POSITIVE INOTROPIC ACTION OF AN ALKALOIDAL FRACTION FROM AJUGA BRACTEOSA WALL ex BENTH

By

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An alkaloidal fraction obtained from *Ajuga bracteosa*, Wall ex Benth shows a positive inotropic action on the frog heart, the isolated rabbit auricle and the electrically driven rat ventricle. The cardiostimulant action was antagonised by dichloroisoprenaline and was absent in preparations taken from reserpinised animals. Catecholamine liberation from stores in the heart is suggested as the mechanism for the cardiostimulant action of the alkaloidal fraction.

Ajuga bracteosa, (*Nilkanthi*), Wall ex Benth (N.O. Labiatae) has been used in the indigeneous system of medicine since very early time. Gokhale and Karandikar (1960) investigated the pharmacological actions of a watery extract of the plant. A protective action against carbon tetrachloride induced liver damage in rats, diuresis in rats, hypotension in cats and dogs and a cardiac stimulant action in the isolated rabbit heart were the significant findings reported by these authors. An alkaloidal fraction was also isolated by these authors from the plant and was found to have a stimulant action on the frog heart.

It is the purpose of the present communication to characterise the cardiac stimulant action of the alkaloidal fraction.

METHODS

Preparation of the alkaloidal fraction.—The genuine whole plant material was obtained from the National Botanical Gardens, Lucknow. Samples were reduced to a moderately coarse powder in a disintegrator and were extracted with 95 per cent ethanol in a Soxhlet extraction apparatus. The alcohol extract was concentrated and repeatedly treated with 2N hydrochloric acid. The acid extract was made alkaline with ammonia and repeatedly extracted with ether. The fraction thus obtained was dried and was tested for alkaloids by routine chemical tests and by filter paper chromatography (Schwerdtfeger, 1953). The fraction gave positive tests for alkaloids and therefore was termed "alkaloidal fraction". For pharmacological testing the

alkaloidal fraction was dissolved in water by adding a few drops of dilute hydrochloric acid. The pH of the resultant solution was 4.5.

Frog (Rana tigrina) heart.—Frog heart was perfused as described by Burn (1952). The perfusion rate was kept constant. The injection volume did not exceed 0.1 ml. In all, 10 preparations were used.

Isolated rabbit auricle.—Ten male white rabbits weighing between 1.5-2 kg were used. Auricles were dissected out and set up in a 40 ml organ bath according to the technique of Burn (1952). Bath fluid was freely oxygenated and maintained at 29°C (± 0.5°C). The preparation was allowed to stabilise for one hr before testing was begun. Drug effects were studied every ten min for two min.

Isolated rat ventricle.—Eight white rats weighing 150 to 200 g were used. Right ventricular strips were prepared and employed in the manner described by Covin and Berman (1959). Bath fluid was maintained at 27°C (± 0.5°C) and aerated with oxygen. The strips were driven electrically by supramaximal square wave pulses delivered every sec for 0.5 m sec. Isotonic contractions were recorded on a smoked drum, Drug effects were studied every ten min for five min.

Reserpinised animals.—Rabbits were given reserpine 3 mg/kg i.p on two successive days and were killed 24 hr after the last injection. Rats were killed 24 hr after a single injection of reserpine 2 mg/kg subcutaneously.

Dichloroisoprenaline (DCI) antagonism.—In antagonism studies DCI was placed in the bath 3 min before addition of the test compounds.

Blood-bathed isolated rat stomach strip.—Stomach strips were obtained from the fundal stomach of reserpinised rats and prepared in the manner described by Vane (1957). The strips were suspended in a continuous stream of blood circulating from a chloralosed cat (Vane, 1958). Oxygenated blood was pumped from a carotid artery at a constant rate of 10 ml per min into a jacketed bath of 10 ml capacity from where it was drained back into the jugular vein. The temperature of the external circulation was kept at 37°C. Five such preparations were used. When bathed in a continuous flow of blood, the rat stomach strip is a suitable test organ for detecting adrenaline-like substances in the circulating blood (Vane, 1960). 0.5 to 1.0 μg of adrenaline or nor-adrenaline injected intravenously into the cat caused relaxation of the stomach strip when the circulating catechol amine reached the external circuit.

RESULTS

Frog heart.—The alkaloidal fraction in a dose of 0.5 mg elicited a positive inotropic response on the frog heart. The response was antagonised by DCI (20 μg) administered one min before the injection of the alkaloidal fraction. The response to calcium chloride was however, not affected (Fig. 1).

