Short communication

Cholera toxin but not pertussis toxin inhibits angiotensin II-enhanced contractions in the rat portal vein

Jisi Zhang a, Jacques C.A. Van Meel b, Martin Pfaffendorf a and Pieter A. Van Zwieten a

a Department of Pharmacotherapy, Academic Medical Centre, University of Amsterdam, Meibergdreef 15, NL-1105 AZ Amsterdam, Netherlands
and b Division of Cardiovascular Pharmacology, Dr. Karl Thomae GmbH, Birkendorferstrasse 65, 7950 Biberach / RSS, Germany

Received 30 October 1992, accepted 10 November 1992

Angiotensin II (Ang II)-enhanced phasic contractions in the rat portal vein were concentration dependently inhibited by cholera toxin (0.1-10 µg/ml) and dibutyryl cyclic AMP (0.1-1 mM), but not by pertussis toxin (1 µg/ml), which suggests that G i is not involved in the Ang II signal transduction pathway. It also seems likely that the effect of cholera toxin is due to its ability to increase cyclic AMP production through G s.

Angiotensin II; Cholera toxin; Pertussis toxin; Portal vein (rat); Dibutyryl cAMP

1. Introduction

In a variety of target tissues angiotensin II (Ang II) receptors are coupled to GTP binding and regulatory guanine nucleotide proteins (G proteins). The effects of Ang II are sensitive to pertussis toxin, an inactivator of inhibitory G protein (G i) (Pfeilschifter and Bauer, 1986; Gaul et al., 1988; Anand-Srivastava, 1989), and to cholera toxin, an activator of stimulatory G protein (G s) (Guillon et al., 1988; Socorro et al., 1990). Recently, Ohya et al. (1991) suggested that the Ang II-induced stimulation of Ca 2+ channels in smooth muscle of guinea-pig portal vein may be mediated by a G protein that is insensitive to both cholera toxin and pertussis toxin.

Isolated rat portal vein preparations display spontaneous myogenic activity which is sensitive to physiologically relevant concentrations of Ang II (Bohr and Uchida, 1967). However, little is known about the receptor-G protein-coupling events subsequent to Ang II receptor activation. In the present study, we investigated the effects of cholera toxin and pertussis toxin on Ang II-enhanced phasic contractions in the rat portal vein, to study the possible role of G i and G s, respectively. Since cholera toxin is able to ADP-ribosylate the α-subunit of G s, thus causing the stimulation of adenylate cyclase and a rise in cyclic AMP (cAMP) formation (Gilman, 1984), we also examined the effect of dibutyryl cyclic AMP (db-cAMP), a lipid-soluble analog of cAMP (Levine, 1969), on Ang II-induced contractions.

2. Materials and methods

Male Wistar rats weighing 300–400 g were killed by stunning and exsanguination. Isolated longitudinal strips (about 8 mm in length) of portal vein were obtained and were mounted vertically in 6-ml organ baths containing a modified Tyrode solution of the following composition (mM); NaCl 124, KCl 4.0, CaCl 2 0.9, MgCl 2 1.1, NaH 2 PO 4 0.42, NaHCO 3 24.9, glucose 5.5. The Tyrode solution was kept at 37°C by a thermostat and at pH 7.4 by gassing with carbogen (95% O 2 and 5% CO 2 ). The passive tension applied to the portal vein was 10 mN. Isometric contractions of the preparations were measured with force transducers (Kyowa, Tokyo, Japan) connected to strain amplifiers (Kyowa, Tokyo, Japan) and recorded on a thermal pen recorder (WKK, Kaltbrunn, FRG). The force and frequency of the spontaneous phasic contractions in each preparation were measured during the last 2.5 min of the 1-h equilibrating period before the addition of Ang II and the toxins. Cumulative concentration-response curves for the effect of Ang II on spontaneous phasic contractions were obtained by increasing the concentrations of Ang II from 10−10 to 3 × 10−8 M at 5-min intervals. Changes in the force and frequency of phasic myogenic contractions caused by Ang II and other

Correspondence to: J.S. Zhang, Department of Pharmacotherapy, Academic Medical Centre, University of Amsterdam, Meibergdreef 15, NL-1105 AZ Amsterdam, Netherlands. Tel. 31-20-566 4977, fax 31-20-691 2401.
drugs were measured as the maximal difference of the average peak force or the number of spontaneous contractions per minute before and after the addition of the drugs, respectively. Only one concentration-response curve for Ang II was made with each preparation. Cholera toxin (0.1, 0.5, 2 and 10 
μg/ml) and pertussis toxin (1 μg/ml) were incubated with rat portal vein preparations for 4 h, respectively, before the Ang II concentration-response curves were made. To study the effect of db-cAMP on the Ang II-induced contraction, rat portal vein preparations were incubated with db-cAMP (0.1, 0.5 and 1 mM) for 1 h in the dark and then concentration-response curves for Ang II were made. Data are presented as mean values ± S.E.M. Student’s t-test for paired or unpaired observations was used for statistical evaluation. P values smaller than 0.05 were considered to be significant. Ang II, cholera toxin and db-cAMP were purchased from Sigma Chemical Company (St. Louis, MO, USA). Pertussis toxin was a gift from Dr. D.J. De Wildt (Bilthoven, The Netherlands). All compound were dissolved in saline.

