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Contractile effect of (+)-glaucine in the isolated guinea-pig ileum

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Abstract

The intestinal effects of (+)-glaucine [(*S*)-1,2,9,10-tetramethoxyaporphine] were studied using the guinea-pig ileum. (+)-Glaucine (10–300 μM) induced ileal contractions. The contraction was not affected by tetrodotoxin, atropine, hexamethonium, propranolol, naloxone, methysergide, N^G-nitro-L-arginine methyl ester, SR141716A (a cannabinoid CB_1 receptor antagonist) or SR140333 (a tackykinin NK_1 receptor antagonist) plus SR48968 (a tackykinin NK_2 antagonist). (+)-Glaucine-induced contraction was reduced by indomethacin, nordihydroguaiaretic acid or bisindolylmaleimide I and abolished by verapamil and nifedipine. These results suggest that (+)-glaucine-induced contraction involves activation of voltage-dependent Ca^{2+} channels and protein kinase C and could be mediated by the release of arachidonic acid metabolites.

Keywords: Glaucine; Intestine; Prostaglandin; Leukotriene; Protein kinase C

1. Introduction

(+)-Glaucine [(*S*)-1,2,9,10-tetramethoxyaporphine] is an aporphinoid alkaloid isolated from the above-ground parts of *Glaucium flavum* Crantz (Papaveraceae), a biennal plant distributed throughout coastal Europe. (+)-Glaucine possesses a wide variety of pharmacological effects including antitussive, antinociceptive, anticonvulsant and neuroleptic-like activities (Petkov and Stancheva, 1980; Berthe et al., 1983; Kasè et al., 1983a; Zetler, 1988). (+)-Glaucine also decreases the volume of the respiratory tract, has depressant effect on blood pressure and heart rate (Kasè et al., 1983b) and possesses anti-inflammatory and antipyretic effects without associated gastric damage (Pinto et al., 1998).

Like the structurally analogue papaverine, (+)-glaucine relaxes vascular and non-vascular smooth muscles. The mechanism of this activity is still elusive and is probably different from that of papaverine (Cortes et al., 1990). The relaxant action of glaucine seems to be related to α_1-adrenoceptor blockade in the rat aorta (Ivorra et al., 1992), to inhibition of Ca^{2+} entry through potential-operated Ca^{2+} channels in the rat uterus (Anselmi et al., 1992) and to a non-selective α-adrenoceptor blocking properties and Ca^{2+} antagonistic activity in the rat vas deferens (Orallo et al., 1993).

Although it has been shown that (+)-glaucine affect upper gastrointestinal transit in vivo in mice (Kasè et al., 1983a,b), there are no studies concerning the effect of (+)-glaucine on isolated gastrointestinal preparations. We have therefore tested the effect of this alkaloid on the isolated guinea-pig ileum and found that it induced smooth muscle contraction.

2. Materials and methods

2.1. Isolated guinea-pig ileum and drugs administration

Guinea-pig (250–400 g) were killed by axphixyation by CO_2. A segment 15–25 mm long was taken from the

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terminal ileum, at least 30 mm from the caecum. The tissue was suspended in an organ bath containing 20 ml of Krebs solution (composition mM: NaCl 119, KCl 4.75, KH_2PO_4 1.2, $NaHCO_3$ 25, $MgSO_4$ 1.5, $CaCl_2$ 2.5 and glucose) gassed with a mixture of 5% CO_2 in O_2 and maintained at 37°C. The ileum was set up vertically and the mechanical activity of the longitudinal muscle was recorded with an isotonic transducer (load 1 g) connected to a recording apparatus. After 1 h equilibration, (+)-glaucine (10–300 μM) was added non-cumulatively to the bath and left in contact with the tissue for 1 min and then washed out. The interval between additions was 10 min. The contractions were expressed as percent of contraction produced by 10 μM histamine. This concentration of histamine produced a maximal contraction of the guinea pig ileum. In some experiments tetrodotoxin (0.3 μM), atropine (1 μM), hexamethonium (100 μM), indomethacin (10 μM), nifedipine (1μM), verapamil (1 μM), nordihydroguaiaretic acid (1μM), phentolamine (1μM), propranolol (10 μM), naloxone (1 μM), methysergide (1 μM), N^G-nitro-L-arginine methyl ester (L-NAME 300 μM), SR141716A (1 μM), SR140333 (0.1 μM) plus SR48968 (1 μM) and bisindolylmaleimide I (0.2 μM) were added to Krebs solution (contact time: 20 min). These concentrations were selected on the basis of previous work (Izzo et al., 1993, 1997, 1998; Kamata et al., 1993; Yu et al., 1993).