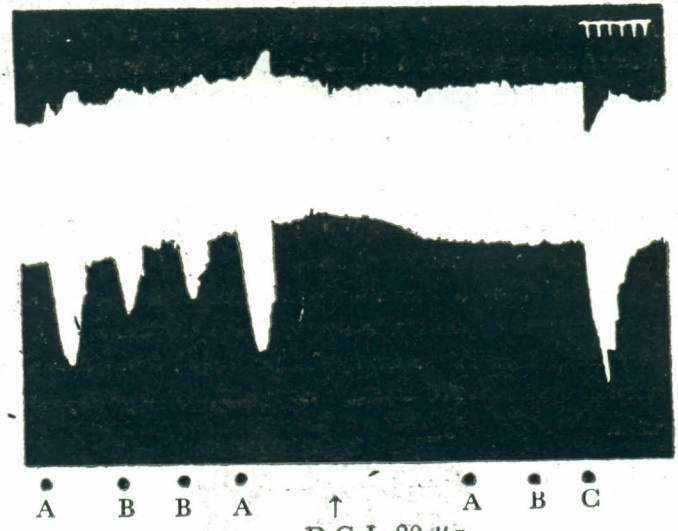

Fig. 1. Responses (at dots) of the frog heart to adrenaline. 0.02 μg (A) and alkaloidal fraction 0.5 mg (B) before and after DCI (20 μg) given at arrow. 1 mg of calcium chloride was given at C. Time 30 sec.

Rabbit auricle.—The alkaloidal fraction in concentrations of 5×10^{-6} to 2×10^{-5} increased the force of contraction of the isolated rabbit auricle. This positive inotropic action was related to the dose of the alkaloidal fraction and it was possible to demonstrate a dose response relationship (Fig. 2). The cardiostimulant action was blocked by DCI 1×10^{-5} (Fig. 2) and was absent in preparations obtained from reserpinised rabbits (Fig. 3).

Rat ventricle.—The alkaloidal fraction (1.3×10^{-5} and 2.6×10^{-5}) had a positive inotropic action on the rat ventricle (Fig. 4). DCI 1.0×10^{-5} blocked the action and the action was absent in ventricles from reserpinised rats.

In all the preparations studied the antagonistic effect of DCI persisted for about 30 min.

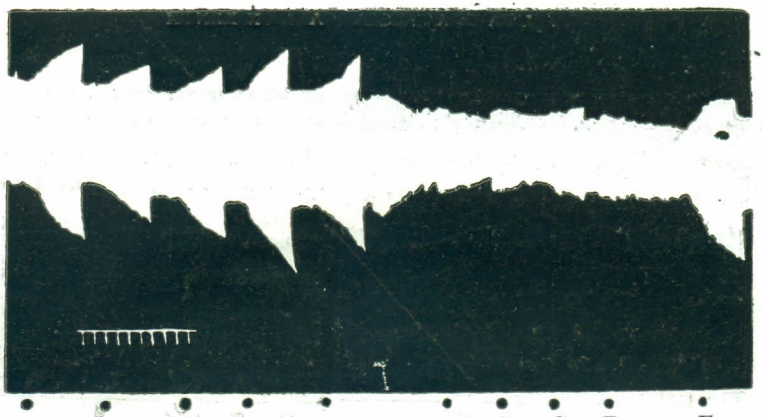

Fig. 2. Responses (at dots) of the isolated rabbit auricle to adrenaline 2.5×10^{-9} (A) and the alkaloidal fraction 5×10^{-6} (B), 1×10^{-5} (C) and 2×10^{-5} (D). DCI 1×10^{-5} was added at at the arrow. Calcium chloride 5×10^{-5} was added in the bath at E. Contact time 2 min. Time 30 sec.

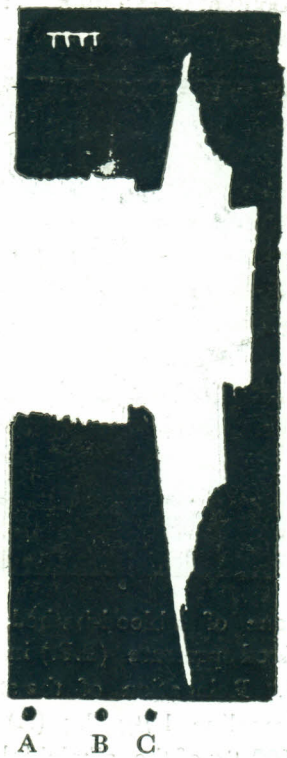

Fig. 3. Responses (at dots) of the isolated auricle from a reserpinised rabbit (reserpine 3 mg/kg i.p. daily for two days) to the alkaloidal fraction 1×10^{-5} (A), 2×10^{-5} (B) and adrenaline 2.5×10^{-9} (C). Contact time 2 min. Time 30 sec.

