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Zacopride, a potent 5-HT_3 antagonist

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Abstract—The substituted benzamide derivative zacopride was found to antagonize competitively the effects of 5-hydroxytryptamine (5-HT) on the guinea-pig ileum, the rabbit vagus nerve and the von Bezold Jarisch reflex in the rat. The potency of zacopride was comparable with that of ICS 205–930 and it is concluded that zacopride possesses 5-HT_3 receptor antagonizing properties.

Zacopride, 4-amino-*N*-(1-azabicyclo[2.2.2]oct-3-yl)-5-chloro-2-methoxybenzamide(E)-2-butenedioate, is a potent gastrokinetic agent and a potent inhibitor of cancer chemotherapy-induced emesis in animals (Alphin et al 1986). The compound is covered under U.S. Patent Number 4,657,911 assigned to Delalande, S.A. Further studies indicate that zacopride, like ICS 205–930 ((3α-tropanyl)-1*H*-indole-3-carboxylic acid ester) MDL 72222 (1αH,3α,5αH-tropan-3-yl-3,5-dichlorobenzoate), and metoclopramide, inhibits the responses to 5-hydroxytryptamine (5-HT) under certain experimental conditions suggesting that zacopride possesses 5-HT_3 antagonizing properties.

Materials and methods

The studies were conducted in rats and on guinea-pig ilea and rabbit vagi by using the methods as described previously by Richardson et al (1985) and Costall et al (1987). Briefly, using the von Bezold Jarisch reflex, rapid bolus injections of 5-HT (0·2–19 $\mu g\ kg^{-1}$) in the rat (Sprague-Dawley) caused dose-related falls in cardiac rate. The potential antagonists were administered i.v. 5 min before 5-HT challenge, and a dose was derived that reduced a submaximal response to 5-HT (2 $\mu g\ kg^{-1}$) by 50% (ID50). Concentration response curves for 5-HT-induced contractions were constructed from data from the guinea-pig (Dunkin-Hartley) ileum (incubated with methysergide 10^{-6} M) in the presence and absence of antagonists; pA_2 values were determined. By using the desheathed rabbit (albino) vagal nerve preparation, 5-HT-induced depolarization of the resting membrane potential was measured in the presence and absence of potential antagonists (30 min pretreatment); pA_2 values were determined. Zacopride, MDL 72222, and ICS 205–930 were synthesized by the Chemical Research Department of the A. H. Robins Company, Richmond, VA and metoclopramide was obtained from Lee Laboratories, Petersburg, VA.

Results and discussion

The activities of the reference 5-HT_3 antagonists, ICS 205–930, MDL 72222, and metoclopramide (Bradley et al 1986), to antagonize the effects of 5-HT on the ileum and vagus nerve are similar to those determined by Richardson et al (1986) and Donatsch et al (1984). Zacopride was approximately equipotent to ICS 205–930 in blocking the response to 5-HT on the isolated rabbit vagus and guinea-pig ileum and approximately three times more potent than ICS 205–930 in blocking the 5-HT-induced von Bezold Jarisch reflex (Table 1). Both compounds were more potent than metoclopramide and MDL 72222 in blocking the response to 5-HT. In assessing the type of antagonism to 5-HT on the guinea-pig ileum, pA_2 values were determined for zacopride and ICS 205–930 to indicate the competitive nature of the antagonism. In contrast, pD_2 values were reported for metoclopramide and MDL 72222 indicating that their antagonism of 5-HT was non-competitive (Donatsch et al 1984; Table 1). Thus in three systems zacopride is shown to be a potent 5-HT antagonist where the similarity of its profile of action to that of ICS 205–930 is indicative of a 5-HT_3 antagonist. Further evidence is obtained from radioligand binding studies where [^{3}H]zacopride binding to rat brain is displaced only by 5-HT_3 receptor antagonists, e.g. ICS 205–930 and GR 38032F. In particular, compounds acting on 5-HT_1, 5-HT_2 and dopamine receptors, e.g. methysergide, methiothepin, ritanserin and fluphenazine, and other transmitter receptor sites, e.g. noradrenergic and cholinergic are ineffective (Barnes et al unpublished). Therefore in both a functional and binding assay zacopride has the characteristics of a potent 5-HT_3 receptor antagonist with a specificity of action to the 5-HT_3 receptor.

Table 1. Anti-5-hydroxytryptamine activity of zacopride, ICS 205–930, MDL 72222, and metoclopramide on the guinea-pig isolated ileum and rabbit vagus nerve and in the von Bezold Jarisch reflex in rats

Compound	No. of Trials	pA_2 $\bar{x} \pm$ s.e.	ID50 ($\mu g\ kg^{-1}$, i.v.) $\bar{x} \pm$ s.e.
Guinea-pig ileum			
Zacopride	6	8·5 ± 0·2	
Metoclopramide	6	3·9 ± 0[ab]	
MDL 72222	6	6·2 ± 0·2[ab]	
ICS 205–930	6	8·3 ± 0·3	
Rabbit vagus			
Zacopride	6	10·1 ± 0·3	
Metoclopramide	6	7·3 ± 0·2	
MDL 72222	6	8·0 ± 0·2	
ICS 205–930	6	10·2 ± 0·3	
von Bezold Jarisch Reflex			
Zacopride	8		0·12 ± 0·01
Metoclopramide	8		188 ± 23
MDL 72222	8		42·0 ± 9·5
ICS 205–930	8		0·42 ± 0·05

[a] pD'_2.
[b] Donatsch et al (1984).

