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5-HT_3 receptor antagonism by anpirtoline, a mixed 5-HT_1 receptor agonist/5-HT_3 receptor antagonist

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1 The aim of this study was to provide evidence that anpirtoline, which is an agonist at 5-HT_{1B} and 5-HT_{1D} receptors and also displays submicromolar affinity for 5-HT_{1A} recognition sites, in addition, acts as an antagonist at 5-HT_3 receptors.

2 In radioligand binding studies on rat brain cortical membranes, anpirtoline inhibited specific binding of [^{3}H]-(S)-zacopride to 5-HT_3 receptor recognition sites (pK_i: 7.53).

3 In N1E-115 neuroblastoma cells in which [^{14}C]-guanidinium was used as a tool to measure cation influx through the 5-HT_3 receptor channel, the 5-HT-induced influx was concentration-dependently inhibited by anpirtoline. In this respect, anpirtoline mimicked other 5-HT_3 receptor antagonists; the rank order of potency was ondansetron > anpirtoline > metoclopramide.

4 The concentration-response curve for 5-HT as a stimulator of [^{14}C]-guanidinium influx was shifted to the right by anpirtoline (apparent pA_2: 7.78).

5 In urethane-anaesthetized rats, anpirtoline inhibited (at lower potency than zacopride and tropisetron) the 5-HT- or phenylbiguanide-induced bradycardia (Bezold-Jarisch reflex), but did not induce this reflex by itself.

6 Intravenous infusion of cisplatin in the domestic pig caused a consistent emetic response which was antagonized by anpirtoline.

7 It is concluded that anpirtoline, which was previously characterized as a 5-HT_1 receptor agonist also proved to be a 5-HT_3 receptor antagonist in several experimental models and, hence, exhibits a unique pattern of properties at different 5-HT receptors.

Keywords: Anpirtoline; 5-hydroxytryptamine receptors; antiemetic effect; domestic pig; 5-HT_3 receptors; [^{14}C]-guanidinium influx

Introduction

Anpirtoline is a novel drug with antinociceptive and antidepressant-like actions in rodents (Schlicker *et al.*, 1992; Swedberg *et al.*, 1992); furthermore, it has been shown to act as an agonist at 5-HT_{1B} and (at lower potency) 5-HT_{1D} receptors and to display submicromolar affinity not only for 5-HT_{1B} but also for 5-HT_{1A} recognition sites in rat brain membranes (Schlicker *et al.*, 1992). Due to the 5-HT_{1B} and 5-HT_{1D} character of the presynaptic 5-HT autoreceptors in the rat and pig brain, respectively, the drug inhibited the electrically evoked [^{3}H]-5-HT release from the 5-hydroxytryptaminergic nerve terminals contained in superfused cortical slices from these species and it was 22 times more potent in rat than in porcine cortical slices. The antinociceptive and antidepressant-like activities of anpirtoline in rats and mice are probably elicited by its agonist effect at 5-HT_{1A} and 5-HT_{1B} receptors (Schlicker *et al.*, 1992).

In addition to the neuropsychopharmacological properties mentioned so far, anpirtoline exhibited an anxiolytic-like potential: in a mouse light/dark aversion test, it reduced the aversive response of mice (Metzenauer *et al.*, 1992). This test is known to be sensitive to both classical (i.e. benzodiazepines) and novel anxiolytic agents such as 5-HT_{1A} receptor agonists and 5-HT_3 receptor antagonists (Costall *et al.*, 1989). Therefore, the possibility had to be considered that anpirtoline might produce the reduction of aversive response not only by acting as an agonist at 5-HT_{1A} receptors but also by an antagonistic property at 5-HT_3 receptors. The aim of the present investigation was to examine this possibility in classical *in vitro* and *in vivo* models as well as in a newly developed assay for antiemetic properties of drugs in the domestic pig (Szelenyi *et al.*, 1994). A preliminary account of the present data has been given at the 2nd International Symposium on 'Serotonin, from Cell Biology to Therapeutics', Houston 1992 (Szelenyi *et al.*, 1992).

