs from the competitive antagonism between atropine and

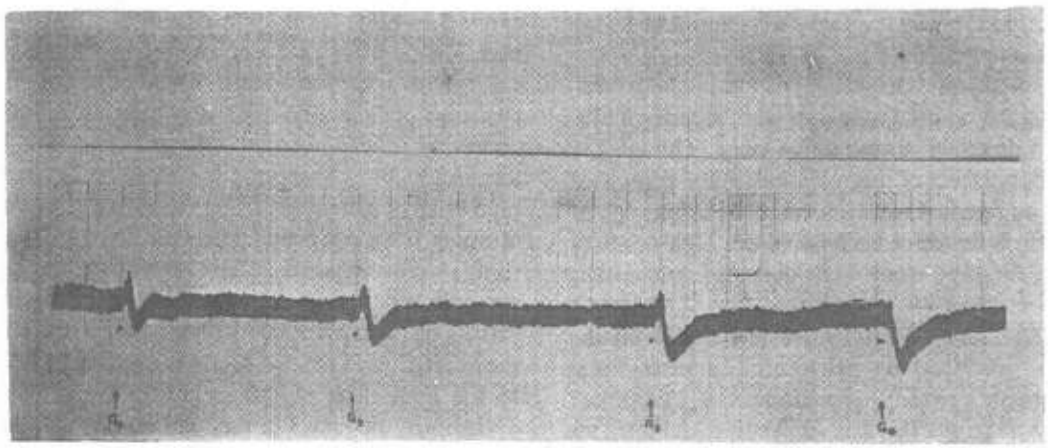

Fig. 5: The effects of an intravenous injection of griffonin (G_1, 2.85mg/kg; G_2, 5.71mg/kg; G_3, 11.42mg/kg; G_4, 22.84mg/kg) on the systemic blood pressure of an anaesthetized cat.

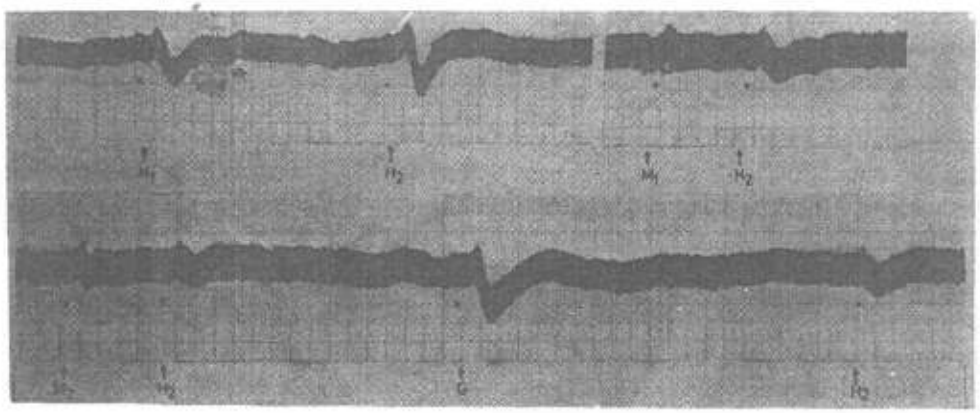

Fig. 6: The effects of an intravenous injection of histamin (H_1, 0.07µg/kg; H_2, 0.14µg/kg) and griffonin (G_1, 11,42mg/kg) on the systemic blood pressure of an anaesthetized cat after the administration of mepyramine (M_1, 1.42µg/kg; M_2, 2.85µg/kg).

