Toxicology and Applied Pharmacology 274 (2014) 180–189

Contents lists available at ScienceDirect

Toxicology and Applied Pharmacology

journal homepage: www.elsevier.com/locate/ytaap

Bidirectional regulation of bakuchiol, an estrogenic-like compound, on catecholamine secretion

Haoping Mao [a,b], Hong Wang [a,b], Shangwei Ma [a,b], Yantong Xu [a,b], Han Zhang [a,b], Yuefei Wang [a,b], Zichang Niu [c], Guanwei Fan [a,b], Yan Zhu [a,b], Xiu Mei Gao [a,b,*]

[a] Tianjin State Key Laboratory of Modern Chinese Medicine, Key Laboratory of Pharmacology of Traditional Chinese Medicine Formulae, China
[b] Institute of Traditional Chinese Medicine, Tianjin University of Traditional Chinese Medicine, Tianjin, China
[c] First Teaching Hospital of Tianjin University of Traditional Chinese Medicine, Tianjin, China

ARTICLE INFO

Article history:
Received 1 August 2013
Revised 31 October 2013
Accepted 1 November 2013
Available online 11 November 2013

Keywords:
Bakuchiol
Catecholamine
Dual effects
Antidepressant-like effect
Anti-stress

ABSTRACT

Excess or deficiency of catecholamine (CA) secretion was related with several diseases. Recently, estrogen and phytoestrogens were reported to regulate the activity of CA system. Bakuchiol is a phytoestrogen isolated from the seeds of *Psoralea corylifolia* L. (Leguminosae) which has been used in Traditional Chinese medicine as a tonic or aphrodisiac. In the present study, bovine adrenal medullary cells were employed to investigate the effects and mechanisms of bakuchiol on the regulation of CA secretion. Further, its anti-depressant like and anti-stress effects were evaluated by using behavioral despair and chronic immobilization stress models. Our results indicated that bakuchiol showed bidirectional regulation on CA secretion. It stimulated basal CA secretion in a concentration dependent manner ($p < 0.01$), while it reduced 300 μM acetylcholine (ACh) ($p < 0.01$), 100 μM veratridine (Ver) ($p < 0.01$) and 56 mM K^+ ($p < 0.05$) induced CA secretion, respectively. We also found that the stimulation of basal CA secretion by bakuchiol may act through estrogen-like effect and the JNK pathway in an extra-cellular calcium independent manner. Further, bakuchiol elevated tyrosine hydroxylase Ser40 and Ser31 phosphorylation ($p < 0.01$) through the PKA and ERK1/2 pathways, respectively. Bakuchiol inhibited ACh, Ver and 56 mM K^+ induced CA secretion was related with reduction of intracellular calcium rise. In vivo experiments, we found that bakuchiol significantly reduced immobilization time in behavioral despair mouse ($p < 0.05$ or 0.01), and plasma epinephrine (E) and norepinephrine (NE) levels in chronic immobilization stress ($p < 0.05$). Overall, these results present a bidirectional regulation of bakuchiol on CA secretion which indicated that bakuchiol may exert anti-stress and the potential anti-depressant-like effects.

Introduction

Catecholamines (CA), which consist of norepinephrine (NE), epinephrine (E) and dopamine (DA), are important neurotransmitters that mediate various physiological effects. Typically, the "fight or flight response" induced by acute stress (Cannon and Paz, 1911) is accompanying excess CA secretion of the sympathetic nervous system which elevates heart rate, blood pressure and blood glucose to help animals escape from threats. Either excess or deficiency of CA secretion disturbs physiological functions. Therefore, pharmacological agents that alter or modify regulation of CA may have potential therapeutic efficacy for treatments of CA related diseases.

Bovine adrenal medulla cells are derived from multipotent neural crest cells in the developing embryo. They share a common sympathoadrenal progenitor with sympathetic neurons. In these cells, rise of intercellular concentration of Ca^{2+} is a prerequisite for secretion and synthesis of CA. Acetylcholine (ACh) induced Na^+ influx by nicotinic ACh receptor-ion channels and veratridine (Ver) induced Na^+ influx by voltage-dependent Na^+ channels increase intercellular concentration of Ca^{2+} by voltage-dependent Ca^{2+} channels; high K^+ directly gates voltage-dependent Ca^{2+} channels to increase Ca^{2+} influx without increasing Na^+ influx (Wada et al., 1985). Because CA secretion mediated by stimulation of these ion channels in adrenal medullary cells was thought to be similar to that of norepinephrine (NE) and epinephrine (E) in the sympathetic neurons, bovine adrenal medullary cells have been widely used as a sympathetic model (Haass et al., 1997; Matsuda et al., 2008; Satoh et al., 2012).

