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Crude extract and purified components isolated from the stems of *Tinospora crispa* exhibit positive inotropic effects on the isolated left atrium of rats

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ABSTRACT

Ethnopharmacological relevance: *Tinospora crispa* has been used in folkloric medicine for the control of blood pressure. We previously found that an extract of *Tinospora crispa* and its constituents effect the heart rate and blood pressure in anesthetized rats.

Aim of the study: The aim was to investigate the effects and mechanisms of the *Tinospora crispa* extract and bioactive components on the rat isolated left atria.

Materials and methods: Air-dried stems of *Tinospora crispa* were extracted with water, followed by partitioning with chloroform, ethyl acetate, and finally by *n*-butanol. The *n*-butanol soluble material was concentrated and dried under reduced pressure and lyophilized to obtain a crude powder (*Tinospora crispa* extract). The active components of *Tinospora crispa* extract were separated by column chromatography and preparative HPLC. The effects and mechanisms of the *n*-butanol extract and the bioactive purified components (adenine, uridine, adenosine, salsolinol, tyramine, higenamine, syringin, (−)-litcubinine, borapetside A, borapetoside B, borapetoside D and borapetoside E) were studied in isolated left atria from normal and reserpinized rats.

Results: *Tinospora crispa* extract caused an increase in the force of contraction of the electrical field stimulated left atrium. This effect was inhibited by propranolol, atenolol, ICI-118,551, phentolamine and atropine. The positive inotropic effect on the reserpenized isolated left atrium of the *Tinospora crispa* extract was significantly inhibited by propranolol, atenolol and ICI-118,551. Phentolamine, on the other hand, caused potentiation and the effect was inhibited when propranolol was also added. Higenamine caused an increase in the force of contraction of the electrical field stimulated left atrium and this effect was significantly inhibited by ICI-118,551 and atenolol but not by phentolamine. Reserpine did not significantly shift the concentration–response curve (*C–R* curve) of the inotropic effect of the higenamine. ICI-118,551 and atenolol caused a parallel shift of the *C–R* curve to the right of about 8 and 33 fold, respectively. At low concentrations salsolinol caused a slight increase in the force of contraction of the left atrium, but at higher concentrations a decrease was observed. The negative inotropic effect of salsolinol was significantly inhibited by propranolol and atropine. In the reserpinized isolated left atrium, the negative inotropic effect of salsolinol was potentiated and again this effect was significantly inhibited by propranolol and atropine. Tyramine caused a positive inotropic effect, and this effect was inhibited by propranolol or by pretreatment of the rat with reserpine. Adenosine caused a negative inotropic effect, while uridine caused a slight positive inotropic effect on the left atrium. This effect was significantly inhibited by DPCPX.

Conclusions: Crude extracs of *Tinospora crispa* exert a positive inotropic effect on the electrical field stimulated isolated left atria that results from the concerted action of 5 bioactive compounds: higenamine, salsolinol, tyramine, adenosine and uridine. Higenamine, salsolinol (at low concentration) and tyramine acted via the adrenergic receptors to increase the force of the atrial contraction, whereas a high concentration of salsolinol acted indirectly by stimulating the release of acetylcholine. Adenosine and uridine acted via the purinergic pathways to cause negative inotropic effects on the isolated left atria.

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1. Introduction

Tinospora crispa (also known as "*Tinospora crispa* (L.) Miers ex Hook. f. & Thoms", "*Tinospora rumphi* Boert" or "*Tinospora tuberculata* Beumee" or Borapet in Thai), belongs to the Family Menispermaceae. Decoctions from the stems of the *Tinospora crispa* (*Tinospora crispa*) have been used in Thai (Kongsaktrakoon et al., 1994) and Indonesian (Borneo) (Dweck and Cavin, 2006) traditional medicine to treat many conditions including hypertension, as a cardiotonic and for good health and well being. However, scientific investigations to test these therapeutic claims are scarce especially on its antihypertensive effect. Three authors have reported on its activity on the cardiovascular system. Mokkhasmit et al. (1971) found that a crude alcohol extract from the stems of *Tinospora crispa* caused an increase in blood pressure with a decrease in heart rate in anesthetized dogs. While Kongkathip et al. (2002) claimed that cycloeucalenol, isolated from a chloroform extract, produced a slight increase in the force of spontaneous contraction of the right atrium and a decrease in the force of contraction of the left atrium. Third, we recently (Praman et al., 2011, 2012) demonstrated that an *n*-butanol extract from the stems of *Tinospora crispa* exerts hypotensive activity, as well as chronotropic (cardiotonic) effects in anesthetized rats. Of the twelve substances isolated from the *n*-butanol extract five: higenamine, salsolinol, tyramine, adenosine and uridine effected blood pressure and heart rate in anesthetized rats *in vivo*. In an attempt to obtain some experimental evidence for the therapeutic claims for the cardiotonic effect of *Tinospora crispa* the present study looked at whether the *n*-butanol extract exhibits an inotropic effect on isolated rat atria, and if so, which metabolites are responsible for the effect and by what mechanism(s).