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Methods

In vitro *studies*

Radioligand binding assays Adult male Sprague-Dawley rats were killed by decapitation and membranes from pooled entorhinal cortices were prepared as described elsewhere (Bolaños *et al.*, 1990). The membranes were incubated for 30 min at 30°C in 0.025 M Tris-HCl, pH 7.4, supplemented with 154 mM NaCl and 0.6 nM [^{3}H]-(S)-zacopride (specific activity: 83 Ci mmol^{-1}; Synthélabo, Rueil-Malmaison, France); the total volume was 0.5 ml. Nonspecific binding, measured in the presence of a saturating concentration (10 μM) of the 5-HT_3 receptor antagonist, tropisetron, corresponded to 45% of total binding. Further details are given elsewhere (Bolaños *et al.*, 1990).

The inhibition of the specific binding of [^{3}H]-(S)-zacopride by anpirtoline, ondansetron and MDL 72222 was examined with 10 different concentrations of the respective drugs, between 0.1 nM and 10 μM. Calculations of IC_{50} and apparent Hill coefficient values were performed as described by McPherson (1983). The equation of Cheng & Prusoff (1973) was used for converting the IC_{50}s into K_is.

Proteins were determined according to the method of Lowry *et al.* (1951) with bovine serum albumin as the standard.

Effects on 5-HT$_3$ receptor channels Mouse neuroblastoma cells of the clone N1E-115 (Amano *et al.*, 1972) at passage numbers 40–50 were grown in Dulbecco's modified Eagle medium supplemented with 10% foetal calf serum (Gibco; for further details of the composition of the culture medium, see Bönisch *et al.*, 1993). Cells were cultured in a humidified atmosphere containing 5% CO_2 at 37°C in vent flasks (Nunc. 750 ml) and fed every third day. Three days before starting experiments, cells were subcultured in 24-well cell culture clusters (Falcon).

Influx of [^{14}C]-guanidinium was studied under two different ionic conditions (buffer A and buffer B). Both were previously shown to be suitable for such investigations, but each exhibited certain advantages and disadvantages compared with the other (see Results and Discussion). Buffer A (previously used by Reiser & Hamprecht, 1989 and Emerit *et al.*, 1993 in NG 108-15 neuroblastoma cells) was composed as follows (in mM): HEPES 20, NaCl 135, KCl 4.5, $MgCl_2$ 1.0, Na_2HPO_4 2.0 and D-glucose. Buffer B (from Reith, 1990; Bönisch *et al.*, 1993) had the following composition (in mM): HEPES-Tris (pH 7.4) 25, choline chloride 135, KCl 5.4, $MgSO_4$ 0.98 and D-glucose 5.5.

The cells were washed and then preincubated for 20 min (1.5 ml per well) with one of these buffers (20°C) containing either no further drug (control) or the drug under investigation. Subsequently, the cells were incubated for 2 min with the respective buffer (A or B) containing 5 μM [^{14}C]-guanidinium chloride (specific activity 59 mCi mmol^{-1}; CEA) in the absence or presence of the drug under study plus, when relevant, 5-HT as stimulant drug. In the experiments with buffer A, substance P (10 μM) was applied simultaneously with 5-HT (see Reiser *et al.*, 1989; Emerit *et al.*, 1993). After termination of incubation (for details, see Bönisch *et al.*, 1993), the cells were dissolved in 0.5 ml 0.1% Triton X-100 and the [^{14}C]-guanidinium content of this solution was determined by liquid scintillation counting. An aliquot of the cell lysate was used for determination of the protein content according to the method of Lowry *et al.* (1951); the mean protein content per well was 218 ± 38 μg ($n = 68$). All experiments were carried out in duplicate or triplicate.

The apparent pA_2 values for anpirtoline against 5-HT was calculated according to Furchgott (1972).

In vivo *studies*

Bezold Jarisch reflex Adult male Sprague-Dawley rats (250–350 g) were anaesthetized with urethane (1.4 g kg^{-1}, i.p.), and a tracheotomy was performed to insert an endotracheal tube. A catheter (0.3 mm internal diameter) was inserted into the abdominal aorta via the femoral artery in order to record the arterial pressure and heart rate. A femoral vein was exposed and cannulated for i.v. drug administrations. 5-HT or the selective 5-HT$_3$ receptor agonist, phenylbiguanide, was injected at a dose of 30 μg kg^{-1} as a bolus in order to induce bradycardia (Bezold-Jarisch reflex).

