The antimigraine drugs ergotamine and dihydroergotamine are potent 5-HT_{1C} receptor agonists in piglet choroid plexus

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1 Fozard & Gray (1989) proposed that migraine is mediated by stimulation of 5-HT_{1C} receptors. We have examined the interaction of two effective anti-migraine agents, ergotamine and dihydroergotamine (DHE), with these receptors. Binding (inhibition of labelling by [³H]-mesulergine) and agonist activity (phosphoinositide hydrolysis) were measured in piglet choroid plexus, a tissue rich in 5-HT_{1C} receptors.

2 The pK_D for [³H]-mesulergine binding was 8.4. Ergotamine and DHE both inhibited [³H]-mesulergine binding with a pK_D of 7.1. This was similar to the potency of *m*-chlorophenylpiperazine (*m*-CPP) (pK_D 7.4) and rather less than that of 5-hydroxytryptamine (5-HT) (pK_D 8.1).

3 Both ergotamine and DHE were full agonists (pEC₅₀s 7.5 and 7.6 respectively) with potencies similar to that of 5-HT (pEC₅₀ 7.7) and greater than that of *m*-CPP (pEC₅₀ 7.1). Mesulergine 10⁻⁷ M produced near-parallel rightward shifts of the concentration-response curves for all these agents of 1.8–2.2 log units, consistent with an action of the agonists at the same receptor.

4 There was no effect of prazosin, spiperone, mepyramine or atropine on the phosphoinositide hydrolysis induced by ergotamine, ruling out an action via α₁-adrenoceptors, 5-HT₂, histamine H₁, or muscarinic receptors.

5 It is concluded that, together with 5-HT, ergotamine and DHE are the most potent 5-HT_{1C} agonists reported so far. These findings do not support the theory that 5-HT_{1C} receptor activation causes migraine.

Keywords: 5-HT_{1C} receptor stimulation; choroid plexus; phospholipase C; ergotamine; dihydroergotamine

Introduction

Fozard & Gray (1989) proposed that a variety of drugs that prevent migraine may do so by blocking cerebral 5-HT_{1C} receptors. Their proposal was based on the observation by Brewerton *et al.* (1988) of a high incidence of migraine-like headache induced by a single oral dose of *m*-chlorophenylpiperazine (*m*-CPP). The incidence of headache was significantly greater in subjects with a personal or family history of migraine. Fozard & Gray (1989) based their argument on the relative high affinity of *m*-CPP for 5-HT_{1C} receptors (Hoyer, 1989), its relative high potency as an enhancer of inositol phosphate levels in pig choroid plexus (Schoeffter & Hoyer, 1989) and its selectivity for 5-HT_{1C} receptors over other 5-HT₁ receptor subtypes and 5-HT₂ receptors (Hoyer, 1989).

The classical antimigraine drugs, ergotamine and dihydroergotamine (Bowman & Rand, 1980), have been reported to possess affinity for the 5-HT_{1C} receptor which is similar to the affinity of *m*-CPP (Hoyer, 1989). In view of this affinity, we investigated whether these ergot analogues possessed agonist properties in the 5-HT_{1C} receptor system of piglet choroid plexus. We found that the ergots are powerful agonists. To establish the relationship between agonist potency and affinity, we labelled the 5-HT_{1C} receptors with [³H]-mesulergine and used the ergots as binding inhibitors. The effects and affinities of the ergots were compared to those of 5-hydroxytryptamine (5-HT) and *m*-CPP.

Methods

Measurement of inositol phosphate accumulation

The method for measuring accumulation of inositol phosphates was a modification of that described by Schoeffter & Hoyer (1989) for choroid plexus from pig. Piglets (Camborough Hi-Bred, 5–9 days old) from a local farm were anaesthetized with halothane and killed by removal of the heart (Kaumann, 1990). The skull was opened, the brain removed and the choroid plexus rapidly dissected and trans-

ferred to Na-Krebs medium at 37°C, gassed with 95% O₂:5% CO₂. The Na-Krebs medium contained (mM): Na⁺ 149, K⁺ 6.4, Mg²⁺ 1.3, Ca²⁺ 0.8, Cl⁻ 128, HCO₃⁻ 26, phosphate 1.4, SO₄²⁻ 1.3 and glucose 10. For each experiment, plexi were collected and pooled from 9 piglets. After the last choroid plexus had been removed (total time less than 2 h), the choroid plexi were cross chopped on a McIlwain tissue chopper set at 300 μm. The slices were washed twice by allowing them to settle under gravity, removing the supernatant and then resuspending them in fresh, gassed Na-Krebs at 37°C. After each resuspension, the slices were incubated for about 10 min before further processing, with gassing over the surface of the suspension. The slices were finally suspended in 6 ml of fresh Na-Krebs and 50 μCi of [³H]-myo-inositol (10–20 Ci mmol⁻¹; stock solution dried under nitrogen and redissolved in Na-Krebs), was added. The slices were then incubated for 1.5 to 2 h at 37°C with constant gassing directed as a jet on to the surface of the suspension to provide good mixing.

