J. Pharm. Pharmacol., 1964, **16**, 369–374 Received December 20, 1963

Research Papers

Effects on anaesthetised animals of an oxytocic glycoside extracted from certain species of *Albizia*

A. LIPTON

The actions on anaesthetised guinea-pigs, rabbits, cats and monkeys of a glycoside, "albitocin", obtained from *Albizia gummifera*, and some other species of *Albizia*, used by East African witchdoctors to accelerate labour and induce abortion, are described. On intravenous injection there is a transient fall in systemic blook pressure and an increase in uterine activity, with the pregnant uterus exhibiting greater sensitivity. Both responses occur in the presence of atropine and antihistamines, and in monkeys after bilateral vagotomy and hexamethonium, but a prolonged small rise in blood pressure occurs on intraperitoneal injection of the drug. Electrocardiograph records in rabbits and monkeys exhibit no consistent acute or chronic changes in the electrical activity of the heart resulting from the injection of the drug even in lethal doses, almost up to the time of death.

THE isolation of an oxytocic glycoside, "albitocin", from *Albizia gummifera* (Gmel.) C. A. Smith, var *gummifera*, and some other species of *Albizia* (extracts of which are used by East African witchdoctors to accelerate labour and induce abortion), the partial chemical characterisation of the active principle, and its actions on uterus and ileum preparations *in vitro*, have been described (Lipton, 1960; Lipton, 1963). The present communication deals with its *in vivo* actions on anaesthetised mammals.

Method

Twenty guinea-pigs, 12 rabbits, 8 cats and 14 vervet monkeys (*Cercopithecus aethiops*) were used. Animals were anaesthetised with urethane (1·5 g/kg), pentobarbitone (20–60 mg/kg) or chloralose (60–100 mg/kg) and were heparinised (1,000 i.u./kg).

Pentobarbitone was also used in the electrocardiograph work, in which recordings were made repeatedly over several days and bemegride (10–12 mg/kg intravenously plus 10–12 mg/kg intraperitoneally), was administered to the monkeys after each ECG recording to avoid the poor survival due to dehydration, salt loss, and anorexia with animals almost continuously anaesthetised, and also the poikilothermia due to barbiturate which has been found to occur in monkeys (Luck, 1953). This reduced the duration of each anaesthesia from 6–8 hr to 20–90 min. These animals remained in good condition throughout the experiments, control animals each surviving up to fourteen pentobarbitone inductions over 8–9 days. 30,000–50,000 i.u. of sodium benzylpenicillin B.P. were administered daily to all surviving animals.

As in the *in vitro* work previously reported (Lipton, 1963) the assumption has been made that the glycoside albitocin is the only new active substance present in the plant extract.

From the Department of Physiology, Makerere College Medical School, Kampala, Uganda. Present address: The Department of Physiology, University of Queensland, St. Lucia, Brisbane, Australia.

The drug was administered in solution in isotonic saline checked for neutrality with Universal indicator, and as shown previously, contained no nitrogen hence no polypeptide or alkaloid spasmogens. Free inorganic ions were removed by dialysis during purification.

Respiration was recorded by Gaddum's (1941) method. Carotid or femoral *arterial blood pressure* was recorded by a Condon (1951) modified manometer, where appropriate, otherwise an ordinary U-tube mercury manometer was used. *Venous pressure* was recorded from the right jugular vein in three monkeys using a miniature volume recorder (Luck, 1952).

UTERINE ACTIVITY

Four different methods were used for recording uterine activity.

In Method A, a 5–8 cm mid-line abdominal incision was made, the skin and body wall stitched to an elliptical brass ring, and raised slightly on threads to keep them clear of the abdominal contents. The uterine cervix was clamped through this ring and a thread was attached to the ventral surface of the uterus 4–6 cm from it, leading to a frontal lever. Alternatively (Method B), a Cushny myograph was used via a ventral abdominal incision, the ends of the lever arms stitched direct to the uterus 5–8 cm apart.

A small steady rhythm due to respiration was observed super-imposed on the large irregular spontaneous uterine contractions.

Because of the laparotomy, cooling and drying of the surface of the uterus occurred to a variable extent, with a consequent increase in activity due to irritation. The attachments of the threads and levers were also foci of irritation, and in some cases the non-specific activity set up by these causes confused the results obtained by injecting the drugs.

In method C an internal catheter was inserted via the uterine cervical os and, with or without a small fluid-filled rubber balloon at its end, was attached to a pressure transducer device connected to an ink-writing galvanometer (Smyth, 1957). This method left the uterus almost undisturbed and such preparations showed a steady base-line trace without the occasional increase in spontaneous activity which characterised methods A and B.

Method D, which could only be applied to animals in an advanced stage of gestation, was the use of a tocograph recording head (Smyth, 1957) which was attached externally to a shaved area of the abdomen, over the uterus, and recordings made with an ink-writing galvanometer.

ELECTROCARDIOGRAPHY

A portable "Sanborn Visette" electrocardiograph was used. The anaesthetised animals were insulated on a sheet of dry polythene, and the electrodes were attached to shaved areas over electrode paste.