Phytoestrogens are plant-derived compounds showing estrogen- or antiestrogen-like effects (Albertazzi and Purdie, 2002). Recent

Abbreviations: CA, catecholamine; NE, norepinephrine; E, epinephrine; DA, dopamine; DOPA, dihydroxyphenylalanine; TH, tyrosine hydroxylase.

* Corresponding author at: Institute of Traditional Chinese Medicine Research, Tianjin University of Traditional Chinese Medicine, #312 Anshan Xi Road, Nankai District, Tianjin 300-193, China. Fax: +86 22 59596110.
E-mail address: gaoxiumei@tjutcm.edu.cn (X.M. Gao).

0041-008X
http://dx.doi.org/10.1016/j.taap.2013.11.001

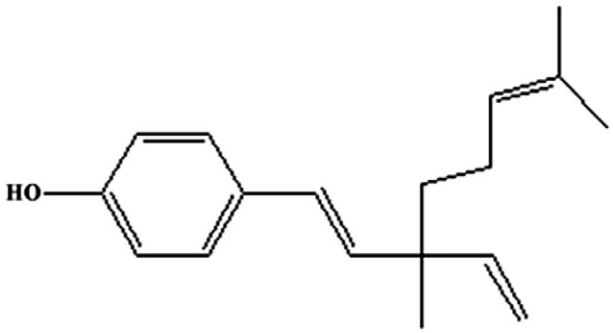

Fig. 1. Chemical structure of bakuchiol.

evidences demonstrate the regulation of estrogen and phytoestrogens on CA system. Estrogen receptors have been found in adrenergic neurons, cholinergic neurons and neuroendocrine cells (Haywood et al., 1999; Milner et al., 2001). Further evidences show that estrogen and phytoestrogens can regulate synthesis, secretion and degradation of CA. Estradiol benzoate elevates the mRNA expression of tyrosine hydroxylase (TH), the rate-limiting enzyme of CA synthesis (Serova et al., 2002). 17β-estradiol (E_2) stimulates CA synthesis via activation of p44/42MAPK (Yanagihara et al., 2006) and decreases monoamine oxidase-A activity (Ma et al., 1993, 1995). Phytoestrogen like daidzein suppresses CA secretion at high concentrations while stimulating CA synthesis at low concentration (Liu et al., 2007). Resveratrol (3,4′,5-trihydroxy-trans-stilbene), a natural polyphenolic compound found in grapes, berries and red wine, was reported to be an agonist for the estrogen receptor (Gehm et al., 1997). It suppresses CA secretion and synthesis induced by ACh (Shinohara et al., 2007). Cimicifugoside, a triterpene glycoside from black cohosh, possesses a steroid backbone structure, so was called phytoestrogen. It did not affect the KCl-induced secretion but inhibited a nicotinic ACh receptor (nAChR) agonist 1,1-dimethyl-4-phenylpiperazinium iodide (DMPP) induced CA secretion in bovine chromaffin cells (Woo et al., 2004).

Bakuchiol (the structure was showed in Fig. 1) is a prenylated phenolic monoterpene isolated from the seeds of *Psoralea corylifolia* L. (Leguminosae) which has been broadly used clinically in Traditional Chinese medicine. Kim KA reported that 10 μM bakuchiol had protective effects against oxidative stress (Kim et al., 2013). Bakuchiol also exerts antibacterial (Hsu et al., 2009), anti-inflammation (Choi et al., 2010; Ferrandiz et al., 1996), anti-tumor (Chen et al., 2010; Yang et al., 2010), immunosuppressive (Chen et al., 2008), and hepatoprotective effects (Cho et al., 2001; Park et al., 2007) and hypoglycemic activity (Krenisky et al., 1999). In addition, our group recently reported that bakuchiol possesses phytoestrogenic activity (Xin et al., 2010) which explains its effect in reducing bone loss in ovariectomized Sprague–Dawley rats (Lim et al., 2009). Meanwhile, bakuchiol analogs inhibit monoamine transporters and regulate monoaminergic functions (Zhao et al., 2008), suggesting a potential interaction of bakuchiol and CA. In the present study, we speculated that phytoestrogen bakuchiol may regulate CA secretion and this action may exert anti-depression or anti-stress activity. We employed chromaffin cells to investigate the effect of bakuchiol on the regulation of CA secretion. To examine the in vivo mental effects of bakuchiol, forced swim test (FST), tail suspension test (TST) and immobilization stress test (IMST) were employed in mice.