After this incubation, 40 ml of Li-Krebs (as Na-Krebs but with 10 mM NaCl replaced by 10 mM LiCl) was added. The slices were mixed with a plastic Pasteur pipette and allowed to settle. The supernatant was discarded and replaced with fresh Li-Krebs and the slices incubated for 5 min at 37°C. This process was repeated twice more and the slices were finally resuspended in an appropriate volume of Li-Krebs containing (final concentrations in incubation vials) 100 μM pargyline (to inhibit monoamine oxidase), 6 μM cocaine (to inhibit possible tissue 5-HT uptake) and 0.2 mM ascorbic acid (to inhibit oxidation of 5-HT) and stirred gently with magnetic stirring ready for distribution to the incubation vials.

Incubations were carried out in Beckman Biovials in Li-Krebs for 1 h at 37°C in a shaking water bath. The total incubation volume was 300 μl. Li was included in the incubation medium to prevent breakdown of inositol monophosphate and hence cause the inositol phosphates to accumulate over the incubation period. Drugs were added in a total volume of 20 μl. Agonists (5-HT, ergots and *m*-CPP) were dissolved in 2 mM ascorbic acid and were added in 10 μl. Mesulergine was dissolved in Li-Krebs containing 0.2 mM ascorbic acid and added in 10 μl. Incubations were started by addition to each vial of 280 μl of the slice suspension. The vial contents were

gassed/mixed with a jet of 95% O₂:5% CO₂ and sealed. Incubations were terminated by addition to each vial of 300 µl of 7% perchloric acid after which the vials were placed on ice for 15 min. The contents of the vials were then centrifuged and 0.55 ml of the supernatant mixed well with 0.625 ml of (triethylamine:1,1,2-trichlorotrifluoroethane, 1:1) to extract the perchloric acid. After centrifugation, 0.4 ml of the top (aqueous) phase was pipetted on to columns containing ion exchange resin (Biorad AG 1-X8, 200–400 mesh, formate form) in 5 ml Milli-Q purified water. The columns were washed with a further 20 ml water to remove inositol. The inositol phosphates were then eluted into scintillation vials with 10 ml of a solution containing 1.05 M ammonium formate and 0.1 M formic acid. ³H content of each vial was then determined by scintillation counting.

Binding studies

Preparation of membranes from piglet choroid plexus and measurement of binding to 5-HT_{1C} receptors with [³H]-mesulergine were essentially as described by Pazos *et al.* (1984) for pig choroid plexus. Incubations were at 37°C for 30 min in a medium containing 50 mM Tris-Cl (pH 7.7 at room temperature), 4 mM CaCl₂, 10 µM pargyline, 0.1% ascorbic acid, and in a total volume of 1 ml. For binding inhibition studies, drugs were added in 250 µl, [³H]-mesulergine (final concentration about 1 nM) in 250 µl and membranes in 500 µl (30–75 µg protein/assay tube), with all incubation tubes on ice. The incubations were started by transfer of the tubes to a waterbath at 37°C and terminated after 30 min by rapid filtration through Whatman GF/C filters (presoaked in polyethyleneimine, 0.5%, for 2 h to reduce non-specific binding) on a Brandel Harvester followed by three rapid washes with 3 ml of ice-cold 50 mM Tris-Cl (pH 7.4 at room temperature), 4 mM CaCl₂. The filters were then transferred to scintillation vials and counted for ³H in Ready Protein scintillation fluid (Beckman). Protein was determined by the method of Bradford (1976).

The saturation binding isotherm for [³H]-mesulergine was determined by use of a range of [³H]-mesulergine concentrations between 0.05 and 80 nM. Mesulergine 1 µM was used to define non-specific binding. At 1 nM [³H]-mesulergine, as used in the binding inhibition experiments, approximately 60% of the total ³H binding was specific. For all binding experiments, data were analysed by non-linear regression with GraFit (Erithacus Software Ltd., Staines, U.K.) on a Tandon PCA20 computer. Both saturation binding and binding inhibition data were well fitted by equations assuming interactions at a single site and yielding a value for the binding equilibrium constant, *K*_D.

