Effect of *Thunbergia Laurifolia* Extract on Extracellular Dopamine Level in Rat Nucleus Accumbens

Watchareewan Thongsaard PhD*,
Charles Marsden PhD**

** Department of Physiology, Faculty of Medicine, Srinakharinwirot University, Bangkok, Thailand*
*** School of Biomedical Sciences, Faculty of Medicine and Health Sciences, University of Nottingham, United Kingdom*

Background: *Thunbergia laurifolia Linn. (TL) is a herbal medicine used as an antidote for several poisonous agents in Thai traditional medicine. TL were reported not only to significantly increase potassium-stimulated dopamine release from rat striatal slices but also potentiated the effect of amphetamine on potassium-stimulated dopamine release.*
Objective: *The present study aim to investigate the effect of TL on extracellular dopamine levels in rat nucleus accumbens in vivo in comparison to cocaine.*
Material and Method: *Single injections of methanol extract of TL (200 mg/kg, ip) and cocaine (10 mg/kg, ip) were performed to determine the levels of extracellular dopamine in vivo in the living brain using the microdialysis technique (collecting time = 20 minutes).*
Results: *Cocaine and TL significantly ($p < 0.05$) increased extracellular dopamine levels in the rat nucleus accumbens in a similar manner, but the effect of cocaine had a faster onset than that of TL.*
Conclusion: *The results are in agreement with a previous in vitro study. It can be summarized that TL stimulated rat dopamine release from the nucleus accumbens both in vitro and in vivo. This brain area is responsible for the reward mechanism of addiction. The clinical significance for the use of TL in humans and the effect of long term treatment with TL on addiction requires further investigation.*

Keywords: *Addiction, Cocaine, Dopamine, Microdialysis, Thunbergia laurifolia*

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Thunbergia laurifolia Linn. is a herbal medicine used as an antidote for several poisonous agents in Thai traditional medicine. The Thai name for this plant is Rang Jued and English name is babbler's bill. Different parts of the plant are used for various medicinal purposes. For example, aqueous extracts of fresh leaves, dried leaves, dried root and bark are used as an antidote for insecticide, ethyl alcohol, arsenic and strychnine poisoning; the dried root is also used as an anti-inflammatory agent and antipyretic[1-4]. The hepatoprotective activity of TL (25 mg/kg, po) against ethanol induced liver injury has also been reported[5] while TL leaf extracts reduced neuronal cell death and memory loss caused by lead poisoning in mice[6]. Aqueous extracts of TL also provided protection against the genotoxicity of *Puraria Mirifica*[7]. This plant has been used extensively as a component of a mixture of crude extracts to treat drug addicted patients[8]. Recently, TL and amphetamine were reported to significantly increase potassium-stimulated dopamine release from rat striatal slices when compared to potassium-stimulated alone[9]. Furthermore, TL potentiated the effect of amphetamine on potassium-stimulated dopamine release when compared to amphetamine alone indicating that TL may stimulate dopamine release in a manner similar to amphetamine. There is extensive evidence linking changes in dopamine function to the pharmacology of addictive drugs and the development of drug dependence[10]. The ability of TL to increase cerebral activity in the anesthetized rat brain detected by functional magnetic resonance imaging (fMRI) has also been demonstrated[11]. The present study was performed to identify whether TL could increase extracellular dopamine levels in freely moving rats in a manner similar to that seen with drugs of addiction.

Correspondence to:
Thongsaard W, Department of Physiology, Faculty of Medicine, Srinakharinwirot University, 114 Sukhumvit 23, Wattana, Bangkok 10110, Thailand.
Phone: 0-2649-5921, Fax: 0-2260-1533
E-mail: watchare@swu.ac.th

Material and Method

Animals

Male Wistar rats, weighing 250-350 g, were obtained from the National Laboratory Animal Center, Mahidol University, Thailand. They were housed in

groups of 4-6 in a room with 12 hours light: 12 hours dark cycle and allowed free access to laboratory pellets (National Laboratory Animal Center, Thailand) and water. All animals were cared for in accordance with the International Guiding Principles for Biomedical Research Involving Animals provided by the National Research Council of Thailand.

Methanol extraction of TL

Thunbergia laurifolia (TL) was obtained from Wutythamawech botanical garden, Nakhorn Prathom, Thailand and the identification confirmed by comparison with the herbarium specimens in the Botany Section, Technical Division, Department of Agriculture, Ministry of Agriculture and Co-operative, Thailand. Methanol extracts of dried leaves of TL (1 mg/ml water) were tested using high performance liquid chromatography (HPLC) with UV detection (Waters, USA) at absorbance of 210 nm, gradient 0-100% acetonitrile from 0-20 min using a Waters' Symmetry C18, 4.6 x 250 mm column and flow rate of 0.8 ml/min. On the day of the experiment, TL solutions were prepared freshly 10 mins prior to injection by dissolving 200 mg/kg TL in 1.0 ml physiological saline (0.9% NaCl) vehicle solution.

