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Noscapine antagonizes vasoconstrictor action of bradykinin in isolated human umbilical artery

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Abstract

Background: It has been demonstrated that noscapine, an antitussive opioid alkaloid, could antagonize bradykinin-induced responses such as bradykinin effects in guinea-pig ileum, cough induced by bradykinin receptor agonist and angiotensin converting enzyme inhibitors, and brain damage after brain edema both in neonatal rat model and in patients with stroke. In the present study, the effect of noscapine on bradykinin-induced constriction of human umbilical artery was investigated.

Methods: Segments of human umbilical cords were obtained from women with normal full term pregnancies. Concentration-response curves for bradykinin (1-1000 nM) were constructed in the absence and presence of noscapine (1-1000 nM). To show the specificity of noscapine for bradykinin-induced constriction in the tissue, the effect of noscapine (10 pM) on vasoconstriction produced by histamine were also examined.

Results: The results showed that noscapine could antagonize the constriction produced by bradykinin in human umbilical artery. It was also demonstrated that noscapine was capable of reducing histamine-induced contractile response.

Conclusion: It is concluded that noscapine can antagonize bradykinin-induced constriction of human umbilical artery in a nonspecific manner. Thus, noscapine is likely to find a clinical application in pathologic conditions accompanied by higher vascular sensitivity to bradykinin in pregnancy.

Keywords: noscapine, human umbilical artery, bradykinin, histamine.

Introduction

The kallikrein-kinin system plays an important role in many physiological and pathophysiological conditions such as homeostasis of circulation, inflammation/ allergy, pain, shock, etc. Two types of kinin receptors are known, bradykinin (BK) B_1 and B_2 receptors. B_2 receptors are constitutively expressed and mediate most physiological actions of kinins, whereas B_1 receptors are highly inducible upon inflammatory stimulation or tissue injury, suggesting that they are involved in inflammation and/or nociception [1]. Numerous BK antagonists have been developed in recent years with the prime aim of treating diseases resulting from excessive BK formation. Such BK receptor antagonists may have novel therapeutic applications in various pathological conditions associated

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with abnormal kinin system [2]. It has been demonstrated, in our laboratory, that noscapine, a non-narcotic antitussive opioid alkaloid, specifically antagonizes the contractile response of BK in guinea-pig ileum [3], interferes with cough induced by a BK B_2 receptor agonist, ie, FR190997, and angiotensin converting enzyme inhibitor (ACEI), ie, enalapril, in guinea-pig [4], and attenuates BK-mediated brain damage after brain edema both in neonatal rats with a hypoxic-ischemic insult [5] and in patients with acute ischemic stroke [6]. Because of noscapine safety [7], the newer medical applications of the drug would be desirable. In the present study, the effect of noscapine on BK-induced constriction of isolated human umbilical artery (HUA) was investigated. Umbilical artery plays an essential role in adequate perfusion and nutrition of the placenta and is sensitive to kinins [8]. It has been shown that BK results in constriction of HUA through B_2 receptors [9]. To show the specificity of noscapine against BK-induced constriction in this tissue, its action on vasoconstriction produced by histamine was also examined.

Methods

Tissue preparation

Segments of human umbilical cords were obtained from women with normal full term pregnancies. Immediately after delivery, a 7-8 cm long segment of the umbilical cord was taken from the placental portion. This area of the cord is known to be nerve free [10-12]. Cords from mothers exhibiting eclampsia, hypertension, diabetes, a Rhesus problem or other overt diseases were not included in the study. Cords from mothers taking medication, such as antihistamines, morphine, adrenergic blockers or anticholinergic drugs during near term period were also excluded. All procedures had Institutional Ethical Committee approval. The cords were immediately placed in cold (4°C) Krebs solution (see below). The arteries were dissected free of the Wharton's jelly within 1 h. Care was taken in the dissection, to minimize handling in order to prevent tissue damage and prostaglandin release [13,14] which could cause constriction of the isolated HUA [15,16]. A magnifying lens was always used during the preparation procedure. The arteries with an intact endothelium (confirmed histologically), were cut into 3-4 mm long ring segments and suspended under 2 g tension in 10 ml organ bath. The organ bath contained Krebs solution of the following composition (in mM): NaCl 118, KCl 4.8, $CaCl_2$ ($2H_2O$) 2.5, $MgSO_4$ ($7H_2O$) 1.2, KH_2PO_4 1.2, $NaHCO_3$ 25 and glucose 10. The temperature was thermostatically maintained at 37°C and the solution was aerated with 95% O_2 and 5% CO_2. A 2-h equilibration period was allowed before the administration of the agonist and during this time Krebs solution was changed every 15 minutes. Responses were measured with isometric tension transducer (F90-NARCO, USA) coupled to a polygraph. Tissues that exhibited spontaneous activity were not utilized in the experiments.