The standard leads were recorded in the order 1, 2, 3, AVR, AVL, AVF, each for 8 to 10 beats without moving the animals or the electrodes.

This was repeated immediately after administration of the drug, and then at regular intervals subsequently during the survival time of the animals. The animals were re-anaesthetised when necessary for this purpose.

Records were taken continuously from two of the dosed rabbits during and just after injection, using Lead 1 for several min, to observe any acute effects on the heart.

Results

ORAL ROUTE

Gastric tube administration of up to 5 mg/kg albitocin in six urethane anaesthetised pregnant guinea-pigs had no effect on the uterine activity recorded by method A, or on the respiration, in up to 8 hr of recording. A dose of albitocin, 0·25 mg/kg, was then dripped onto the exposed uterus and immediately caused a series of powerful contractions, persisting for 30–120 min.

It was concluded that the guinea-pig pregnant uterus would respond *in situ* as *in vitro*, to direct application of the extract, but the oral dose was either destroyed in the digestive tract, or was absorbed from it too slowly or in inadequate amounts.

INTRAVENOUS ROUTE

Doses containing from 0·1–8·0 mg/kg albitocin produced, in all animals, falls in blood pressure proportional to the dose and lasting up to 1 min, and sometimes brief hyperpnoea (Figs 1–3).

In 8 pregnant guinea-pigs, 2 pregnant and 2 non-pregnant rabbits, 1 pregnant and 2 non-pregnant cats, 2 pregnant and 1 post-partum monkey, intravenous injection of up to 8 mg/kg albitocin gave an increase in the rate and force of spontaneous uterine contractions where present, or

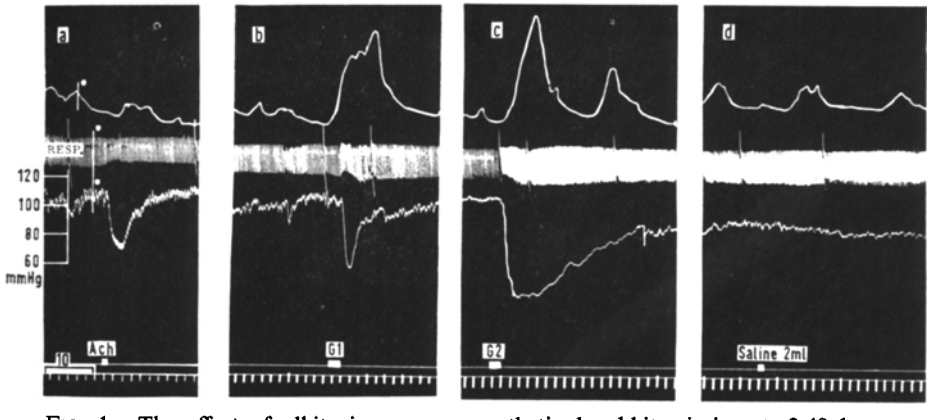

FIG. 1. The effect of albitocin on an anaesthetised rabbit, virgin, wt, 2·48 kg. pentobarbitone anaesthesia. Uterus by method B. All doses intravenous, Ach = 2 μg/kg acetylcholine chloride. G1 = 0·3 mg/kg and G2 = 0·4 mg/kg albitocin. Upper trace, uterus; middle trace, respiration; lower trace blood pressure. Time: 10 sec.

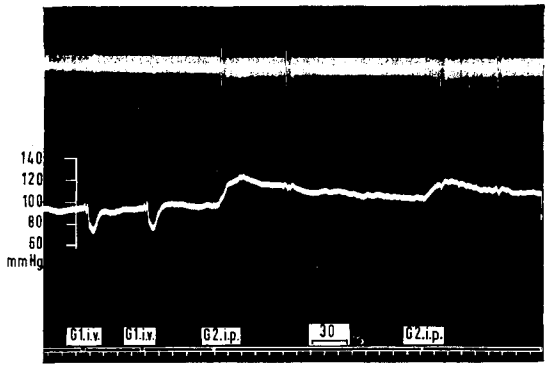

FIG. 2. Effect of route of administration on response to albitocin in an anaesthetised cat. Male, wt 2 kg, pentobarbitone anaesthesia. G1 = 1·25 mg/kg albitocin i.v. G2 = 0·25 mg/kg albitocin i.p. Upper trace, respiration; lower trace, blood pressure. Time: 30 sec.

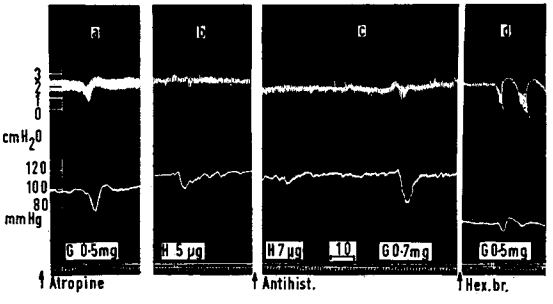

FIG. 3. Comparison of albitocin with other depressant drugs in the monkey (*Cercopithecus aethiops*), wt 3·2 kg, pentobarbitone anaesthesia. Both cervical vagi cut, atropine sulphate 2·5 mg/kg. All doses intravenous. G = albitocin; H = histamine acid phosphate. Antihist = 2 mg/kg promethazine HCl. Hex.br. = 15 mg/kg hexamethonium bromide. Upper trace, venous pressure; lower trace, blood pressure. Time: 10 sec.

produced powerful contractions in quiescent uteri, all responses were proportional to the doses (Fig. 1b, c).

