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Short communication

Excitatory action of prostanoids on the ferret isolated vagus nerve preparation

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Abstract

We have investigated the actions of various prostanoid receptor agonists on an isolated preparation of the ferret cervical vagus using a grease-gap extracellular recording technique. The potency ranking for depolarization was BW245C (5-(6-carboxyhexyl)-1-(3-cyclohexyl-3-hydroxypropyl) hydantoin; DP-selective, EC_{50}=0.14 μM)>prostaglandin E_2 (nonselective EP agonist)>U-46619 (11α, 9α-epoxymethano-15*S*-hydroxyprosta-5*Z*,13*E*-dienoic acid; TP agonist)>prostaglandin $F_2\alpha$ (FP receptor agonist). Sulprostone (EP_1/EP_3-selective), fluprostenol (FP-selective) and cicaprost and iloprost (both IP-selective) had minimal effects. It is likely that DP, EP_2/EP_4 and TP receptors are present on the vagal fibres of the ferret.

Keywords: Vagus; Prostanoid; Prostaglandin; DP receptor; 5-HT_3 receptor; Depolarization; Neurone; Nervous; (Ferret)

1. Introduction

Prostaglandins are mediators of inflammatory pain, and the analgesic effects of nonsteroidal anti-inflammatory drugs (NSAIDs) result from suppression of prostaglandin biosynthesis through inhibition of fatty acid cyclo-oxygenase. There is evidence for the involvement of both prostaglandin E_2 and prostacyclin acting via prostanoid EP_1 and IP receptors, respectively (Coleman et al., 1994; Kiriyama et al., 1997; Smith et al., 1998; Stock et al., 2001). Dorsal root ganglia and c-afferents of the vagus are some of the sensory structures activated by endogenous prostanoids (Bley et al., 1998; Smith et al., 1998).

While the isolated dorsal root ganglion preparation is widely used to study pain mechanisms, the ability of endogenous prostanoids to depolarize the vagus may indicate that these agents have other pathophysiological roles. This applies to the cervical region of the vagus, which represents an interface between the central nervous system, heart, lungs, liver and gastrointestinal tract (Paintal, 1973), and a prostanoid-induced change in membrane potential could be relevant to a modulation of any/all of the systems.

In this context, we have recently shown that DP, EP_1/EP_3 and TP (thromboxane) receptor agonists induce emesis in the ferret and *Suncus murinus* (Kan et al., 2002, 2003). Because the vagus nerve is integral to the emetic reflex (Andrews, 1992), we proceeded to investigate whether an isolated preparation of the ferret cervical vagus nerve can be depolarized by prostanoid agonists. BW245C (5-(6-carboxyhexyl)-1-(3-cyclohexyl-3-hydroxypropyl) hydantoin), fluprostenol and cicaprost are selective agonists for DP, FP and IP receptors, respectively, while sulprostone is selective for EP_1 and EP_3 receptors, and iloprost is selective for EP_1 and IP receptors; U-46619 (1α, 9α-epoxymethano-15*S*-hydroxyprosta-5*Z*,13*E*-dienoic acid) is selective for TP receptors (Town et al., 1983; Coleman et al., 1994; Dong et al., 1986). Comparisons were made with the fast excitatory actions of 5-hydroxytryptamine (5-HT), and the 5-HT_3 receptor-selective agonist *m*-chlorophenylbiguanide was also tested (Kilpatrick et al., 1990).

2. Materials and methods

The method is essentially that of Ireland and Tyers (1987) and is briefly described. Male ferrets (1.4–2.4 kg) were killed by pentobarbital sodium 80 mg/kg, i.p. The cervical trunks of both vagus nerves were excised (2–2.5

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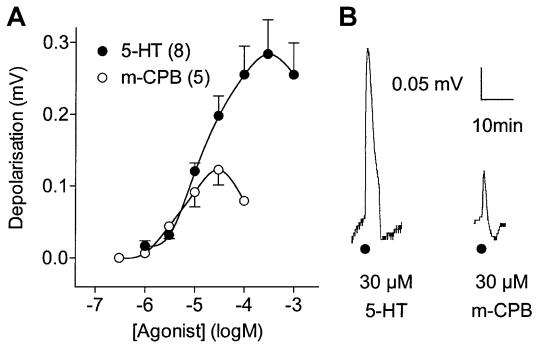

Fig. 1. Ferret isolated vagus nerve: (A) log concentration–response curves for 5-HT and *m*-chlorophenylbiguanide (m-CPB) and (B) representative experimental recordings. Results represent the mean±S.E.M.; number of preparations used is shown in parentheses.