Individual protocols

Ascending concentrations of the vasoconstrictor agonist, ie, BK (1-1000 nM), were added to the organ bath cumulatively to generate a concentration-response curve. Subsequent concentration-response curve for BK was constructed in the presence of noscapine, as an inhibitor. A washout period of at least 90 min was allowed between constructing the two concentration-response curves. During the washout period, Krebs solution was changed every 15 min. When inhibitor was used, different concentrations of noscapine (1, 10, 100 and 1000 nM) were added to the bath and tissue allowed to equilibrate for 30 min before the agonist concentration-response curve was repeated. In each individual preparation, concentration-response curves for BK in the absence and presence of only one concentration of noscapine were constructed. To show the specificity of the inhibitory action of noscapine for BK-induced constriction, the effect of noscapine (10 pM) against histamine-induced vasoconstriction was also assessed.

Agonist potency was expressed as pEC50

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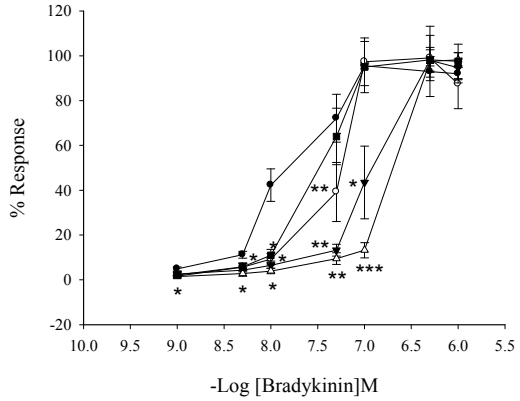

Fig. 1. Mean constrictor concentration-effect curves to BK alone (●), on human isolated umbilical artery and in the presence of 1 (Δ), 10 (▼), 100 (❍) and 1000 (■) nM noscapine. Each point represents arithmetic mean ± S.E.M of six experiments. *p<0.01, **p<0.001, ***p<0.0001, significant difference between BK alone and in the presence of noscapine.

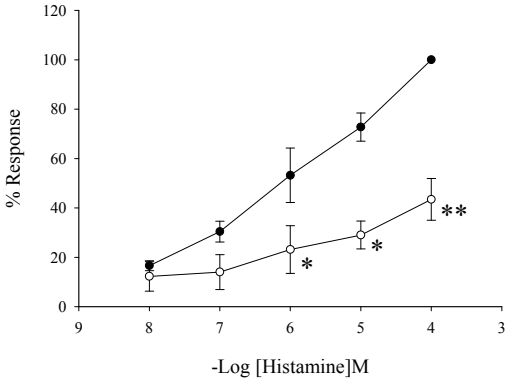

Fig. 2. Mean constrictor concentration-effect curves to histamine alone (λ), on human isolated umbilical artery and in the presence of 10 pM noscapine (μ). Each point represents arithmetic mean ± S.E.M. of six experiments. *p<0.05, **p<0.01.

value which defined as the negative logarithm of the concentration required to produce a response equivalent to 50% of the maximal response elicited by the agonist. pEC50 values for BK alone and in presence of different concentrations of noscapine were used to compare the differences between the inhibitory responses caused by noscapine and control.

Drugs

BK and histamine was obtained from Sigma Chemical Company, Germany. Noscapine was obtained from Temad Pharmaceutical Company, Tehran, Iran. BK was dissolved in distilled water and final drug solutions were made up in saline (0.9% w/v NaCl). A 10 mg/ml noscapine stock solution was prepared in 0.1 M acetic acid and diluted with saline to obtain final solutions. Histamine solutions were also made up in saline.

Table 1. pEC50 values for bradykinin (BK) in the absence and presence of noscapine (1, 10, 100 and 1000 nM).