2. Materials and methods

2.1. Plant material

Stems of *Tinospora crispa* (15 kg) were collected from Phangnga Province, Thailand. Botanical identification of the plant was carried out by Prof. Poungpen Sirirugsa, Department of Biology, Prince of Songkla University, Thailand, where a voucher specimen has been deposited (Collecting No. 2548-02).

2.2. Preparation of Tinospora crispa extract

Preparation of *Tinospora crispa* extract followed our previously reported procedure (Praman et al., 2011). Briefly, air-dried stems of *Tinospora crispa* (15 kg) were simmered in hot filtered water for a period of 3 h. The clear solution was collected and heated at 50 °C to reduce the volume to 30%. The concentrated solution was extracted with chloroform, followed by ethyl acetate, and finally with *n*-butanol. The *n*-butanol extract was evaporated under reduced pressure, and the residue lyophilized to give 150.6 g of a crude brown powder (0.01% yield).

2.3. Isolation of the cardioactive constituents from the stem extract of Tinospora crispa

The bioassay-guided isolation followed the isolation protocol previously reported (Praman et al., 2012).

2.4. Pharmacological studies of the Tinospora crispa extract and purified compounds on isolated left atria

Adult female Wistar rats in estrus (250–280 g) were supplied from the Southern Animal Facility, Faculty of Science, Prince of Songkla University. They were maintained in a controlled environment (24–26 °C), with a 12 h light/dark cycle and allowed access to standard food and tap water *ad libitum*. The preparation of the animals followed the Prince of Songkla University guidelines for the approved Care and Use of Experimental Animals.

Normal or reserpinized rats (these rats were injected with reserpine at a dose of 5 mg/kg, i.p., once a day, starting two days before the experiment) were killed by decapitation with a guillotine. The thorax was opened, the heart was rapidly removed and placed in a Krebs Heinseleit solution saturated with carbogen (95% O_2+5% CO_2) at 37 °C, and allowed to beat for a few seconds to expel intra-atrial chamber blood. Both the left and the right atria were excised from the ventricles, and then the left atrium was separated. The left atrium was mounted between two platinum electrodes approximately 10 mm apart (left and right) and placed in a 20-ml organ bath, one end was fixed at the bottom and the other end connected to a force-displacement transducer (FT03C) connected to a Grass polygraph, under a basal tension of 0.7 g and equilibrated for 50 min. After the equilibration, the atrium was stimulated with several trains at 3 Hz, 5 ms pulse duration with a 1 V increase in voltage for each step until the threshold voltage (3–5 V) was reached. Preparations were allowed to equilibrate for another 20 min, then a 10–20% suprathreshold voltage (5–7 V) at frequency of 3 Hz was applied (this rhythmic contraction was continued throughout each set of experiments), allowing for 5 min of continuous contraction, followed by a cumulative challenge with *Tinospora crispa* extract before and after pre-incubation (at least 40 min for each concentration of any possible co-reactant) such as: propranolol (10^{-8}–10^{-7} M), ICI 118,551 (10^{-8}–10^{-6} M), atenolol (10^{-8}–10^{-6} M), phentolamine (10^{-6} M), atropine (10^{-6} M), DPCPX (10^{-6} M) alone or in various combinations as required. Each concentration of the *Tinospora crispa* extract was left for 2–3 min by which time the response had reached a plateau. A similar protocol was used for the pure compounds isolated from the *Tinospora crispa* extract: higenamine, salsolinol, tyramine, adenosine and uridine. Each atrium was used with only one drug and one antagonist except when the experiments needed a combination of other antagonists.

The organ bath contained Krebs Henseleit solution (pH 7.4) of the following composition (mM) NaCl 118.3, KCl 4.7, $CaCl_2$ 1.9, $MgSO_4.7H_2O$ 0.45, KH_2PO_4 1.18, $NaHCO_3$ 25.0, glucose 11.66, Na_2EDTA 0.024 and ascorbic acid 0.09, maintained at 37 °C, and was continuously bubbled with carbogen (95% O_2 and 5% CO_2).

2.5. Drugs

The following drugs were used: acetylcholine chloride, adenosine, atropine sulphate, atenolol, phentolamine hydrochloride, propranolol hydrochloride, reserpine, salsolinol, uridine and 1,3-dipropyl-8-cyclopentylxanthine (DPCPX, adenosine A_1 receptor antagonist) were from Sigma, U.S.A. ICI-118,551 was from Tocris Bioscience, UK. Borapetoside A, B, D and E, higenamine, litcubinine and syringine were previously isolated from *Tinospora crispa* extract. Atenolol, ICI-118,551, borapetoside B, borapetoside D, uridine and *Tinospora crispa* extract were dissolved in distilled water, DPCPX, reserpine, borapetoside A, borapetoside E, higenamine, litcubinine, syringin and tyramine were dissolved in 10% DMSO, and the remainder were dissolved in a solution containing NaCl 9 g/l, NaH_2PO_4 0.19 g/l and ascorbic acid 0.03 g/l. Each drug was prepared at a concentration of 10^{-1}–10^{-2} M, and the total volume added to the 20 ml-organ bath medium did not exceed 200 μl.

2.6. Data analysis

Data are expressed as a mean ± S.E.M. in which *n* indicates number of left atrium specimens used as replicates. Each left atrium

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