2.2. Drugs

Drugs used were: atropine sulphate, hexamethonium bromide, naloxone hydrochloride, N^G-nitro-L-arginine methyl ester (L-NAME) hydrochloride, nifedipine, nordihydroguaiaretic acid, phentolamine hydrochloride, propranolol hydrochloride, tetrodotoxin and verapamil hydrochloride from Sigma (Milan, Italy); methysergide maleate, mepyramine hydrochloride from RBI (Milan, Italy), bisindolylmaleimide I from Inalco (Milan, Italy). SR 141716A, SR140333 and SR48968 were a gift from SANOFI-Reserche (Montpellier, France). (+)-Glaucine bromidrate was synthesized by methoxylation of boldine (isolated from *Peumus boldus*) with diazomethane, and bromide acid to increase the solubility in water, in Indena Laboratories (Milan, Italy). Indomethacin, nifedipine, bisindolylmaleimide I, nordihydroguaiaretic and SR 141716A were dissolved in dimethyl sulphoxide (DMSO), SR140333 in DMSO/water (50%), v/v). The other drugs were dissolved in distilled water. DMSO (less than 0.01%) did not modify (+)-glaucine-induced contractions.

2.3. Statistics

Results are expressed as means ± S.E.M and analysed by analysis of variance (ANOVA). Probability less than 0.05 was regarded as significant.

3. Results

(+)-Glaucine (10–300 μM) evoked a contraction of the guinea pig ileum. Lower concentrations (1 μM) were inactive ($n = 8$) or produced contractions (3 μM) in 5 cases out of 8 experiments. An effective threshold concentration was 10 μM (17 ± 3% contraction, $n = 92$), while the optimum concentration for (+)-glaucine-induced contraction was 100 μM (49 ± 3% contraction, $n = 92$). The contraction induced by (+)-glaucine was rapid in onset (latency between 3 and 8 sec) and reached a maximum within 1 min; however, when (+)-glaucine was allowed to stay in contact with ileum, the contraction was sustained, with rapid oscillations. When the tissue bath was drained and Krebs was added, there was rapid relaxation of the muscle to base-line resting tension. The contractile response of the guinea-pig ileum to the whole concentration-curve to (+)-glaucine (10–300 μM) was not significantly modified in the presence of tetrodotoxin (0.3 μM), atropine (1 μM), hexamethonium (100 μM), SR140333 (0.1 μM) plus SR 48968 (1 μM), mepyramine (1 μM), methysergide (1 μM), phentolamine (1 μM), propranolol (10 μM), naloxone (1 μM), N^G-nitro-L-arginine methyl ester (L-NAME 300 μM), SR 141716A (1 μM). Table 1 shows the effect of the above mentioned drugs on (+)-glaucine (30 μM)-induced contractions.

(+)-Glaucine was significantly ($P < 0.01$) less effective in contracting the guinea-pig ileum in the presence of indomethacin (10 μM), nordihydroguaiaretic acid (1 μM) or bisindolylmaleimide (0.2 μM), (Fig. 1). A combination of indomethacin and nordihydroguaiaretic acid further on inhibited the contractions induced by (+)-glaucine (Fig. 1). In fact, the curve representing the contractile effect of (+)-glaucine in the presence of indomethacin plus nordihydroguaiaretic acid was significantly ($P < 0.05$) different from the curve representing the contractile effect of (+)-glaucine in the presence of indomethacin. A further significant inhibitory effect was achieved in the presence of

Table 1
Influence of various drugs on (+)-glaucine (30 μM)-induced contractions in the guinea-pig ileum
Results are expressed as % of variation ± S.E.M of the corresponding control response. No statistical differences were observed.

Treatment	Variation (%)
Tetrodotoxin 0.3 μM	−5 ± 3
Atropine 1 μM	+1 ± 4
Hexamethonium 100 μM	+6 ± 4
SR140333 0.1 μM + SR48968 1 μM	−14 ± 6
Mepyramine 1 μM	−1 ± 3
Methysergide 1 μM	−10 ± 5
Phentolamine 1 μM	0 ± 4
Propranolol 10 μM	−4 ± 2
Naloxone 1 μM	+2 ± 3
L-NAME 300 μM	+5 ± 3
SR141716A 1 μM	+4 ± 4

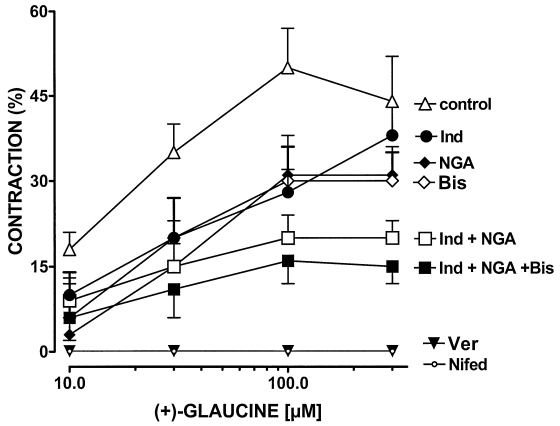

Fig. 1. Contractile effect of (+)-glaucine alone (control), or in the presence of indomethacin 10 μM (Ind), nordihydroguaiaretic acid 1 μM (NGA), bisindolylmaleimide 0.2 μM (Bis), indomethacin 10 μM plus nordyhidroguaiaretic acid 1 μM (Ind+NGA), indomethacin 10 μM plus nordyhydroguaiaretic acid 1 μM plus bisindolylmaleimide I 0.2 μM (Ind+NGA+Bis), nifedipine 1 μM (Nifed) or verapamil 1 μM (Ver). The ordinates show the percentage of contraction compared to histamine 10 μM. Each point represent the mean of 8–10 experiments; vertical lines show S.E.M.