Concentration of noscapine (nM)	pEC50 values for BK
-	8.81 ± 0.16
1	7.55 ± 0.06*
10	7.73 ± 0.3**
100	8.46 ± 0.25
1000	8.33 ± 0.25

* p< 0.01 **p< 0.0001 vs pEC50 value for BK in absence of noscapine

Statistical analysis

All data are expressed as arithmetic means ± standard error of mean (SEM) of n experiments. Student's t-test used for analysis and differences were considered significant when $p<0.05$. All statistical analyses were carried out using SigmaPlot software, and EC50 values are expressed as pEC50.

Results

Noscapine alone in concentrations used in this study had no effect on the tone of HUA rings. Cumulative addition of BK (1-1000 nM) resulted in concentration-dependent constriction of HUA rings. Noscapine (1, 10, 100 and 1000 nM) antagonized the constriction produced by BK (Fig. 1). Although the presence of ascending concentrations of noscapine did not affect the BK-induced maximal response but the lowest concentration (1nM) caused the greatest shift of BK concentration-response curve to the right. On the contrary, the highest concentration of noscapine used in the experiment (1μM) produced the least inhibition against BK-induced constriction of HUA rings (Fig. 1).

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The pEC50 values for BK in the absence and presence of noscapine are presented in Table 1. Noscapine (10 pM) caused an inhibition against histamine-induced constriction in HUA ring segments as well (Fig. 2).

Discussion

The results presented in this study indicated that noscapine might antagonize the constriction produced by BK and also histamine in HUA ring segments. It has been previously reported that noscapine, being used clinically as an antitussive drug, could antagonize BK-induced contraction of isolated guinea-pig ileum in a non-competitive manner while it did not inhibit the responses to acetylcholine, histamine, serotonin or angiotensin II [3]. Moreover, in another study, noscapine was able to suppress cough induced by an ACEI and an agonist of BK B_2 receptor. Decreased degradation of BK has been proposed as a major mechanism involved in ACEI-induced cough [17]. This antitussive effect of noscapine was not mediated through the μ, κ or δ opioid receptors and it was proposed that noscapine might interfere with the BK cough mediation [4]. It has also been shown that noscapine could reduce brain injury after hypoxic-ischemic insult in neonatal rats as a model of brain edema [5] and was effectively able to attenuate the postischemic brain edema and reduce the mortality rate in patients with stroke [6]. BK is known to contribute to neural tissue damage as well as to long lasting disturbances of the blood-brain barrier function through activation of B_2 receptors [18-20] and a BK B_2 receptor antagonist, LF 16-0687 Ms, was demonstrated to enhance the neurological recovery in a rat model of temporary focal cerebral ischemia [21]. In the present study, noscapine antagonized BK-induced constriction of isolated HUA. This BK action was previously demonstrated to be exerted through B_2 receptors [9]. The antagonism against BK was inversely dependent on noscapine concentrations. In nearly all experiments performed in our lab, noscapine was shown to have an inverted U shape biphasic response depending on the concentration range used in the experiment. Obtaining the greatest inhibitory effect of noscapine, although non-specific, with the lowest concentration of the drug resembled the findings presented by Zausinger et al. [21] and Plensila et al. [22] who have achieved beneficial results when using a lower dose of specific non-peptide competitive B_2 receptor antagonist, LF 16-0687 Ms. Furthermore, Relton et al. [23] when using a selective peptide-based B_2 receptor antagonist, CP-0596, demonstrated that the higher dose of B_2 antagonist failed to affect brain edema after induction of a focal lesion. With regard to pEC50 values and maximal responses of BK in presence of different concentrations of noscapine, it seems that noscapine caused a competitive inhibition of BK action in HUA. However, noscapine attenuated histamine contractile response as well and did not show specificity against BK-induced constriction in this human tissue. So it can be deduced that noscapine may play a physiologic antagonistic role against BK in HUA.

BK is a vasoactive kinin that is present in fetal circulation and has an important role in local regulation of vascular tone in the placental and umbilical blood vessels [8,24]. Significant potentiation of BK-induced vasoconstriction in HUA from pregnancy-induced hypertension (PIH) has been previously reported [25]. Thus, vascular sensitivity to BK is likely to be enhanced in hypertensive pregnancy. Regarding the safety of noscapine [7] and its very potent inhibitory effects against BK-induced HUA constriction, future investigations could be proposed for possible application of the drug in pathological conditions, such as PIH, in pregnancy.

Conclusion

It is concluded that noscapine can antagonize BK-induced constriction of HUA in a nonspecific manner. Thus, further studies are required to elucidate if noscapine could find a clinical application in pathologic conditions accompanied by higher vascular sensitivity to BK in pregnancy.

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