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Effect of BW755C on prostaglandin synthesis in the rat stomach

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Recently Whittle *et al.* [1, 2] have shown that the dual inhibitor of cyclooxygenase and lipoxygenase, BW755C [3-amino-1-(3-trifluoromethylphenyl)-2-pyrazoline], inhibits prostaglandin (PG) production in inflammatory exudates of rats, but, contrary to other non-steroidal anti-inflammatory drugs (NSAID) like indomethacin, does not influence PGI_2 formation by rat gastric mucosa *ex vivo* and is not ulcerogenic. The authors concluded that some NSAID can selectively inhibit PG biosynthesis in different tissues *in vivo* and, furthermore, that there is a relationship between the production of gastric erosions and inhibition of gastric mucosal PGI_2 formation by NSAID. We have further investigated the tissue selectivity of BW755C by comparing its effects on PGI_2 synthesis in three different regions of rat stomach, forestomach, corpus mucosa and antrum mucosa, both *in vitro* and *ex vivo*.

Materials and methods

In the *in vitro* experiments whole cell preparations of gastric tissue were prepared as described by Knapp *et al.* [3] (90 mg wet wt/incubate for forestomach and corpus mucosa, 30 mg/incubate for antrum mucosa). Mucosal tissue of corpus and antrum was separated from the underlying smooth muscle layer, while the forestomach preparation consisted of the whole stomach wall. The tissues were washed in ice-cold Krebs–Henseleit bicarbonate buffer [3] and then incubated in the absence or presence of various concentrations (10^{-5}–10^{-3} mol/l) of BW755C at 37° for 10 min. BW755C had been obtained from Wellcome Research Labs. (Beckenham, U.K.) and was freshly dissolved before use. In addition, incubations were performed with indomethacin (10^{-7}–10^{-3} mol/l; Merck, Sharp & Dohme, Rahway, NJ) as a standard PG synthesis inhibitor [4]. Under the experimental conditions used PG release by gastric tissue was linear for at least 10 min. At the end of the incubation period the medium was removed and aliquots were analysed for their content of 6-keto-$PGF_{1\alpha}$, the stable hydration product of PGI_2, using a highly sensitive and specific radioimmunoassay [5].

In the *in vivo* experiments BW755C (100 mg/kg) or indomethacin (2.5 mg/kg), suspended in 0.25% (w/v) carboxymethylcellulose, were administered orally to rats (1.0 ml/kg). Controls received the solvent only. Groups of rats were killed 30 min or 180 min after administration of BW755C and 180 min after administration of indomethacin. Tissue of the forestomach, corpus mucosa and antrum mucosa was rapidly isolated, washed in ice-cold Krebs–Henseleit bicarbonate buffer and incubated as whole cell preparations at 37° for 10 min [3]. The amount of 6-keto-$PGF_{1\alpha}$ released into the incubates was determined as

Table 1. Inhibition of synthesis of 6-keto-$PGF_{1\alpha}$ by tissue from three different regions of rat stomach by indomethacin and BW755C *in vitro**

Gastric region	Indomethacin IC_{50} (x 10^{-5} mol/l)	BW755C IC_{50} (x 10^{-5} mol/l)
Forestomach	3.2 ± 1.2	2.0 ± 0.6
(*n*)	(8)	(4)
Corpus mucosa	3.7 ± 1.5	20.8 ± 4.7
(*n*)	(8)	(4)
Antrum mucosa	4.7 ± 1.3	18.0 ± 4.7
(*n*)	(8)	(6)

* Results are the mean ± S.E.M. derived from *n* dose–response curves.

Table 2. Effect of oral administration of BW755C (100 mg/kg) and indomethacin (2.5 mg/kg) on synthesis of 6-keto-$PGF_{1\alpha}$ *ex vivo* by forestomach, gastric corpus mucosa and antrum mucosa

	Time after drug administration				
	30 min		180 min		
Gastric region	Control	BW755C	Control	BW755C	Indomethacin
Forestomach	1949 ± 152	179 ± 33†	2599 ± 261	501 ± 76†	572 ± 23†
(*n*)	(4)	(4)	(4)	(4)	(6)
Corpus mucosa	774 ± 104	680 ± 174	723 ± 152	938 ± 134	246 ± 15§
(*n*)	(6)	(6)	(6)	(6)	(6)
Antrum mucosa	367 ± 72	90 ± 15‡	388 ± 29	151 ± 32†	128 ± 14†
(*n*)	(6)	(6)	(6)	(6)	(6)

* Controls received the solvent only. Results are the mean ± S.E.M. and are given in pg/mg wet weight/10 min incubation at 37°.
† $P < 0.001$.
‡ $P < 0.005$.
§ $P < 0.02$.

described for the *in vitro* experiments. Results from *in vitro* and *in vivo* experiments were confirmed using inhibition of ADP-induced aggregation of human platelets as a bioassay system for PGI_2 determination [6].

Results and discussion

The effects of BW755C and indomethacin on PG synthesis by rat gastric tissue *in vitro* are shown in Table 1. Both compounds inhibit dose-dependently PG formation by forestomach as well as gastric corpus mucosa and antrum mucosa incubated *in vitro* as whole cell preparations. The results show that *in vitro* BW755C, like indomethacin, is an effective inhibitor of PG synthesis by rat gastric tissue, although in corpus and antrum indomethacin is the more potent compound (Table 1). While the IC_{50}-values for indomethacin are very similar for all three gastric regions, BW755C is a more potent inhibitor of PG synthesis in the forestomach than in the mucosal preparations of gastric corpus and antrum. In the latter two tissues BW755C inhibits PG synthesis with almost equal potency. Thus, the *in vitro* data shown in Table 1 indicate some tissue selectivity even within the stomach for the inhibition of PG synthesis by BW755C.

The results of the *in vivo* experiments with BW755C are shown in Table 2. In agreement with the data of Whittle *et al.* [2] no inhibition of PGI_2 formation (determined as 6-keto-$PGF_{1\alpha}$) was observed in gastric corpus mucosa at 30 and 180 min after drug administration. The lack of effect at 30 min shows that there is not even a short lasting inhibition of PGI_2 synthesis in corpus mucosa by BW755C *in vivo*. On the other hand, long-lasting inhibition of PG synthesis was found in antrum mucosa and in the forestomach (Table 2). These results are in contrast to the inhibitory effect of BW755C on PG synthesis observed in all three gastric regions *in vitro*. From the *in vitro* data (Table 1) it can be concluded that gastric corpus mucosal cyclooxygenase is not insensitive to inhibition by BW755C. Thus, *in vivo* pharmacokinetic properties of the compound may contribute to its lack of inhibitory effect on PG synthesis by gastric corpus mucosa.

Contrary to BW755C, indomethacin (2.5 mg/kg) inhibited *in vivo* synthesis of 6-keto-$PGF_{1\alpha}$, determined 3 hr after drug administration, in all three gastric regions (Table 2). Since indomethacin, but not BW755C, is ulcerogenic [2], our data support for gastric corpus mucosa the conclusion of Whittle *et al.* [2] that there is a correlation between inhibition of gastric mucosal PG formation and production of gastric erosions. However, such a correlation does not obviously exist for antrum mucosa and forestomach. In these gastric regions both indomethacin and BW755C strongly reduce synthesis of 6-keto-$PGF_{1\alpha}$ *in vivo*. However, administration of NSAID is usually not accompanied by erosion formation in antrum and forestomach. Thus, in these regions inhibition of PG formation alone does not induce mucosal lesions, and other protective factors besides mucosal PGI_2 formation may, therefore, be responsible for the increased resistance to erosion formation in these parts of the stomach [7].

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