Drugs

Ergotamine, dihydroergotamine, 5-HT hydrochloride, atropine sulphate and prazosin were from Sigma, Mepyramine maleate was from May and Baker, *m*-CPP and spiperone were obtained from Research Biochemicals Incorporated. Mesulergine was a gift from the Sandoz Company, Basle. [³H]-mesulergine was obtained from Amersham International and [³H]-myo-inositol from New England Nuclear.

Results

Both ergotamine and dihydroergotamine were full agonists. Their potency was almost as high as that of 5-HT and 3 times higher than that of *m*-CPP (Figure 1, Table 1). Mesulergine, 10⁻⁷ M, caused surmountable blockade of the effects of all the agonists, shifting the concentration-effect curves in nearly parallel manner and to similar extent (Figure 1). The similarity of the concentration ratios for these agonists (Table 1)

Figure 1 Effects of agonists on the accumulation of inositol phosphates in slices of piglet choroid plexus in the absence (open symbols) and presence (closed symbols) of mesulergine 10⁻⁷ M. (○, ●), 5-hydroxytryptamine; (△, ▲), ergotamine; (◇, ◆), dihydroergotamine; (□, ■), *m*-chlorophenylpiperazine; (▽, ▼), basal accumulation. Each panel shows a separate experiment carried out on pooled choroid plexi from 9 piglets. Each point is the mean of triplicate determinations; vertical bars show s.e.mean. Where not shown, the error bar is smaller than the symbol.

suggests the involvement of the same receptor. Spiperone 10⁻⁷ M had no effect on the response to 5-HT 10⁻⁶ M or ergotamine 10⁻⁶ M (Table 2), ruling out the involvement of 5-HT₂ receptors. The effects of micromolar concentrations of ergotamine were also not altered by the histamine H₁ antagonist, mepyramine (10⁻⁶ M), the muscarinic antagonist atropine (10⁻⁶ M) or the α₁-adrenoceptor antagonist, prazosin (10⁻⁶ M) (Table 2).

[³H]-mesulergine bound in a saturable manner to sites with a *pK*_D of 8.4 and density of -log₁₀(*K*_D), M

Table 1 Effects of agonists on inositol phosphate accumulation and inhibition of binding of [³H]-mesulergine in piglet choroid plexus

Drug	Inositol phosphate accumulation			Binding <i>pK</i> _D ^c
	<i>n</i> (piglets)	<i>pEC</i> ₅₀ ^a	log(CR) ^b	
5-HT	27	7.7	1.8	8.1
Ergotamine	9	7.5	2.0	7.1
Dihydroergotamine	9	7.6	2.2	7.1
<i>m</i> -CPP	9	7.1	1.9	7.4

m-CPP = *m*-chlorophenylpiperazine.

^a *pEC*₅₀ = -log₁₀(*EC*₅₀), M.

^b CR = concentration ratio of agonist with 10⁻⁷ M mesulergine.

^c *pK*_D = -log₁₀(*K*_D), M.

Table 2 Effects of receptor antagonists on 5-hydroxytryptamine (5-HT)- and ergotamine-induced inositol phosphate accumulation in piglet choroid plexus

Condition	Inositol phosphate accumulation (d.p.m.)
<i>Experiment 1</i>	
Basal	12,044 ± 99
5-HT (1 µM)	223,483 ± 26,235
5-HT (1 µM) + spiperone (0.1 µM)	250,843 ± 29,976
Ergotamine (1 µM)	205,994 ± 4,980
Ergotamine (1 µM) + spiperone (0.1 µM)	195,221 ± 7,853
Ergotamine (1 µM) + atropine (1 µM)	227,011 ± 4,697
Ergotamine (1 µM) + mepyramine (1 µM)	227,683 ± 17,225
<i>Experiment 2</i>	
Basal	8,584 ± 315
Ergotamine (3.3 µM)	68,815 ± 8,324
Ergotamine (3.3 µM) + prazosin (1 µM)	78,835 ± 12,233

0.52 ± 0.05 pmol mg⁻¹ protein (mean ± s.e.mean, *n* = 10; experiments not shown). 5-HT, ergotamine, dihydroergotamine and *m*-CPP inhibited binding in a fashion consistent with interaction of a single site with p*K*_D values shown in Table 1.

Discussion

Our data are consistent with 5-HT_{1C} receptor-mediated phospholipase C stimulation by both ergotamine and dihydroergotamine. Our binding affinity estimates for 5-HT, ergotamine, dihydroergotamine, *m*-CPP and [³H]-mesulergine in piglet choroid plexus membranes agree with the corresponding affinities of the compounds for 5-HT_{1C} receptors of choroid plexus of adult pigs reported by Hoyer (1989). Mesulergine antagonized with similar affinity the effects of the four agonists. This evidence, taken together, is consistent with an interaction of the four agonists with 5-HT_{1C} receptors labelled and blocked by mesulergine.