cm length) and transferred to ice-cold Krebs–Henseleit solution (NaCl 118 mM, KCl 4.75 mM, $CaCl_2$ 2.5 mM, $MgSO_4$ 1.2 mM, KH_2PO_4 1.2 mM, $NaHCO_3$ 25 mM and glucose 11 mM) containing 3 μM indomethacin. The nerve was desheathed under a dissecting microscope and then carefully mounted on filter paper strips in a Perspex "grease gap" chamber, contained in a water jacket at 27 °C. Silver/silver chloride recording electrodes with a cotton–wool wicks were placed in contact with the surface of the nerve in each half of the chamber, and the potential difference between them was recorded with a MacLab® GP amplifier/4s interface/PowerMac® 7200/120 computer (sampling rate 40 Hz). Each half of the nerve was superfused at 1.5–2.0 ml/min with Krebs–Henseleit solution containing 3 μM indomethacin, aerated with 95% O_2/5% CO_2 and maintained at 27 °C. Drugs were applied in the superfusate to one-half of the nerve. Preparations depolarized to less than 1.2 mV by 20 mM KCl were abandoned. 5-HT-receptor agonists were added noncumulatively (3 min contact/15 min interval), and prostanoid receptor agonists were added cumulatively (5–20 min contact). EC_{50} values for 5-HT, *m*-chlorophenylbiguanide and BW245C were determined by GraphPad Prism® software (version 3.0c, GraphPad Software, San Diego, U.S.A.). Concentrations of prostanoids eliciting 0.15 mV depolarization ($EC_{0.15}$ mV) were used to estimate relative potencies. Dose–response relationships (using raw data) were also analysed using repeated-measures one-way analysis of variance (ANOVA) coupled with preplanned contrasts, as necessary (SuperANOVA® version 1.11, Abacus Concepts, California, U.S.A.). Differences were considered significant when $P<0.05$.

5-Hydroxytryptamine hydrochloride (5-HT), *m*-chlorophenylbiguanide hydrochloride and indomethacin were from Sigma–Aldrich, Saint Louis, U.S.A. BW245C [5-(6-carboxyhexyl)-1-(3-cyclohexyl-3-hydroxypropyl) hydantoin], prostaglandin E_2, prostaglandin $F_{2\alpha}$ tromethamine salt, fluprostenol and U-466191(1α, 9α-epoxymethano-15*S*-hydroxyprosta-5*Z*,13*E*-dienoic acid) were from Cayman Chemical, Michigan, U.S.A. Sulprostone, cicaprost and iloprost were gifts from Schering Aktiengesellschaft, Berlin, Germany.

3. Results

5-HT and *m*-chlorophenylbiguanide produced depolarizations that were rapid in onset and offset (Fig. 1B). The maximum depolarization induced by 5-HT was 0.29±0.03 mV at 300 μM and responses were lower on increasing the concentration to 1 mM, resulting in a bell-shaped concentration–response curve (Fig. 1A). *m*-Chlorophenylbiguanide had similar potency as 5-HT for depolarizations of 0–0.1 mV but had a lower maximum than 5-HT maximum (~44%) and also appeared to have a bell-shaped concentration–response curve (Fig. 1A). The EC_{50} values for 5-HT and *m*-chlorophenylbiguanide were 14.8±0.3 and 5.4±1.6 μM, respectively. Four prostanoids, BW245C, U-46619, prostaglandin E_2 and prostaglandin F_{2a} produced clear and consistent depolarizations of the vagus preparation (ANOVA main effect, $P<0.002$ for all). Responses to single concentrations of BW245C, U-46619 and prostaglandin $F_{2\alpha}$ reached a steady level within 5 to 8 min, whereas at least 15 min was required for prostaglandin E_2. The depolarizations persisted on continuous washing, often for more than 1 h,

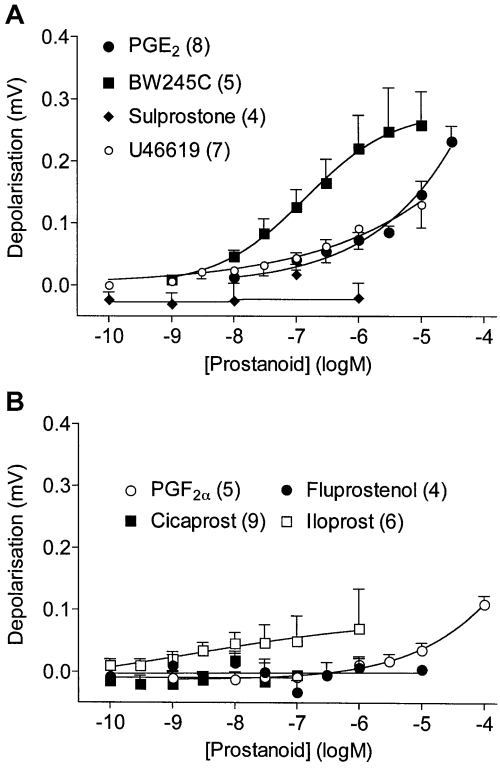

Fig. 2. Log concentration–response curves for prostanoids on ferret isolated vagus nerve: (A) prostaglandin E_2 (PGE_2), BW245C, sulprostone and U46619 and (B) prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), fluprostenol, cicaprost and iloprost. Results represent the mean±S.E.M.; number of preparations used is shown in parentheses.