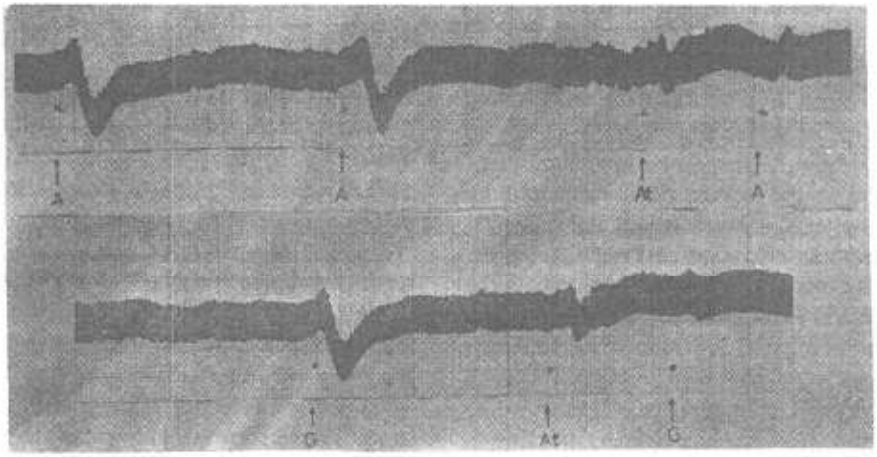

Fig. 7: The effects of an intravenous injection of acetylcholine (A, 0.14µg/kg) and griffonin (G, 11.42mg/kg) on the systemic blood pressure of an anaesthetized cat after the administration of atropine (At, 5.7µg/kg).

acetylcholine. This result shows that though griffonin behaved like acetylcholine in the experiments, there are dissimilarities in the way the two drugs interacted with the muscarinic receptors.

The contractions produced by griffonin on the guinea-pig ileum and the rabbit duodenum were slightly inhibited by high doses of mepyramine only. Though the slight inhibitory effect of mepyramine on the responses to griffonin suggests possible histaminic properties of griffonin, this is doubtful because of the high doses of mepyramine required since high doses of antagonists become non-specific.

Hexamethonium did not have any effect on the contractions produced by griffonin suggesting that griffonin does not have any nicotinic actions. The lack of nicotinic action of griffonin is further indicated by the absence of stimulatory effect of griffonin on all the skeletal muscle preparations used. [illegible] doses exerts nicotinic actions at different sites such as ganglia and the neuromuscular junction, in addition to its muscarinic effects [4]; griffonin was expected to have similar effects. However, muscarine and pilocarpine have only muscarinic actions and it is possible that griffonin which is a natural plant product like muscarine and pilocarpine, behaves more like these two drugs rather than like acetylcholine. Griffonin did not have any anti-nicotinic actions either. This result differs from that obtained by Aloka [1]. Aloka observed that griffonin had an antagonistic effect on nicotine-induced contractions of the guinea-pig ileum. The differences in these results may be due to various factors. For example, the purity of griffonin used in the experiments may influence the results obtained. In the present study, the authenticity and purity of the griffonin used were determined by thin layer chromatography, infra-red spectroscopy and melting point determination methods. The results obtained from these tests compared favourably with results obtained with an authentic sample of griffonin provided by Professor Dwuma-Badu and also with literature values.

The contractions produced by griffonin on the pregnant rat uterus were not antagonized by dibenamine, an α-adrenoceptor blocker. The pregnant uterus contains α- and β -adrenoceptors. Stimulation of the α-receptors causes contraction whilst stimulation of the β- receptors causes relaxation of the uterus. Thus the inability of dibenamine to inhibit the contractions produced by griffonin shows that griffonin does not stimulate α-adrenoceptors. Histamine has no effect on the rat uterus. Therefore griffonin cannot be said to have contracted the uterus through histaminic receptor stimulation.

Results obtained from the Langendorff's rabbit heart perfusion and the anaesthetized cat experiments indicate that griffonin has dose-related depressor effects on the cardiovascular system. The hypotensive effect of griffonin is not due to histaminic action nor mediated through β-adrenoceptor stimulation since mepyramine and propranolol failed to have any effect on the hypotensive responses produced by griffonin.

Atropine abolished the hypotensive effects of griffonin and acetylcholine suggesting that griffonin exerted its effect through muscarinic receptor stimulation. The depressor effect observed in the anaesthetized cat agrees with the results obtained from the rabbit perfused heart experiment in which the amplitude of contractions was depressed by griffonin. The negative ionotropic and chronotropic effects observed with the high dose of griffonin (16mg) were not antagonized by atropine, but those produced by the low dose of griffonin (8mg) were antagonized. The high dose of griffonin did not only produce negative ionotropic and chronotropic effects but also produced irregular heart beats. Death of the heart always ensued following exposure of the heart to the high dose of griffonin. Failure of atropine to antagonize the effect of the high dose may probably be due to toxic effect of this dose of griffonin on the heart.