Emesis by cisplatin Details of the induction of emesis by cisplatin and the evaluation of antiemetic drugs in the domestic pig have been described elsewhere (Szelenyi *et al.*, 1994). Briefly, 12–15 week-old domestic pigs of either sex (28–40 kg; on average about 30 kg in each of the various treatment groups) were housed in individual boxes of about 10 m^2 with warmed acryl-coated floor. They were maintained under constant environmental conditions (temperature 22 ± 1°C; humidity 50–60%; light/dark-rhythm (12/12 h). They received standardized pellet food for breeding pigs (hemo-Schweinefutter, Hemo, Heilbronn, Germany) twice daily (08 h 00 min and 14 h 00 min). Water was available *ad libitum*. On the day of the experiment (at 09 h 00 min), they were anaesthetized with ketamine 10 mg kg^{-1}, i.m. and xylazine 2 mg kg^{-1}, i.m., and a cannula was inserted into a superficial vein on one of the extremities. Cisplatin was administered intravenously with a total administration time of 15 min. Anpirtoline was given intravenously 15 min prior to cisplatin infusion. After removal of the cannula, the animals were placed in their boxes for recovery from anaesthesia (on an average, 30 min) and for subsequent observation of emesis (during 24 h). Food and water were available *ad libitum*. Emesis was characterized by expulsion of solid and/or liquid material from the gastrointestinal tract (ingested food, gastric juice contaminated by bile, etc.).

Statistical analysis

Results are given as means ± s.e.mean. For comparison of mean values, Student's *t* test was used.

Drugs

Anpirtoline HCl and cisplatin were synthetized by the Department of Chemical Research, ASTA Medica AG, Frankfurt, Germany. In addition, the following drugs were used: tropisetron (formerly ICS 205 930; Sandoz, Basel, Switzerland); ondansetron HCl (Glaxo, Greenford, Middlesex, UK), MDL 72222 (1αH, 3α, 5αH-tropan-3yl-3,5-dichlorobenzoate; Marion Merrell-Dow, Strasbourg, France); zacopride HCl (Synthélabo, Rueil-Malmaison, France, or Research Biochemical Inc., Natick, MA, U.S.A.); 5-hydroxytryptamine creatinine sulphate (5-HT; Merck, Darmstadt, Germany); phenylbiguanide (1-phenylbiguanide; Aldrich, Steinheim, Germany); substance P (SP), metoclopramide, urethane (Sigma, Deisenhofen, Germany). Stock solutions were prepared with dimethylsulphoxide (tropisetron, MDL 72222, metoclopramide) or saline (other drugs); in the case of cisplatin, solution in saline at 70–75°C was followed by gradual cooling to room temperature. The solvents, which were applied in the corresponding control experiments, did not affect the parameters investigated. Ketamine (Ketavet, Parke-Davis, Freiburg, Germany) and xylazine (Rompun, Bayer, Leverkusen, Germany) were used in their commercial form.

Results

Receptor binding studies

Competition experiments with [^{3}H]-(S)-zacopride to label 5-HT$_3$ receptor binding sites in membranes from the rat entorhinal cortex revealed that anpirtoline was a potent inhibitor of the specific binding of the radioligand (Figure 1). The pK_i value of anpirtoline was 7.53 ± 0.13 (Hill coefficient: 0.85 ± 0.10; $n = 3$). Comparison with typical 5-HT$_3$ receptor antagonists indicated that anpirtoline was slightly more potent than MDL 72222 ($pK_i = 7.31 \pm 0.11$; $n = 3$), but less potent than ondansetron ($pK_i = 8.50 \pm 0.09$; $n = 3$; Figure 1).

Effects on 5-HT$_3$ receptor channels

N1E-115 neuroblastoma cells were used to measure [^{14}C]-guanidinium influx through the 5-HT$_3$ receptor channel. When experiments were carried out with buffer A (see Methods), 5-HT induced only a weak influx of [^{14}C]-guanidinium (Reiser & Hamprecht, 1989; Emerit *et al.*, 1993). However, this response was potentiated by substance P (SP), 10 μM, which given alone caused only negligible [^{14}C]-guanidinium influx (Reiser & Hamprecht, 1989; Emerit *et al.*, 1993). Anpirtoline (up to 3 μM), ondansetron (up to 100 nM) and metoclopramide (up to 10 μM) by themselves did not induce [^{14}C]-guanidinium influx, but they caused a