Drugs

Cocaine hydrochloride (Macfarlan Smith Limited, UK) was used to represent a drug of addiction acting on dopaminergic neurons. The authors hold the official license for handling and experimenting on drugs of addiction under the Faculty of Medicine, Srinakharinwirot University, Bangkok, Thailand issued by Office of the Narcotics Control Board, Thailand.

Surgery and microdialysis probe implantation

Concentric-style microdialysis probes used in the microdialysis study were prepared and stored free from dust and other airborne particles until required. Before starting surgery, the microdialysis probe was connected to a small infusion pump via a swivel which was connected to the Portex tubing (0.25 mm id) and artificial cerebrospinal fluid (aCSF) was perfused continuously through the probe at a flow rate of 0.5 ml/min. The probes were implanted vertically into the nucleus accumbens according to the atlas of Paxinos and Watson[13].

Sample collection

The rate at which the aCSF was perfused through the probe was changed from 0.5 ml/min to 1.5 ml/min on the following day and left to run for at least 30 min before starting the experiment. Four basal samples (30 µl each) were collected at 20 min intervals and the mean value of the basal level was used to represent the value of 100% dopamine release. The samples were collected continuously at 0, 20, 40, 60, 80, 100, and 120 minutes. In the first experiment, animals were injected with cocaine hydrochloride (10 mg/kg, ip) after collection of four basal samples and the samples following injection were collected at the time intervals given above. In the second experiment, after collection of the four basal samples, the animals received 200 mg/kg, ip TL and the samples were collected at the same time intervals. Extracellular levels of dopamine in the samples were measured immediately after collection.

At the end of the experiment, the animal was killed with an overdose of pentobarbital and the brain removed, fixed overnight in 10% formaldehyde and sectioned to check the placement of the probe in the nucleus accumbens. The microdialysis data were only used from animals in which the dialysis probe was correctly positioned within the nucleus accumbens.

Sample analysis

High performance liquid chromatography with electrochemical detector (HPLC-ECD) was used to analyze the levels of dopamine in the samples and compared to the standard (2 pmole/20 ml) and data presented as % of basal level. Quantification of the levels in the experimental samples was obtained by comparing the area under the peak (obtained from the integrator) to that of the standard.

Statistical analysis

The data were presented as mean $\pm$ SEM and analysed using ANOVA and a post-hoc (Newman-Keuls multiple comparison) test with $p < 0.05$ considered significant.

Results

The chromatograms of TL extract recorded by HPLC with UVdetection from 4 extraction batches indicate 5 distinct peaks at 2.336-2.422, 2.622-2.812, 3.719-3.817, 4.061-4.086 and 4.164-4.249 mins after injection onto the HPLC. The results indicated that the four extraction batches were of identical composition and suggested the presence of at least 5 different compounds in the TL methanol extracts. The identification of each compound has not yet been established. The largest peak was that measured between 2.336-2.422 mins (Table 1) indicating a major compound or a group of major compounds was/were

extracted from the methanol extract.

The percentages of basal dopamine release from the rat nucleus accumbens after cocaine (10 mg/kg, ip) and TL (200 mg/kg, ip) are shown in Fig. 1. Both cocaine (10 mg/kg, ip) and TL (200 mg/kg ip) significantly ($p < 0.05$) increased dopamine release from the rat nucleus accumbens but the onset of the increase was faster with cocaine with the increase seen in the first sample collected after injection while with TL the increase was not observed until collection of the second sample post injection (Fig. 1). These results indicate that both cocaine and TL increase dopamine release but that the onset of the increase is faster with cocaine than with TL. Cocaine increased the percentage of dopamine release at the mean value of 195 $\pm$ 8% (n = 8) while TL stimulated release by a mean value of 148 $\pm$ 12% (n = 8). The significant effect of cocaine on dopamine release persisted for 60 min post injection and then returned to basal values while the TL induced increase lasted for 40 mins before recovery to the basal level.

Table 1. The chromatographic peaks obtained from four TL extracts recorded by HPLC with UV detection

Extraction	Time (Min)				
	Peak 1	Peak 2	Peak 3	Peak 4	Peak 5
Lot 1	2.336	2.622	3.719	4.061	4.170
Lot 2	2.381	2.654	3.817	4.086	4.201
Lot 3	2.422	2.722	-	-	4.249
Lot 4	2.367	2.812	-	4.073	4.164

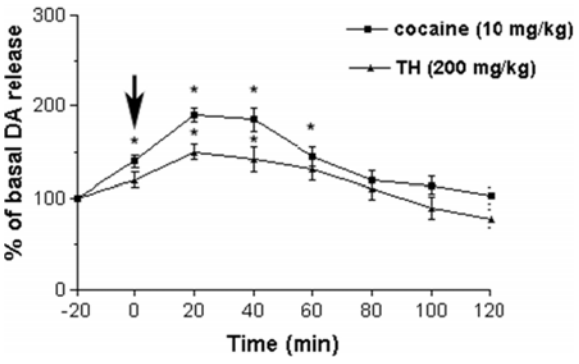

Fig. 1 Effect of cocaine (10 mg/kg, ip.) and *Thunbergia laurifolia* (TL; 200 mg/kg, ip.) on the levels of dopamine (DA) in perfusates from rat nucleus accumbens. The perfusion rate through the dialysis tubing was 1.5 ml/min and dopamine was measured in 20 min samples (30 ml). Data are expressed as a percentage of the mean basal release before administration of cocaine or TL. Administration is indicated by the arrow. Each value is the mean $\pm$ SEM (n = 8). *$p < 0.05$ using ANOVA and post-hoc (Newman-Keuls multiple comparison) test

