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Journal of Ethnopharmacology 101 (2005) 227–232
Journal of ETHNO-PHARMACOLOGY
www.elsevier.com/locate/jethpharm

Barakol suppresses norepinephrine-induced inhibition of spontaneous longitudinal smooth muscle contractions in isolated rat small intestine

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Received 19 August 2004; received in revised form 23 March 2005; accepted 27 April 2005
Available online 25 July 2005

Abstract

The present study aimed to investigate the purgative effects of barakol, the purified extract of *Cassia siamea* Lam., on the longitudinal smooth muscle contractions of the rat ileum. The extract increased the force of spontaneous muscle contractions in a concentration-dependent manner (EC_{50} = 0.3 mM). Saxitoxin (0.3 μM) abolished the stimulatory effects of barakol, a result indicating a neural mechanism of action. In addition, atropine (10 μM) but not propanolol (10 μM) or phentolamine (10 μM), partially inhibited barakol-induced smooth muscle contractions suggesting that cholinergic nerves were involved. The motor effects of barakol were further examined in muscle strips treated with catecholamines to suppress spontaneous contractile activity and decrease muscle tone. Norepinephrine or dopamine (10 μM) decreased the amplitude of spontaneous contractions by 72% and 18%, respectively. Pretreatment of the tissues with barakol (1 mM) significantly decreased the inhibitory effect of norepinephrine by 60%, but not that of dopamine. Its ability to potentiate atropine- and saxitoxin-sensitive contractions and inhibit the antimotility actions of norepinephrine suggests that barakol may increase longitudinal smooth muscle contractions by decreasing the inhibitory effect of norepinephrine on excitatory cholinergic motor neurons. Barakol may produce a purgative action in small intestine which may be clinically important in patients with intestinal hypomotility disorders.

Keywords: *Cassia siamea*; Intestinal motility; Constipation

1. Introduction

Barakol is a biologically active constituent extracted from the leaves and flowers of *Cassia siamea* Lam. (or *Senna siamea* Lam. Irwin & Barneby) of the family *Caesalpiniaceae*. This plant is widely cultivated in south-east Asia including Thailand and traditionally used to treat insomnia, diabetes, fever, hypertension and constipation (Satyavati et al., 1979; Kinghorn and Balandrin, 1992). Alcoholic extracts of *Cassia siamea* have been shown to possess central nervous system (CNS) depressant activity, decrease spontaneous locomotor activity, increase smooth muscle tone (Arunlakshana, 1949) and decrease blood pressure (Mokasmit, 1981). Barakol was first isolated by Hassanali-Walji et al. (1969) and its chemical structure (3α, 4-dihydro-3α, 8-dihydroxy-2, 5-dimethyl-1, 4-dioxaphenalene or 2,5-dimethyl-3αH-pyrano-[2,3,4-de]-1-benzopyran-3α,8-diol) was identified by Bycroft et al. (1970). In animal models, barakol produces hypotension (Suwan et al., 1992), suppresses serotonergic activity as shown by decreasing 5-hydroxytryptophan-induced head shake behavior (Tongroach et al., 1992) and possesses anxiolytic activity on the elevated plus maze, a behavioral test for anxiolytic drugs (Thongsaard et al., 1996). Barakol also suppresses K^+-stimulated endogenous dopamine release from striatal slices of the rat brain (Thongsaard et al., 1997). These data suggest that barakol can alter CNS activity. However, little is known about its

Abbreviations: CNS, central nervous system; DA, dopamine; ENS, enteric nervous system; NE, norepinephrine

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doi:10.1016/j.jep.2005.04.017

effects on the peripheral nervous system, another potential site of barakol action.

Gastrointestinal secretory and motor functions are regulated by the enteric nervous system (ENS; Guyton and Hall, 1996). The myenteric ganglionated plexus (also termed Auerbach's plexus) within the ENS modulates propulsive motor activity along the intestinal tract. Stimulation of enteric cholinergic neurons within the gut wall as well as those originating in the vagus increases intestinal motility by promoting peristalsis. On the other hand, noradrenergic neurotransmission in the gut is generally associated with decreased motility (Lange, 1996; Curry and Tatum-Butler, 1996). Decreases in intestinal motility induced by psychological stress or idiopathic constipation may be mediated by norepinephrine (NE) and possibly the related catecholamine, dopamine (DA; Tsukada et al., 2002). Catecholamines may reduce motility through direct actions on intestinal myocytes or by suppressing excitatory neurotransmission to smooth muscle (McEvoy, 2000). As barakol modulates dopaminergic transmission in the CNS, it is possible that it may alter the inhibitory effects of catecholamines on intestinal motor function. Therefore, the aim of the present study was to investigate the effect of barakol on spontaneous longitudinal smooth muscle contractions in the isolated rat ileum. An additional objective was to determine the mechanism by which barakol alters the inhibitory actions of NE and DA on smooth muscle contractility.