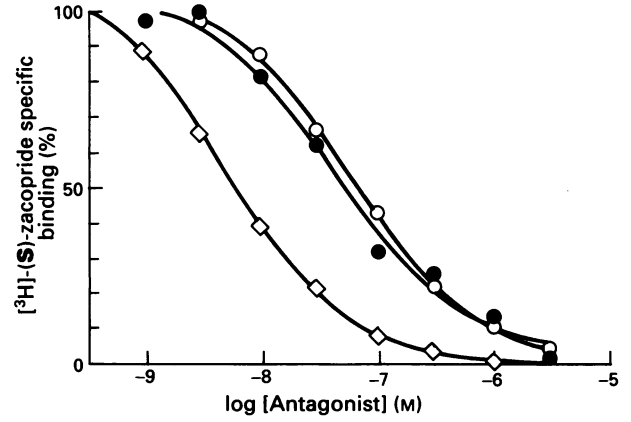

Figure 1 Concentration-dependent inhibition by anpirtoline (●), ondansetron (◇) and MDL 72222 (○) of the specific binding of [^{3}H]-(S)-zacopride (0.6 nM) to 5-HT$_3$ sites in membranes of rat entorhinal cortex. Non-specific binding was determined in the presence of 10 μM tropisetron. Each point is the mean of triplicate determinations in a typical experiment (100% corresponds to 13.2 fmol [^{3}H]-(S)-zacopride specifically bound mg^{-1} membrane protein). This experiment was repeated 3 times for the calculation of pK_i values indicated in the text.

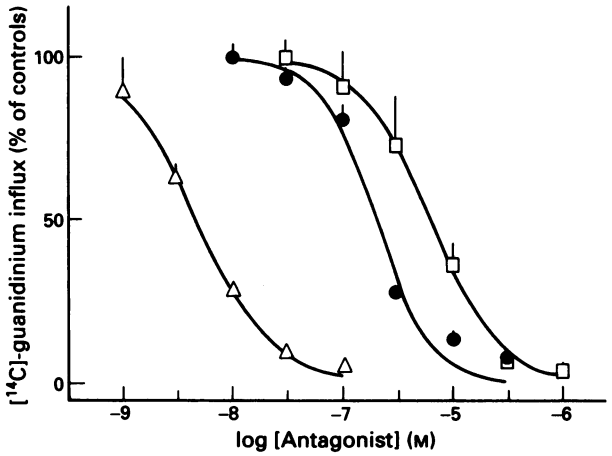

Figure 2 Inhibition of influx of [^{14}C]-guanidinium induced by 5-hydroxytryptamine (5-HT, 10 μM) plus substance P (SP, 10 μM) in N1E-115 cells by anpirtoline (●) in comparison to ondansetron (Δ) and metoclopramide (□). Each antagonist was present for 20 min before and during the 2-min exposure to 5-HT plus SP. The responses are expressed as percentages of the effect elicited by 10 μM 5-HT plus 10 μM SP in parallel control experiments carried out in the absence of antagonists. All experiments were performed with buffer A (see Methods). Each point is the mean with s.e.mean of 3 experiments.

concentration-dependent inhibition of the influx evoked by 10 μM 5-HT plus 10 μM SP (Figure 2). At the highest concentration applied, they virtually abolished evoked influx. The pK_i values for anpirtoline, ondansetron and metoclopramide calculated from the pIC_{50} values (see the concentration-response curves shown in Figure 2) were 7.44 ± 0.23, 9.31 ± 0.06 and 7.21 ± 0.18, respectively. 5-HT applied at increasing concentrations in the presence of 10 μM SP caused a concentration-dependent increase in [^{14}C]-guanidinium influx (Figure 3). The concentration-response curve for 5-HT (EC_{50}: 1.20 ± 0.14 μM) was concentration-dependently shifted to the right by anpirtoline (mean apparent pA_2: 7.78; Figure 3).