The depressor response was instantaneous and usually compensated in less than 1 min, but the uterine response had a latency of up to 1 min, and sometimes continued for 3–5 min.

Control intravenous doses of saline, and of acetylcholine and histamine giving comparable depressor effects to the doses of albitocin had no effect on the uterus (Fig 1a, d), and doses of antihistamines (pyranisamine maleate and promethazine hydrochloride), and atropine, sufficient to minimise the depressor responses to histamine and acetylcholine respectively, had no effect on the depressor (Fig. 3) or uterine responses to albitocin.

DEPRESSOR ACTION

Three adult male monkeys were examined in an attempt to determine the character of the transient blood pressure fall occurring on intravenous injection.

Atropine, bilateral vagotomy, and massive doses of antihistamines had no effect on the arterial blood pressure fall resulting from up to 0·7 mg/kg of albitocin, even when all these were applied together (Fig. 3, a, b, c). The venous blood pressure showed only a corresponding small change at the time of the arterial pressure fall.

In one animal, after atropine, bilateral vagotomy, and antihistamines, 20 mg/kg of hexamethonium bromide was also injected, and after stabilisation of the arterial blood pressure at a lower level, 0·5 mg/kg albitocin gave a smaller but still quite distinct and reproducible fall (Fig. 3, d).

INTRAPERITONEAL ROUTE

In contrast to the above results, an interesting reversal of the depressor effect occurred in all the animals if administration of the drug was by the intraperitoneal route, this was a prolonged rise in arterial blood pressure from doses as low as 0·2–0·5 mg/kg albitocin (Fig. 2).

ELECTROCARDIOGRAPHY

Four adult male rabbits (doses from 2·3–2·7 mg/kg albitocin) showed no acute or chronic differences in ECG from normal before dosing up to a short time before death 1–7 days later. A control animal dosed with saline also showed no effects throughout.

In two other rabbits lead 1 was recorded continuously during drug administration, and one showed a brief T wave reversal lasting about 30 sec which then returned to normal. The other showed only a brief tachycardia.

Similar results were obtained with seven non-pregnant adult female monkeys (doses from 3·3–6·6 mg/kg albitocin). Only one, which had had 3·3 mg/kg on each of two successive days, survived, the others all died within 72 hr. In three cases the monkeys were so moribund after a few hr, that although they were conscious there was no need for anaesthesia for restraint. The only change observed was bradycardia when the animals were almost on the point of death.

One monkey received a total of 37 mg/kg albitocin over 3 hr in gradually increasing dosage, while all ECG leads were recorded. This animal died 24 hr later without any changes in ECG until the last few min before death.

Discussion

These results demonstrate that albitocin or extracts containing it can act powerfully on the uterus *in vivo*, in moderate doses, causing marked increase in activity in all the species tried, as it does *in vitro* (Lipton, 1963), and the greater sensitivity of the gravid uterus to the drug which has been demonstrated *in vitro* is also apparent *in vivo*, both in the

minimal dose required for an effect, and in the relative increase in activity with larger doses.

In all the animals, the extracts, on intravenous injection, gave a transient fall in blood pressure proportional to the dose, which persisted, like the uterus response, in the presence of atropine and antihistamines, while bilateral vagotomy and hexamethonium also failed to prevent the depressor response in the monkeys. This would appear to localise the cause to a direct action of the drug on the peripheral vessels or heart. On the other hand, intraperitoneal injection of the extracts gave a persistent rise in blood pressure, the mechanism of which is under investigation.

The drug caused no consistent changes in the electrical activity of the hearts of the rabbits or monkeys (Dr G. Shaper, personal communication) even with lethal doses, so that its action is not like that of the common cardiac glycosides.

These results provide further justification for the known use of extracts of these plants by African witchdoctors to accelerate labour and procure abortion.

Acknowledgements. The author wishes to express his appreciation of the generosity of the Makerere College Research Grants Committee and the Wellcome Foundation for grants in support of the work. Grateful acknowledgement is also due to Professor C. P. Luck for his advice and guidance, and to Mrs. T. Walker for technical assistance.

References

Condon, N. E. (1951). *Brit. J. Pharmacol.*, **6**, 19–20.
Gaddum, J. H. (1941). *J. Physiol.*, **99**, 257–264.
Lipton, A. (1960). Thesis for Ph.D. London University.
Lipton, A. (1963). *J. Pharm. Pharmacol.*, **15**, 816–824.
Luck, C. P. (1953). *South African J. med. Sci.*, **18**, 131–140.
Luck, C. P. (1952). *J. Sci. Instru.*, **29**, 333–334.
Smyth, C. N. (1957). *J. Obstet. Gynaec., Brit. Emp.*, **64**, 59–66.