The EC₅₀ values of 5-HT, ergotamine and dihydroergotamine roughly agree with the corresponding *K*_D values (Table 1), suggesting that there is negligible receptor reserve under our conditions. Some 5-HT_{1C} receptor reserve has been reported for the effects of 5-HT in rat choroid plexus (Sanders-Bush & Breeding, 1990).

Ergotamine and dihydroergotamine are known to interact with other receptors which stimulate phospholipase C, including 5-HT₂ (Hoyer, 1989) and α₁-adrenoceptors (Megens *et al.*, 1986). Since these receptors might be present in piglet choroid plexus, we examined the effects of antagonists on the ergotamine-induced inositol phosphate accumulation in choroid plexus slices. Neither 10⁻⁷ M of the 5-HT₂ antagonist spiperone (p*K*_D at 5-HT₂ receptors, 8.8; p*K*_D at 5-HT_{1C} receptors, 5.9; Hoyer, 1989) nor 10⁻⁶ M of the α₁-adrenoceptor antagonist prazosin (p*K*_D 10.0; Watson & Abbott, 1991) inhibited the effect of micromolar concentrations of ergotamine, ruling out roles for either of these receptors. The

absence of α₁-adrenoceptors on choroid plexus is consistent with the findings of Conn & Sanders-Bush (1986) in rat choroid plexus. We also tested whether two further phospholipase C-linked receptors, muscarinic and histamine H₁ receptors, might be involved in the effect of ergotamine in this tissue. However, neither atropine 10⁻⁶ M (p*K*_D at muscarinic receptors, 9.3; Fisher, 1985) nor mepyramine 10⁻⁶ M (p*K*_D at histamine H₁ receptors 9.1; Hill, 1990) altered ergotamine-induced inositol phosphate accumulation.

The high agonistic potency of the antimigraine drugs ergotamine and dihydroergotamine at 5-HT_{1C} receptors would appear to be inconsistent with the hypothesis of Fozard & Gray (1989) that activation of these receptors induces migraine. Indeed, it might be expected from the data presented here that these agents would be more potent inducers of migraine than *m*-CPP. However, the two ergots only occasionally cause headache (Bowman & Rand, 1980), possibly related to abuse (Andersson, 1975).

One possible explanation for this apparent inconsistency might be that the ergots are poorly brain penetrant. The chemical nature of ergotamine and dihydroergotamine makes it unlikely that they would readily cross the blood-brain barrier (Ziegler, 1990). However, there is some evidence for brain penetration of both substances although the amounts detected are small. For example, 20–50 ng g⁻¹ of [³H]-ergotamine and 10 ng g⁻¹ of [³H]-dihydroergotamine have been reported in rat brain following 0.5–1 mg kg⁻¹ oral or i.v. administration (Eckert *et al.*, 1978). These amounts of the two drugs, if evenly distributed, would suffice to yield concentrations greater than 10 nM at cerebral 5-HT_{1C} receptors and could well cause phospholipase C stimulation as seen in the piglet choroid plexus. Interestingly, 1 mg kg⁻¹ i.p. dihydroergotamine increases wakefulness and modifies sleep patterns in rats, consistent with a central site of action (Loew *et al.*, 1978). Ergotamine has also been measured in human cerebrospinal fluid after oral administration of a therapeutic dose (Ala-Hurula *et al.*, 1979) although some concern has been expressed about the specificity of the analytical method used in this study (Sanders *et al.*, 1986).

These data suggest that some headache might be expected with ergots if the hypothesis of Fozard & Gray (1989) is correct for, given the high agonistic potency of the compounds, even a small amount of brain penetration should result in some activation of cerebral 5-HT_{1C} receptors. It is conceivable that the therapeutic effects of the two ergots, presumably exerted through cerebral vascular constriction (Bowman & Rand, 1980), overshadow some possible 5-HT_{1C} receptor stimulation. The present data therefore suggest a need to regard the hypothesis of Fozard & Gray (1989) with some caution though they are not sufficient to reject it. In addition, *m*-CPP also causes a considerable release of hypothalamic 5-HT (Pettibone & Williams, 1984) which could play an additional role in the production of headache.

We conclude that ergotamine and dihydroergotamine, together with 5-HT, are the most potent agonists of 5-HT_{1C} receptors described so far (Schoeffter & Hoyer, 1989; Hoyer, 1989).

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