In experiments performed with buffer B, 5-HT induced a pronounced [^{14}C]-guanidinium influx even in the absence of SP (Bönisch *et al.*, 1993). This response was resistant to tetrodotoxin but susceptible to blockade by 5-HT$_3$ receptor antagonists at concentrations higher than those required in experiments with buffer A or in experiments on other 5-HT$_3$ receptor models (Bönisch *et al.*, 1993). In agreement with these findings, anpirtoline, ondansetron and metoclopramide concentration-dependently inhibited the 5-HT-induced [^{14}C]-guanidinium influx at lower potency than in the experiments with buffer A (results not shown). The pK_i values (6–8 experiments) for anpirtoline, ondansestron and metoclopramide were 6.60 ± 0.10, 7.38 ± 0.07 and 5.49 ± 0.01, respectively; hence, the rank order of potency of the three compounds was identical to that found with buffer A (see above). In the experiments with buffer B, we also found a rightward shift by anpirtoline (3 μM; 6 experiments) of the concentration-response curve for 5-HT (6 control experiments) as a stimulator of [^{14}C]-guanidinium influx (apparent pA_2: 6.27; result not shown).

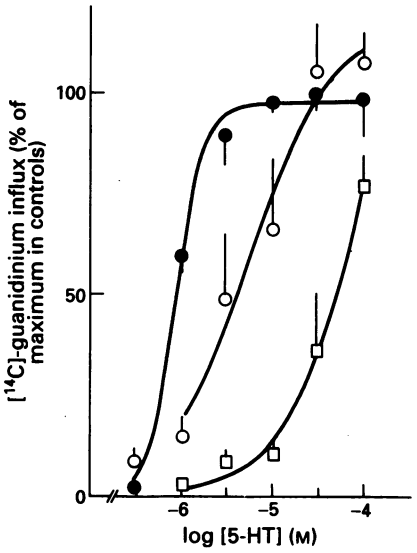

Figure 3 Concentration-response curves for the 5-hydroxytryptamine (5-HT; plus 10 μM substance P, SP)-induced influx of [^{14}C]-guanidinium in N1E-115 cells in the absence (●) or presence of 0.1 μM (○) or 1 μM anpirtoline (□). Anpirtoline was present for 20 min before and during 2-min exposure to 5-HT plus SP. The responses are expressed as percentages of the maximum effect elicited by 30 μM 5-HT plus 10 μM SP) in the absence of anpirtoline. Each point is the mean with s.e.mean of 6 experiments carried out with buffer A (see Methods).

Inhibition of Bezold-Jarisch reflex

Under control conditions, the heart rate of urethane-anaesthetized rats was in the range of 300–350 beats min^{-1}. 5-HT and phenylbiguanide (used as agonists in the laboratories in Paris and Frankfurt/M, respectively) at the submaximally effective dose of 30 μg kg^{-1} induced an immediate decrease in heart rate by 72.4 ± 6.3% (n = 13) and 63.4 ± 7.7% (n = 6), respectively; this effect lasted only for a few seconds. The bradycardia was reproducible when these 5-HT receptor agonists were repeatedly injected (up to five times) at intervals of 10 min (results not shown).

Anpirtoline, zacopride, tropisetron and MDL 72222, given alone did not cause a change in heart rate. However, 5 to 30 min after intravenous injection, all 5-HT$_3$ receptor antagonists dose-dependently inhibited the 5-HT- and/or phenylbiguanide-induced bradycardia (most results not shown; as a representative example, Figure 4 illustrates the decrease of 5-HT-induced bradycardia 5 min after injection of zacopride or anpirtoline). The doses of zacopride and anpirtoline which 5 min after injection induced 50% inhibition of the bradycardic response (ID_{50}) to 5-HT were 0.25 and 13.3 μg kg^{-1}, respectively. When a 15-min interval elapsed between the injection of either zacopride or anpirtoline and that of 5-HT,

the ID_{50} values of the 5-HT$_3$ receptor antagonists were 0.35 and 42.7 $\mu g\,kg^{-1}$, respectively. Anpirtoline also inhibited the phenylbiguanide-induced bradycardia (determined 5, 15 and 30 min after injection; ID_{50}: 3.6, 13.6 and 33.6 $\mu g\,kg^{-1}$, respectively), and this property was shared by tropisetron (ID_{50} determined 15 and 30 min after injection: 2.4 and 2.7 $\mu g\,kg^{-1}$, respectively) and MDL 72222 (ID_{50} determined 5, 15 and 30 min after injection: 28.1, 51.3 and 67.7 $\mu g\,kg^{-1}$, respectively). After 15 min of exposure, the ratios of the potencies of zacopride, tropisetron and MDL 72222 in antagonizing the 5-HT or phenylbiguanide-induced bradycardia related to the corresponding potency of anpirtoline were 0.008, 0.176 and 3.77, respectively.