Discussion

The chromatograms of TL extracts produced, using HPLC with UV detection, indicated that among four extraction batches, there were five distinct peaks. The largest peak was found between 2.336 and 2.422 min. The HPLC chromatograms of four methanol (80%) extracts of TL (1 mg/ml water), obtained using UV detection at an absorbance of 210 nm was shown in our previous report[11]. Methanol extracts of the dried aerial parts of TL have been reported to contain 9 compounds, including two benzyl alcohol glucosides, three iridoid glucosides, two aliphatic alcohol glucosides, and two flavonoid C-glucosides[14,15]. It remains to be determined which of these compounds in the dried leaves are responsible for pharmacological effects in the brain.

The present in vivo study used cocaine instead of amphetamine to compare the effects with TL due to the limitation of the use of amphetamine in animal models. Cocaine also exerts an effect on dopaminergic neuron resulting in increased extracellular dopamine levels indicative of increased release and so cocaine (10 mg/kg ip) was used to examine the stimulating effects of a drug of abuse on extracellular dopamine in freely moving rats using microdialysis in the nucleus accumbens. The results were in agreement with those previously reported[16,17]. The present result showing that TL also increased extracellular dopamine levels in the nucleus accumbens supports a previous study from our laboratory on the effect of a crude water extract of TL on in vitro dopamine release[9]. In the earlier study TL significantly increased potassium-stimulated dopamine release from rat striatal slices when compared to potassium-stimulated alone[9] indicating that TL exertes a similar effect in the striatum and nucleus accumbens to some drugs of addiction such as cocaine and amphetamine. Furthermore, the increases in blood oxygen level-dependent (BOLD) following TL administration indicates that the plant extract can enhance neuronal activity in specific areas of the brain responsible for reward and locomotion. Moreover, the decrease in blood pressure produced by TL administration in the fMRI study supports the view that the change in functional activity reflects a true

regional neuronal response rather than any change in blood flow caused by a change in systemic blood pressure[11]. Similar changes in BOLD have been reported with cocaine administration[12]. The nucleus accumbens is an area of the mesolimbic dopaminergic system responsible for the reward mechanisms associated with drug addiction[18] and has been suggested to play a key role in brain reward processes[19]. There is also considerable evidence in the literature to support such a role in drug reinforcement mechanisms[20]. Intravenous self-administration studies utilizing 6-hydroxydopamine lesioning and micro-injection techniques suggest that the nucleus accumbens is a site initiating the rewarding effects of psychostimulant drugs[21].

Conclusion

The present study demonstrates a dopaminergic neuronal effect of TL by increasing extracellular dopamine levels in the rat nucleus accumbens in vivo using microdialysis and that this effect is similar to that seen with cocaine. In summary the results indicates that a methanol extract of TL exertes similar effects on dopaminergic function to cocaine in the nucleus accumbens, an area of the mesolimbic dopaminergic system responsible for the reward mechanisms associated with addiction. The question whether long term treatment with TL can cause addiction or not remains to be evaluated.

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Potential conflicts of interest

None.

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ผลของสารสกัดรางจืดต่อระดับโดปามีนในนิวเคลียสแอคคัมเบนส์

วัชรีวรรณ ทองสะอาด, *Charles Marsden*

ภูมิหลัง: *รางจืดเป็นพืชสมุนไพรที่ใช้อย่างแพร่หลายในการถอนพิษสารเคมีต่างๆ มีรายงานว่าสารสกัดรางจืดออกฤทธิ์ต่อเนื้อเยื่อสมองของหนูทดลองโดยมีผลเพิ่มการหลั่งสารโดปามีน เช่นเดียวกับผลจากสารเสพติด*
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ผลการศึกษา: *การทดลองพบว่ารางจืดกระตุ้นการหลั่งสารสื่อประสาทโดปามีนในสมองส่วนนิวเคลียสแอคคัมเบน เช่นเดียวกับผลของโคเคน เมื่อเปรียบเทียบเป็นเปอร์เซ็นต์ของค่าตั้งต้น*
สรุป: *ผลการทดลองสามารถสรุปได้ว่ารางจืดมีผลกระตุ้นการหลั่งสารสื่อประสาทโดปามีนจากสมองส่วนที่ควบคุมพฤติกรรมการเคลื่อนไหวและความพึงพอใจ คล้ายผลของสารเสพติดโคเคน ผลของรางจืดต่อสมองคน ในการก่อให้เกิดภาวะการเสพติดหรือผลต่อระบบอื่นๆ ยังต้องมีการศึกษาต่อไป*