2. Materials and methods

2.1. Drugs and chemicals

Barakol was extracted and purified from *Cassia siamea* in our laboratory as previously described (Thongsaard et al., 2001). Briefly, a *Cassia siamea* plant was collected from the Ladkrabang area of Bangkok, Thailand in August. The herbarium specimen was authenticated by Wongpakam S., a plant taxonomist, deposited, and given voucher specimen number A011432 by the Department of Botany, Faculty of Science, Chulalongkorn University, Bangkok, Thailand. The leaves and flowers were cut into small pieces and boiled twice in 0.5% sulfuric acid for 30 min. The mixture was blended, filtered, and alkalinized with concentrated sodium bicarbonate and subsequently extracted with chloroform. The chloroform extract was further concentrated with 5% acetic acid and neutralized with 25% ammonium hydroxide. The crude barakol extract was obtained as greenish crystallized yellow needles with 0.3% yield. Concentrated hydrochloric acid was finally added to obtain barakol hydrochloride and the mixture was dried by vacuum filtration to form yellowish crystals of anhydrous barakol hydrochloride. The purity of the compound was confirmed by thin layer chromatography on silica gels and nuclear magnetic resonance. When anhydrous barakol hydrochloride was dissolved in water, it converted to barakol in a solution at pH 3–4 in the stock concentration of 50 mM. Prior to each experiment, barakol was freshly dissolved in normal saline solution, kept on ice and in the dark to prevent oxidation, and used within 3 h after preparation.

Arterenol bitartrate (norepinephrine), atropine sulfate, acetylcholine, dopamine hydrochloride, propanolol, phentolamine and saxitoxin were purchased from Sigma Chemical, St. Louis, MO, USA. Other chemicals and analytical grade salts were purchased from Fisher Scientific, Loughborough, UK. All drugs were dissolved in distilled water and maintained in concentrated stock solutions. Aliquots were diluted immediately before the start of each experimental session.

2.2. Animals

Male Wistar rats (250–300 g) were obtained from the National Laboratory Animal Center, Thailand. They were housed in stainless-steel cages in a room with a 12 h light:12 h dark cycle and allowed access to food (National Laboratory Animal Center, Thailand) and tap water ad libitum. All animals were sacrificed by decapitation. After a laparotomy incision, a portion of the ileum was removed and placed in an oxygenated physiological salt solution approximating the composition of rat extracellular fluid (composition in mM: 118 NaCl, 4.7 KCl, 2.5 $CaCl_2$, 0.5 $MgCl_2$, 25 $NaHCO_3$, 1.0 NaH_2PO_4, 11 D-glucose; pH 7.4).

2.3. Measurement of smooth muscle contractility

Ileal segments were longitudinally cut along the antimesenteric border and placed in oxygenated ice-cold physiological salt solution. They were pinned out as a flap, with the mucosa uppermost and a 3×10 mm muscle strip was cut parallel to the longitudinal muscle layer of the ileum. The strip therefore contained the longitudinal smooth muscle, from which isometric recordings were made, as well as the circular smooth muscle and both the myenteric and submucosal plexuses.

Intestinal strips were oriented in the plane of the longitudinal muscle and mounted in 15 ml organ baths containing physiological salt solution that was gassed with 95% O_2 and 5% CO_2 and maintained at 37 °C. Tissues were mounted under an initial tension of 9.8 millinewtons (mN). Strips were equilibrated for 45 min and the bathing media was changed every 15 min. Mechanical activity was recorded isometrically with a strain gauge force transducer (Maclab Model FT-100, AD Instruments, NSW, Australia) connected to a BRIDGE amplifier and a MacLab® 4S A/D converter (AD Instruments) and monitored with a 400 MHz PowerPC MacIntosh computer.

The average peak amplitude and frequency of contractions occurring after administration of each drug or drug concentration was determined and compared to average contraction amplitudes measured prior to drug administration. Due to the acidity of the barakol solution, the effect of 0.1 M hydrochloric acid was determined on spontaneous muscle contractions as a drug-free vehicle control. Hydrochloric acid, in a