Antiemetic effect

After cisplatin infusion at a dose of 2 mg kg^{-1} within 15 min in the domestic pig, emesis was induced in all animals. This dose of cisplatin corresponds roughly to the usual therapeutic dose in man. In the control experiments, the average number of vomits was 10.3 per pig within the first 4 h period (Figure 5). In the subsequent 20 h, the emetic response to cisplatin was clearly less: on the average, only about four further vomits were observed (see difference between open and closed circles in Figure 5), yielding a total number of 14.2 vomits during the whole observation period of 24 h.

Anpirtoline which was injected at doses of 1–10 mg per pig (about 0.033–0.33 mg kg^{-1} on the average) produced a dose-dependent inhibition of the cisplatin-evoked emesis in the first 4-h period after infusion of the anticancer drug (Figure 5). In the subsequent 20 h, the number of vomits was not significantly decreased compared to the control experiments: the difference between the open and closed circles in Figure 5 was 3–6 vomits after each dose of anpirtoline investigated. However due to the pronounced antiemetic effect in the first 4 h, anpirtoline 5 and 10 mg significantly decreased the total number of vomits in the whole 24-h period of observation.

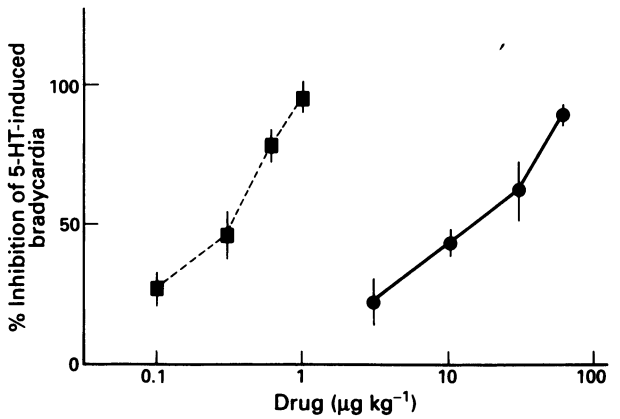

Figure 4 Inhibitory effect of anpirtoline (●) and zacopride (■) on the 5-hydroxytryptamine (5-HT; 30 $\mu g\,kg^{-1}$, i.v.)-induced bradycardia in urethane-anaesthetized rats, determined 5 min after i.v. injection of the respective antagonist. Each point is the mean with s.e.mean of 4–6 experiments.

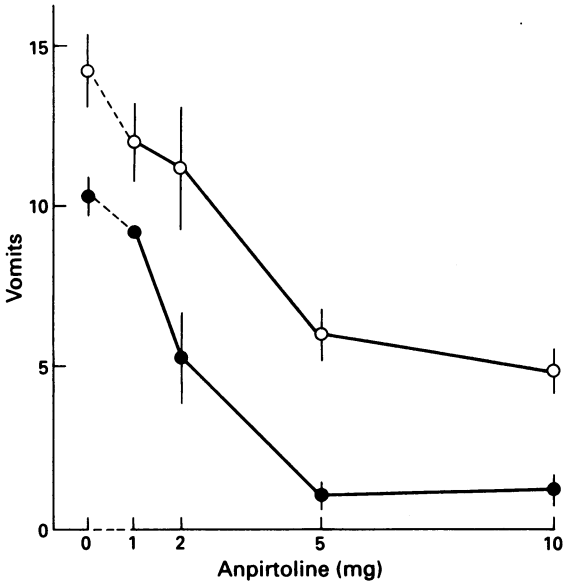

Figure 5 Emesis induced within the first 4 h (●) and 24 h (○) following i.v. infusion of cisplatin and antagonism by anpirtoline. Pigs were treated with the latter drug (i.v. injection) 15 min prior to cisplatin-infusion (2 mg kg^{-1}). The doses of anpirtoline indicated are those per animal (average body weight: 30.2 ± 1.3 kg). Each point represents the mean number of vomits per animal with s.e.mean (3–11 pigs per dose) during the respective observation period. Anpirtoline significantly (at least $P<0.05$, compared to the respective controls) decreased the number of vomits both in the first 4 h (in the dose-range of 2–10 mg) and in the whole 24 h period (at the doses of 5 and 10 mg) after infusion of cisplatin.