indomethacin plus nordihydroguaiaretic acid plus bisindolylmaleimide ($P < 0.05$ vs. indomethacin plus nordihydroguaiaretic acid, Fig. 1). Nifedipine (1 μM) and verapamil (1 μM) completely abolished the contractions induced by (+)-glaucine (Fig. 1).

4. Discussion

(+)-Glaucine is an aporphinoid derivative structurally related to papaverine. Both compounds relax several smooth muscles including the vas deferens (Orallo et al., 1993), uterus (Anselmi et al., 1992), trachea (Kasè et al., 1983b) and aorta (Ivorra et al., 1992). However, while papaverine is able to relax gastrointestinal smooth muscles, in the present study we have demonstrated that (+)-glaucine caused a contractile response in the isolated guinea-pig ileum. The response was not affected by tetrodotoxin, indicating that (+)-glaucine exerts a direct effect on intestinal smooth muscle and that activation of sodium channels is not essential for (+)-glaucine to induce contraction. In addition the contractile effects of (+)-glaucine were not affected by antagonism of α-adrenoceptors (phentolamine), β-adrenoceptors (propanolol), opiate (naloxone), cannabinoids CB_1 (SR141716A), muscarinic (atropine), nicotinic (hexamethonium), histamine H_1 (mepyramine), 5-hydroxytriptamine (methysergide) or tackykinins NK_1 and NK_2 (SR140333 and SR48968) receptors.

In the present study, we have shown that indomethacin and nordihydroguaiaretic acid, which inhibit cyclooxygenase and lipoxygenase respectively, reduced the contractile effect of (+)-glaucine. This indicates that the contractile effect of (+)-glaucine could be due to the release of prostaglandins and leukotrienes. In addition, the effect of indomethacin and nordihydroguaiaretic acid was additive, thus indicating that probably the release of prostaglandins is not secondary to the release of leukotrienes and vice versa. The involvement of eicosanoids could explain why (+)-glaucine produces relaxation in some tissues and contracts guinea-pig ileal smooth muscles. Indeed some eicosanoids (i.e., prostaglandin E_2) can produce contractions of gastrointestinal smooth muscle and relaxation of airway and vascular smooth muscle (Schrör and Schröder, 1994).

Smooth muscle can be activated by membrane depolarization and action potentials. Both types of activation involve an increase in cytosolic free Ca^{2+}, brought about either by release of Ca^{2+} from intracellular stores or by influx of Ca^{2+} through voltage-dependent Ca^{2+} channels (Makhlouf, 1987). In the present study, we have demonstrated that the Ca^{2+} channel antagonists nifedipine and verapamil completely inhibited the contractile response induced by (+)-glaucine, indicating that the stimulatory effect (+)-glaucine involves voltage-dependent Ca^{2+} channels. Ca^{2+} entry into the cells could be secondary to receptor activation by eicosanoids as Ca^{2+} influx from the extracellular space is one of the mechanisms to explain the contractile effect of some eicosanoids. In contrast, Ca^{2+}-antagonistic properties for (+)-glaucine have been described both in the central nervous system (Ivorra et al., 1992) and in some peripheral tissues (Anselmi et al., 1992; Orallo et al., 1993).

Another aspect investigated in the present study was the possible role of protein kinase C in the contractile response elicited by (+)-glaucine. We have found that the protein kinase C inhibitor bisindolylmaleimide I reduced the contractile effect of glaucine in the guinea-pig ileum. These findings suggest that protein kinase-C dependent mechanisms play an important role in the action of (+)-glaucine. When tested after indomethacin and nordihydroguaiaretic acid, bisindolylmaleimide further on reduced (+)-glaucine-induced contractions, thus indicating that activation of protein kinase C is, at least in part, independent from the action of arachidonic acid metabolites.

In summary, (+)-glaucine has been characterised as a spasmogen agent on the isolated guinea-pig ileum. This effect involves activation of voltage-dependent calcium channels, protein kinase C and could be mediated, at least in part, by arachidonic acid metabolites. This broadens the spectrum of actions of (+)-glaucine and opens the possibility to investigate the effect of this alkaloid in the treatment of gut hypomotility disorders.

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