3. Results

Under the experimental conditions used, the force of the spontaneous phasic contractions of the rat portal vein amounted to 2.7 ± 0.2 mN (n = 36), at a frequency of 5.7 ± 0.3 contractions/min (n = 36). Ang II concentration dependently increased the force of the spontaneous phasic contractions (fig. 1).

Incubation of rat portal vein with cholera toxin (0.1 and 0.5 μg/ml) decreased the force of the spontaneous phasic contractions to 56 ± 10% (P < 0.05) and 44 ± 6% (P < 0.05) but increased their frequency to 183 ± 21% (P < 0.05) and 200 ± 12% (P < 0.05) of the control values, respectively. Higher concentrations of cholera toxin (2 and 10 μg/ml) completely inhibited the spontaneous activity of the rat portal vein. As shown in fig. 1, cholera toxin counteracted the Ang II-induced increase in contractile force in a concentration-dependent manner. A low concentration of 0.1 μg/ml cholera toxin caused a rightward shift of the concentration-response curve for Ang II, without depressing the maximal response. Higher concentrations of cholera toxin impaired the maximal response, and a very high concentration (10 μg/ml) almost fully suppressed the effect of Ang II on contractile force. However, under our experimental conditions, pretreatment of the rat portal vein with 1 μg/ml pertussis toxin for 4 h did not affect either the spontaneous activity or the phasic contractile response to Ang II (fig. 2). db-cAMP 0.1 mM slightly but not significantly decreased the force of the spontaneous contractions (to 82 ± 9% of the control value, P > 0.05) without altering the frequency of the contractions, whereas 0.5 and 1 mM abolished the spontaneous activity of the rat portal vein preparations. The Ang II-induced enhancement of the force of contraction was concentration-dependently inhibited by db-cAMP; in the presence of db-cAMP 0.1, 0.5 and 1 mM the maximal responses induced by Ang II were 14 ± 0.8, 12.1 ± 1 (P > 0.05), 4.7 ± 0.5 (P < 0.05) and 1.3 ± 0.6 (P < 0.05) mN of control, respectively.

4. Discussion

Molecular cloning of AT1 receptors in both rat vascular smooth muscle cells and bovine adrenal zona glomerulosa cells has revealed that these AT1 receptors possess a seven transmembrane domain structure similar to that of G-protein-coupled receptors (Catt and Abbott, 1991). The stimulation by Ang II of spontaneous phasic contractions of the rat portal vein preparation is known to be mediated by AT1 receptors.
(Zhang et al., 1992). Little is known, however, about the process of signal transduction following AT₁ receptor activation in this preparation.

An inhibitory effect of cholera toxin on Ang II-stimulated inositol phosphate production has been observed both in cultured vascular smooth muscle cells and in rat glomerulosa cells (Guillon et al., 1988; Socorro et al., 1990). However, a pertussis toxin-sensitive G protein (Gₛ) has been reported also to be involved in Ang II receptor-mediated signal transduction in various tissues (Pfeilschifter and Bauer, 1986; Gaul et al., 1988; Anand-Srivastava, 1989). In the present study, we found a striking difference between the effects of cholera toxin and pertussis toxin on the Ang II-induced stimulation of phasic contractions. Cholera toxin was a potent inhibitor of the Ang II effect, whereas pertussis toxin was ineffective. The concentration of pertussis toxin used (1 μg/ml), with the same incubation time, has been shown to be effective with respect to the ribosylation of various substrates (Trachte, 1990; Muller et al., 1990).

Our data show that db-cAMP inhibited the effect of Ang II in the rat portal vein in a concentration-dependent manner. As cholera toxin interacts directly with Gₛ, thereby increasing cellular cyclic AMP (cAMP), it is suggested that the inhibition of the effects of Ang II by cholera toxin may result from its ability to increase cAMP production rather than from a blockade of an Ang II signal transduction pathway. However, the mechanism of action of cholera toxin is complex. Guillon et al. (1988) proposed that cholera toxin inhibits Ang II-induced inositol phosphate accumulation in rat glomerulosa cells by inhibiting hormone-receptor binding via an increase in intracellular cyclic AMP. Socorro et al. (1990) suggested that the signalling pathway triggered by Ang II is sensitive to cholera toxin, both at the level of the receptor and at the level of G protein-phospholipase C coupling. Furthermore, it is reported that cholera toxin may also ADP-ribosylate other proteins (Milligan, 1988). Since our present data do not rule out the possibility that cholera toxin also exerts its effect on Ang II receptors and other proteins, the precise mechanism by which cholera toxin inhibits Ang II-enhanced phasic contractions remains to be investigated further. Our experiments with pertussis toxin indicate that Gₛ probably does not play a role in the signal transduction triggered by stimulation of AT₁ receptors in the rat portal vein.

References