Discussion

The present experiments provided evidence that anpirtoline, in addition to its properties as a ligand at 5-HT$_{1A}$, 5-HT$_{1B}$ and 5-HT$_{1D}$ receptors (Schlicker *et al.*, 1992), acts as an antagonist at 5-HT$_3$ receptors. The latter belong to the superfamily of ligand-gated ion channels and they are almost exclusively located on neurones (Kilpatrick *et al.*, 1990; Hoyer, 1990; Maricq *et al.*, 1991). An exception to this rule is the enterochromaffin cell which, at least in the guinea-pig small intestine, also appears to be endowed with 5-HT$_3$ receptors (Gebauer *et al.*, 1993). The antagonistic property of anpirtoline at 5-HT$_3$ receptors can be derived from its abilities (1) to displace at intermediate nanomolar affinity [^{3}H]-S-zacopride from 5-HT$_3$ recognition sites in membranes of rat entorhinal cortex and (2) to counteract specific effects which are known to be mediated by 5-HT$_3$ receptors in classical and newly developed *in vitro* and *in vivo* assay systems. In particular, anpirtoline inhibited the [^{14}C]-guanidinium influx, in N1E-115 neuroblastoma cells (Reiser *et al.*, 1989; Emerit *et al.*, 1993; Bönisch *et al.*, 1993), the Bezold-Jarisch reflex in rats (Fozard, 1984; McQueen & Mir, 1989) and the cisplatin-induced emesis in the domestic pig (in this species, this represents a novel assay for evaluation of antiemetic drugs; Szelenyi *et al.*, 1994). The potency of anpirtoline in these test systems was less than that of zacopride, tropisetron, and ondansetron but higher than that of metoclopramide, and it was in a similar range to that of MDL 72222.

Previously published results of competition experiments using radioligands for the specific labelling of 5-HT$_{1A}$, 5-HT$_{1B}$ and 5-HT$_2$ recognition sites revealed that anpirtoline also bound to these 5-HT receptor types in the rat brain (Schlicker *et al.*, 1992). The drug exhibited the lowest affinity for cortical 5-HT$_2$ sites ($pK_i = 5.83$), its affinity for hippocampal 5-HT$_{1A}$ sites was intermediate ($pK_i = 6.82$), and it had approximately the same high affinity for striatal 5-HT$_{1B}$ sites ($pK_i = 7.55$) as found here for entorhinal cortical 5-HT$_3$ sites ($pK_i = 7.53$).

[^{14}C]-guanidinium is a suitable tool to study cation influx through the 5-HT$_3$ receptor channel in certain cell lines. This has first been shown in experiments with incubation buffer containing 135 mM Na^+ (as buffer A of the present experiments) in NG 108-15 cells by Reiser & Hamprecht (1989) and Emerit *et al.* (1993) and was recently confirmed in N1E-115 cells by Barann *et al.* (1993). Thus, 5-HT receptor agonists induced [^{14}C]-guanidinium influx and 5-HT receptor antagonists counteracted this effect at potencies similar to

those found in other 5-HT$_3$ receptor models. However, as already mentioned above, the disadvantage of this ionic condition was that stimulation with 5-HT had to be carried out in the presence of SP in order to obtain a reliably high [^{14}C]-guanidinium influx (Reiser & Hamprecht, 1989; Emerit *et al.*, 1993; Barann *et al.*, 1993). Conversely, under the ionic conditions applied by Reith (1990) and Bönisch *et al.* (1993), in which Na^+ was omitted and replaced by cholinium$^+$, a reasonably high influx of [^{14}C]-guanidinium could be induced without SP as an auxiliary drug, but the disadvantage was that the potencies of 5-HT receptor agonists and antagonists were 1–1.5 log units lower than in other 5-HT$_3$ receptor models. Under both ionic conditions, the involvement of 5-HT$_3$ receptors was beyond doubt, but the mechanisms underlying the interaction of SP with 5-HT$_3$ receptors and the decrease in potency of 5-HT$_3$ receptor agonists and antagonists by omission of Na^+ are unknown. On the basis of those previous results, one would expect that in the experiments with buffer A containing 135 mM Na^+ and with 5-HT plus SP as a stimulus, the pK_i and pA_2 values of anpirtoline should be in the same range as its affinity for 5-HT$_3$ receptors in entorhinal cortical membranes, whereas in the experiments with buffer B (absence of Na^+ ions and SP), these values should be 1–1.5 log units lower. In fact, this was observed in the present study, indicating that anpirtoline acts as a 5-HT$_3$ receptor antagonist in this 5-HT$_3$ receptor model.