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Zacopride: anxiolytic profile in rodent and primate models of anxiety

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Abstract—Zacopride, a substituted benzamide derivative, was compared with diazepam in three models of experimental or provoked anxiety. The drug's action (i) in reducing aversion to a brightly lit environment was assessed in mice using a two compartment black and white test box system, (ii) in disinhibiting a suppressed behaviour was measured in the rat social interaction test under high light/unfamiliar conditions and (iii) in antagonizing a defensive response in the marmoset was assessed using the threat of a human presence. Both zacopride and diazepam enhanced exploratory behaviour and social interaction in the mouse and rat models and antagonized the defensive response in the marmoset, zacopride being 100 times more potent than diazepam. It is concluded that the 5-HT_3 receptor antagonist, zacopride, alters rodent and primate behaviour in a manner consistent with that of an anxiolytic agent.

The development of selective 5-HT_3 receptor antagonists based on a tropane (e.g. MDL 72222), indole (e.g. ICS 205-930) or carbazolone (GR38032F) nucleus has played a crucial role in the characterization of 5-hydroxytryptamine (5-HT) receptors in peripheral systems (see review by Fozard 1984; Richardson et al 1985; Bradley et al 1986; and Brittain et al 1987). In addition, such compounds are being used to assess the functional significance of 5-HT_3 receptors, which appear to be involved with gastric emptying and emesis (Buchheit et al 1985; Miner & Sanger 1986; Costall et al 1986, 1987a). Furthermore, 5-HT_3 receptor antagonists from the above series have profiles of anxiolytic action in rodent and primate models (Costall et al 1987b; Jones et al 1987; Tyers et al 1987) and high affinity binding sites have recently been found for the 5-HT_3 receptor antagonist GR65630 (3-(5-methyl-1*H*-imidazol-4-yl)-1-(1-methyl-1*H*-indol-3-yl)-1-propanone) in the rat brain (Kilpatrick et al 1987). The data indicates that 5-HT_3 receptor antagonists may have potential to influence both peripheral and central 5-HT function.

Zacopride, a substituted benzamide derivative (USA patent 4657911 assigned to Delalande S.A.), has been shown to enhance gastric emptying and to antagonize the emesis induced by cytotoxic therapy (Alphin et al 1986; Smith et al 1986). Subsequent studies have shown that zacopride can antagonize the actions of 5-HT on the vagus nerve, von Bezold Jarisch reflex and ileum (Smith et al 1988) and in both the in-vivo and in-vitro tests the potency of zacopride as a 5-HT_3 receptor antagonist is comparable with that of ICS 205-930 and GR38032F. In the present study we use zacopride to investigate whether the anxiolytic action of the 5-HT_3 receptor antagonists can be extended to compounds from the substituted benzamide series.

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We report that zacopride alters behaviour in mouse, rat and marmoset models in a manner consistent with a potent and effective anxiolytic agent.

Materials and methods

Influence on behaviour in the mouse black: white test box

Naive B.K.W. male albino mice, 30–35 g, were used. 10 mice were normally housed in each cage and kept for two weeks on a 12 h light/dark cycle with lights off at 0700 h. Tests for changes in behaviour which are known to be influenced by anxiolytic agents were conducted between 1300 and 1800 h in a quiet darkened room illuminated with a red light. Mice were taken from a dark holding room in a dark container to the dark testing room where, after a 1 h period of adaptation to the new environment, they were placed individually into the centre of the white, brightly lit area of the test box. The apparatus used for the detection of changes in exploratory behaviour consisted of an open-topped box (45 × 27 × 27 cm high) lined into 9 cm squares, two-fifths painted black and illuminated under a dim red light (1 × 60W) and partitioned from the remainder of the box which was painted white and brightly illuminated with a 60W light source located 17 cm above the box. An opening 7·5 × 7·5 cm located at floor level in the centre of the partition allowed access between the two compartments. The mice were observed over a 5 min period by remote video-recording and four behaviours noted, (i) the number of exploratory rearings in the white and black sections, (ii) the number of line crossings in the white and black areas, (iii) the time spent in the white and black areas and (iv) the latency of the initial movement from the white to the black area.

Mice were used once only in treatment groups of five. Results were analysed using single-factor analysis of variance and where appropriate followed by Dunnett's procedure for comparing all treatments with control.

Influence on rat social interaction

Male Sprague-Dawley rats, 225–275 g, were normally housed in groups of five and kept on a 12 h light/dark cycle with lights on at 0800 h. Tests were conducted between 1300–1800 h in an illuminated room. The apparatus used for the detection of changes in rat social interaction and exploratory behaviour consisted of an opaque white Perspex open-topped box (45 × 32 cm and 20 cm high) with 15 × 16 cm areas marked on the floor. Two naive rats, from separate housing cages, were placed into the box (with a 100W bright white illumination 17 cm above) and their behaviour observed over a 10 min period by remote video