and therefore, a cumulative dosing schedule was employed. BW245C was the most potent agent and had a sigmoid concentration–response curve (EC_{50} and $EC_{0.15\ mV}$=0.14 μM and 0.18 μM, respectively), with a maximum response of 0.28±0.06 mV (Fig. 2). U-46619 and prostaglandin E_2 showed concave-upwards curves between 0.01 and 10/30 μM. $EC_{0.15\ mV}$ values were ≥3 and 11.2 μM, respectively, making these agonists about 17 and 62 times less potent than BW245C. Prostaglandin $F_{2\alpha}$ (1–100 μM), sulprostone (0.1–1000 nM), fluprostenol (0.1–3000 nM) and cicaprost (0.1–100 nM) did not depolarize or hyperpolarize the vagus (Fig. 2B; ANOVA main effect, P>0.05). Several of the preparations on which iloprost (0.1–300 nM) was tested showed a steady upward drift, and in two of these, distinct depolarization was seen at 300 nM. However, there was no statistically significant change from baseline recordings (P>0.05).

4. Discussion

In several species, depolarization of the vagus by 5-HT is predominantly mediated via 5-HT_3 receptors, with a small component due to 5-HT_4 receptors (Coleman and Rhodes, 1995; Bley et al., 1994). There are, however, differences in agonist profiles on the 5-HT_3 receptor systems in rat and ferret vagus preparations. 5-HT is a more potent full agonist on the vagus of the rat (EC_{50}=0.63 μM) compared to the ferret (13 μM), while the selective 5-HT_3 receptor agonist 2-methyl-5-HT is a low-potency partial agonist in the rat (EC_{50}=6.3 μM) but a full agonist on the ferret (EC_{50}=6.6 μM) (Newberry et al., 1992). In our study on the ferret vagus, the EC_{50} value (14.8±0.3 μM) of 5-HT was similar to that of Newberry et al. (1992). *m*-Chlorophenylbiguanide, another 5-HT_3-selective ligand, behaved as a partial agonist on vagi from both rat (Kilpatrick et al., 1990) and ferret (our study) but was approximately 130 times more potent on the former. There is also an apparent species difference between the ferret and rat with respect to the nature of antagonism and potencies of 5-HT_3 receptor blockers, and it has been suggested that the 5-HT_3 receptors on the ferret vagus are unique to that species (Newberry et al., 1992). Unfortunately, there is no binding data available to indicate the receptor affinity of prostanoids on ferret tissues or in ferret recombinant systems. However, there are functional in vitro studies on ferret airways demonstrating the responsiveness of the trachea to prostaglandin E_1, E_2, D_2 and $F_{2\alpha}$ (Chand and Eyre, 1978; Deffebach et al., 1990; Ullman et al., 1990) and U-46619 (McKenniff et al., 1991), suggesting the existence of DP, EP, FP and TP receptors in this species. The situation regarding IP receptors is less defined because there are no direct in vitro studies, although the prostacyclin metabolite 6-keto-prostaglandin $F_{1\alpha}$ is produced from the ferret isolated trachea (Ullman et al., 1990), and both cicaprost and iloprost are capable of modulating the emetic reflex and have other behavioural actions in this species (see Kan et al., 2002).

BW245C is considered to have high specificity for DP receptors in other species (recombinant receptors from man and mouse: Abramovitz et al., 2000; Kiriyama et al., 1997) and was the most potent prostanoid to depolarize the ferret vagus (EC_{50}=140 nM); similar potency is seen for relaxation of rabbit jugular (EC_{50}=70 nM) and saphenous veins (EC_{50}=38 nM) and human pulmonary vein (EC_{50}=50 nM), and for inhibiting histamine release from mast cells (Chan et al., 2000). DP receptors, which couple well to Gs, have been cloned and characterized from man (Boie et al., 1995), rat (Wright et al., 1999) and mouse (Hirata et al., 1994); the rat and mouse receptors show 90% amino acid identity overall rising to 93% in the transmembrane domains, while the corresponding values for human and rat are 73% and 88%. Taken together, we do not expect major differences in the pharmacology of DP receptors in the ferret compared to other species, and we therefore tentatively attribute the action of BW245C to an activation of DP receptors.