Methods

Isolation and primary culture of bovine adrenal medullary cells. Adrenal glands were obtained from the city slaughterhouse. Bovine chromaffin cells were isolated as described previously (Mao et al., 2009). All the cells from different bovines (both male and female) were mixed together before planting. Primary cells between 2 and 7 days of culture were used for experiments.

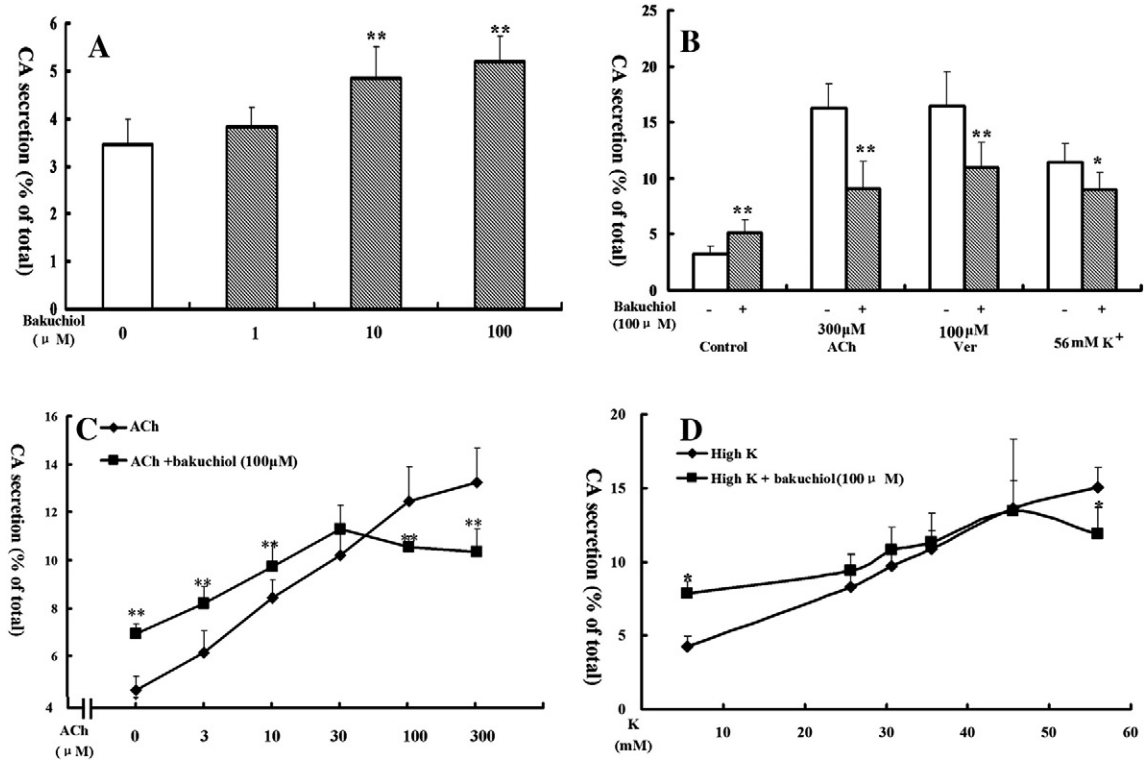

Fig. 2. Bidirectional effects of bakuchiol on CA secretion on bovine adrenal medullary cells. (A) Effects of 1–100 μM bakuchiol on basal CA secretion; (B) effects of 100 μM bakuchiol on various secretagogues induced CA secretion. (C) Effects of 100 μM bakuchiol on CA secretion induced by various concentrations of ACh and (D) effects of 100 μM bakuchiol on CA secretion induced by various concentrations of K^+. Data were expressed in means ± SD based on three independent experiments in triplicate (n = 7–9 per group). *$p < 0.05$, **$p < 0.01$.