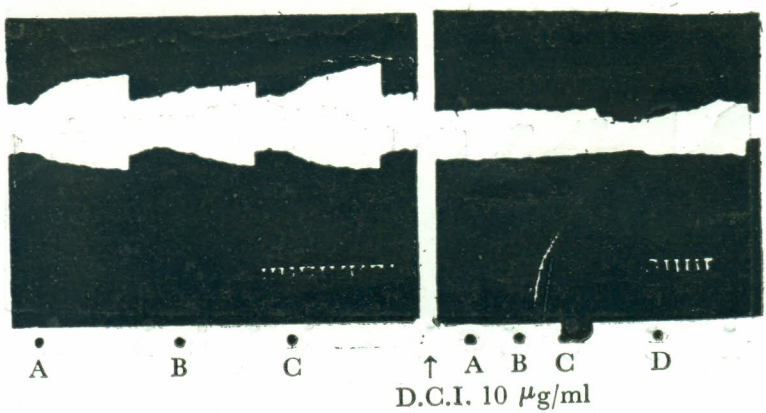

Fig. 4. Responses (at dots) of the electrically driven (1 per sec for 0.5 msec supramaximal shocks) isolated rat ventricle to adrenaline 1 x 10^{-6} (A), alkaloidal fraction 1.3 x 10^{-5} (B) and 2.6 x 10^{-5} (C) before and after DCI 1 x 10^{-5} given at arrow. Calcium chloride 2.5x10^{-5} was given at D. Contact time 5 min. Time 30 sec.

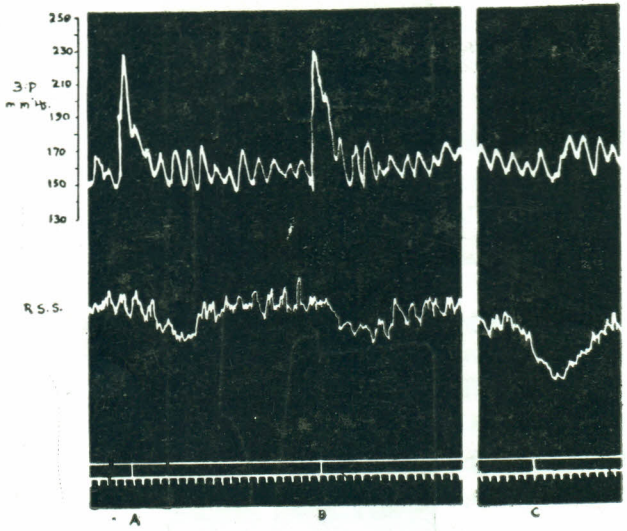

Fig. 5. Record of the tone of a blood-bathed isolated rat stomach strip (R.S.S.) and blood pressure (B.P.) in a cat (3.25 kg anaesthetised with chloralose). Relaxation of the stomach strip to intravenous injection of adrenaline 1 μg / kg (A) and the alkaloidal fraction 3 mg / kg (B). 500 μg of the alkaloidal fraction was injected directly into the external circuit at (C). Time 30 sec.

Blood bathed isolated rat stomach strip.—The alkaloidal fraction 3 mg/kg when injected intravenously into cats produced a rise of blood pressure and relaxation of the stomach strip. Five hundred μg of the alkaloidal fraction when injected directly in the external circuit also caused a relexation of the stomach strip (Fig. 5).

DISCUSSION

The present study has shown that the alkaloidal fraction from *Ajuga bracteosa* has a positive inotropic action on the frog heart, the isolated rabbit auricle and the electrically driven isolated rat ventricle. That an adrenergic mechanism is involved in this stimulant action is indicated by the observation that the action was annuled by DCI which specifically blocks the β-adrenergic receptors in the heart (Moran and Perkins, 1958).

In general a drug can produce adrenergic effects in either of two ways i.e., (i) by activating the adrenergic receptors, or (ii) by liberating the adrenergic transmitter from stores in the effector organ. Paasonen and Krayer (1958) showed that reserpine depleted the nor-adrenaline stores in the heart and therefore drugs which exert adrenergic effects on the heart by liberating nor-adrenaline from the stores no longer act in preparations obtained from reserpinised animals. The alkaloidal fraction had no cardiostimulant action in preparations from reserpinised animals and therefore it appears that its action is mediated through liberation of cathecholamines from available stores in the heart.

An attempt was made to obtain direct evidence for the catecholamine liberating property of the alkaloidal fraction by using the isolated blood-bathed rat stomach strip technique described by Vane (1958). In such a preparation 3 mg/kg of the alkaloidal fraction injected intravenously in the cat produced a rise of blood-pressure and relaxation of the stomach strip. However, an injection of 500 μg of the alkaloidal fraction directly into the external circuit also produced a relaxation of the stomach strip and as such no conclusions could be drawn from these experiments. This failure to obtain a direct evidence for the catecholamine liberating property of the extract, however, is in no way contrary to the conculsions drawn regarding the nature of the adrenergic action of the alkaloidal fraction. Vane (1960) for example, could not obtain evidence of increase in circulating catechol amines after the administration of tyramine or ephedrine or amphetamine, drugs which are known to exert adrenergic effects through liberation of catecholamines.

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