Zacopride, tropisetron, MDL 72222 and anpirtoline, when injected in rats 5–30 min before 5-HT or the preferential 5-HT$_3$ receptor agonist, phenylbiguanide, inhibited the Bezold-Jarisch reflex. This reflex is initiated in the rat by activation of 5-HT$_3$ receptors on vagal afferents projecting the autonomic nuclei in the medulla oblongata (Sanger, 1992); in this brain area, an increased activity of the parasympathetic nerve fibres supplying the sinus node is induced, finally leading to a decrease in heart rate (McQueen & Mir, 1989; Sanger, 1992). Anpirtoline was slightly more potent in inhibiting the phenylbiguanide-induced bradycardia than that evoked by 5-HT, and this may be related to slight differences in the potencies of these agonists. However, the series of experiments with 5-HT and phenylbiguanide resembled each other in that the potency of anpirtoline in antagonizing the response to either agonist declined by a factor of about 3.5 (in detail 3.2 with 5-HT and 3.8, respectively, with phenylbiguanide) from the 5th to the 15th min after injection of anpirtoline. The potency of anpirtoline in antagonizing the response to phenylbiguanide further decreased from the 15th to the 30th min by a factor of 2.5. In contrast, only very slight time-dependent decreases in potency occurred when zacopride, tropisetron and MDL 72222 were applied. These findings suggest that the 5-HT$_3$ receptor antagonistic properties of anpirtoline were of shorter duration than those of the latter drugs.

Investigations carried out in the domestic pig revealed that this species is suitable for evaluating the emetogenic property of anticancer drugs and the ability of certain compounds to counteract this effect (Szelenyi *et al.*, 1994). Evidence has accumulated that 5-HT plays a crucial role in the mechanisms underlying vomiting and nausea induced by anticancer drugs (Fozard, 1987; Andrews *et al.*, 1988; Leslie & Reynolds, 1991; Schwörer *et al.*, 1991; Mitchelson, 1992; Sanger, 1992). The antiemetic properties of selective 5-HT$_3$ receptor antagonists were proved by a large number of experiments with cisplatin and other anti-cancer treatments (Aapro, 1991). In more detail, prevention of cisplatin-induced emesis has been demonstrated using, e.g. tropisetron, granisetron, ondansetron, zacopride and MDL 72222 in ferrets (Costall *et al.*, 1986; 1987; Stables *et al.*, 1987; Sanger, 1992). Investigations in the domestic pig confirmed the antiemetic effect of these 5-HT$_3$ receptor antagonists in these animals treated with a cisplatin dose of 2 mg kg^{-1} (Szelenyi *et al.*, 1994). This dose proved to be most suitable, because it consistently induced emetic responses without other toxic signs in the 24 h following its infusion. In agreement with the 5-HT$_3$ receptor blocking property of anpirtoline (see above), we found in the present investigation in domestic pigs that this drug given in a single dose 15 min prior to cisplatin shared the antiemetic property of other 5-HT$_3$ receptor antagonists. At the doses of 5 and 10 mg, anpirtoline almost abolished vomiting in the first 4 h, whereas it did not decrease the sporadic vomiting in the subsequent 20 h. In the context of this time course, it should be noted that, as a rule, delayed emesis has been found to be resistant to 5-HT$_3$ receptor antagonism (Aapro, 1991). Accordingly, the domestic pig treated with cisplatin may represent an animal model in which delayed emesis can be studied.

In conclusion, evidence has been presented that anpirtoline exhibits a unique pattern of properties at different 5-HT receptors in that it is not only a 5-HT$_1$ receptor agonist (Schlicker *et al.*, 1992), but also acts as a 5-HT$_3$ receptor antagonist. The latter property is responsible for its antiemetic effect and may contribute to the recently demonstrated anxiolytic-like action of the drug (Metzenauer *et al.*, 1992).

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