Prostaglandin $F_{2\alpha}$ has been reported to be active to modulate methacholine-induced changes in albumin transport and lysozyme secretion in the ferret trachea at concentrations as low as 0.01 μM (Deffebach et al., 1990); it also contracts the trachea at threshold concentrations of 0.1–1.0 μM (Chand and Eyre, 1978). However, in the present study, prostaglandin $F_{2\alpha}$ was virtually inactive, with weak depolarizations being only observed at concentrations 10 and 30 μM. It is likely that the action of prostaglandin $F_{2\alpha}$ at high concentrations is mediated via DP rather than FP receptors because fluprostenol, a highly selective FP receptor agonist in both functional assays (Welburn and Jones, 1978; Dong and Jones, 1982; Dong et al., 1986) and radioligand binding assays (Kiriyama et al., 1997; Abramovitz et al., 2000), was inactive and prostaglandin $F_{2\alpha}$ has low potency DP actions in other systems. For example, in human and rat recombinant DP receptor/adenylate cyclase assays, prostaglandin $F_{2\alpha}$ is about 1000 and 1600 times less potent, respectively, than BW245C (Coleman et al., 1990; Sharif et al., 2000).

The log concentration–response curve for prostaglandin E_2 on the ferret vagus appears to have two components. The more sensitive component (0.1–0.3 μM) may be due to activation of an EP receptor, and is consistent with its threshold concentration range (0.1–1 μM) to relax the ferret trachea (Chand and Eyre, 1978). However, the EP receptor on the ferret vagus is unlikely to be either an EP_1 or EP_3 subtype in view of the inactivity of sulprostone, which has activity in the ferret to affect behaviour (Kan et al., 2002). At higher concentrations of prostaglandin E_2 (3–30 μM), we suspect that additional activation of the DP receptor may occur because, in the previously mentioned human and rat DP receptor/adenylate cyclase assays, prostaglandin E_2 was about 500 and 800 times less potent, respectively, than BW245C (Wright et al., 1999). We believe that the inactivity

of cicaprost and iloprost on the ferret vagus is due to the absence of IP receptors rather than these prostacyclin analogues being unacceptable ligands for the ferret IP receptor, based on our previous studies in the ferret where the compounds modified behaviour (Kan et al., 2002). Indeed, cicaprost is considered to have a selective IP agonist profile in functional assays across several species. For example, it is a highly potent inhibitor of human, horse, pig, rabbit and rat platelet activation and a highly potent relaxant of circular smooth muscle in human, dog and rabbit mesenteric arteries (Armstrong et al., 1989) and pig carotid artery and guinea pig aorta (Jones and Chan, 2001). Furthermore, ligand binding studies on recombinant IP receptors show Ki values between 10 and 20 nM for human (Abramovitz et al., 2000), rat (Sasaki et al., 1994) and mouse (Kiriyama et al., 1997) IP receptors. Nevertheless, it is possible that the ferret vagus has a novel IP receptor but prostacyclin excited neuronal IP receptors in the rat vagus preparation (EC_{50}=3.8 nM; Smith et al., 1998) and our previous studies on the same preparation have shown the potency ranking of cicaprost, iloprost and another prostacyclin analogue, carbacyclin (EC_{50}=0.23, 7.9 and 81 nM, respectively) to be similar to rankings found for IP receptor systems in platelets and blood vessels from several species (Rudd et al., 2000).

There is some evidence that thromboxane A_2 and U-46619 can stimulate pulmonary vagal afferents in the rabbit and other species (see Wacker et al., 2002). U-46619 is a fairly selective TP receptor agonist, and it is likely that its depolarizing action on the ferret vagus over the 0.1–10 μM concentration range is due to activation of TP receptors; this is similar to its potency to contract the ferret isolated trachea (EC_{50}=0.8 μM; McKenniff et al., 1989). Smith et al. (1998) reported that carbocyclic thromboxane A_2 depolarized the rat vagus with an IC_{50} of about 0.1 μM. However, the log concentration–response curve was quite shallow and the maximum depolarization was about 0.08 mV at most. Carbocyclic thromboxane A_2 may be a poor choice for a typical TP receptor agonist because it behaves as a full agonist on some TP systems and a partial agonist on others (Armstrong et al., 1983).

In conclusion, our studies have provided supporting evidence for the ferret vagal 5-HT_3 receptors having a unique pharmacological profile. Furthermore, the action of BW245C, prostaglandin E_2, and U-46619 to depolarize the vagus suggests the presence of prostanoid DP, EP and TP receptors, respectively. The clinical significance of these findings is unknown and further investigations are required to confidently identify the receptors.

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