[illegible] the reduced force and irregular contractions produced by the high dose of griffonin (16mg) probably because noradrenaline is predominantly an α -receptor stimulant. Adrenaline on the other hand has equal α- and β-adrenoceptor stimulant actions and the increase in the rate of contraction and the restoration of the heart to normal rhythm is due to the β–effect. However, the irregular contractions and the reduced rate of contraction reappeared after the effect of adrenaline had worn off. The failure of adrenaline to increase the force of contraction of the heart suggests that the high dose of griffonin (16mg) impairs the contractile ability of the myocardium.

The muscarinic effect of griffonin observed on the isolated smooth muscle preparations, the anaesthetized cat and on the rabbit perfused heart was not seen on the guinea-pig auricle preparation. This again portrays a dissimilarity between griffonin and acetylcholine. Griffonin was expected to produce negative ionotropic and chronotropic effects on the auricles similar to the effects it produced on the perfused rabbit heart. The failure of griffonin to have any effect on the contractions of the auricles cannot be readily explained. This finding however lends support to a similar result reported by Aloka [1]. Griffonin was expected to have marked negative ionotropic and chronotropic effects on the auricles because muscarinic receptors on the ventricles are rather sparse as compared to the auricles [5]. The observed differences may probably be due to the existence of different muscarinic receptor types.

In recent years, muscarinic acetylcholine receptors have been classified into M_1 and M_2 subclasses chiefly on the basis of the differences in affinities for the antagonist pirenzepine [14]. M_1-receptors were defined as having a high affinity for pirenzepine whilst M_2-receptors were defined as having a lower affinity. Apart from this, studies have shown that muscarinic receptors couple to two second messenger systems, namely, adenylate cyclase inhibition and phosphatidyl inositol turnover [6, 18] and on the basis of studies in the central nervous system, it was suggested that pirenzepine-sensitive (M_1) receptors

stimulate phosphatidyl inositol turnover whilst the M_2-receptors inhibit adenylate cyclase [11]. M_1-receptors are distributed in the brain and autonomic ganglia and M_2-receptors are found in the heart, intestines and submandibular glands [13,16].

The classification does not seem to apply to muscarinic receptors in all tissues. In the chick heart cells pirenzepine had a higher affinity at cyclase-coupled receptors than at receptors coupled to phosphatidyl inositol turnover [7]. In mammalian heart, muscarinic receptors inhibit adenylate cyclase and stimulate phosphatidyl inositol turnover.

Mizushima, Uchida, Zhou, Kagiya and Yoshida [17] have proposed that cardiac M_2 muscarinic receptors can be further classified into $M_{2\alpha}$ and $M_{2\beta}$ based on relationship to different agonist binding sites, namely, superhigh (SH), high (H) and low (L) agonist binding sites on the basis of their affinity for carbachol.

More recently five muscarinic acetylcholine receptor subtypes have been identified [3]. It is now well established that odd-numbered muscarinic acetylcholine receptors (M_1, M_3 and M_5 receptors) couple preferentially to phosphatidyl inositol turnover via several possible G proteins, and that the even-numbered receptors (M_2 and M_4 receptors) couple preferentially to adenylyl cyclase inhibition via G_i or G_o [2.. 19]

The initial increase in the force of contraction produced by the low dose of griffonin (8 mg) was not observed in the anaesthetized cat. Similarly, the irreversible abnormal contractions of the perfused rabbit heart produced by griffonin (16 mg) were not observed in the intact animal preparation with doses of griffonin up to 22.8mg/kg. Possibly in the intact animal, the doses administered were not high enough to affect the heart because the final concentration presented to the heart may be affected by various factors such as distribution and metabolism. In the simple isolated heart preparation, the concentration of the drug in the biophase is considered to be the same as that administered.

The reduction of the rate of perfusion produced by griffonin was due to constriction of the coronary vessels as a result of coronary muscarinic receptor stimulation. Acetylcholine causes constriction of coronary vessels and this effect is mediated through muscarinic receptors [5].

CONCLUSION

Results obtained in this study show that griffonin is predominantly a muscarinic drug devoid of nicotinic actions. At high dose levels, griffonin is toxic to the isolated rabbit heart.

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