Evidence that central 5-HT_{1A}-receptors play a role in the von Bezold-Jarisch reflex in the rat

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1 The effects of intracisternal (i.c.) application of putative 5-hydroxytryptamine (5-HT)_{1A} antagonists on the reflex bradycardia evoked by injection of phenylbiguanide (i.v.) were investigated in anaesthetized, atenolol-pretreated rats.

2 Intracisternal application of spiperone (100 µg kg⁻¹) reversibly attenuated the reflex bradycardia whilst the same dose given i.v. had no effect. The bradycardia was also attenuated by i.c. methiothepin (200 µg kg⁻¹), (±)-pindolol (100 µg kg⁻¹) and buspirone (200 µg kg⁻¹) but was not attenuated by antagonists selective for α₁-adrenoceptors (alfuzosin; 100 µg kg⁻¹), 5-HT₂-receptors (BW 501C67; 100 µg kg⁻¹) or dopamine D₂-receptors ((-)-sulpiride; 100 µg kg⁻¹) given i.c.

3 It is concluded that the 5-HT_{1A}-receptor antagonist action of intracisternally applied spiperone, methiothepin, (±)-pindolol and buspirone is responsible for the ability of these drugs to attenuate reversibly the excitation of cardiac vagal motoneurons caused by activation of the von Bezold-Jarisch reflex.

Introduction

Investigation of the role of 5-hydroxytryptamine (5-HT)_{1A}-receptors in central cardiovascular regulation has led to the suggestion that central 5-HT pathways may be involved in the regulation of cardiac vagal motoneurons in anaesthetized cats (Ramage & Fozard, 1987; Ramage *et al.*, 1988). In this respect D-lysergic acid, which has a high affinity for all the 5-HT₁-receptor subtypes (Hoyer, 1988), has been shown in earlier studies to increase vagal drive in the cat by an action on 'medullary vagal centres' (Cervoni *et al.*, 1963). Furthermore, boutons containing 5-HT-like immunoreactivity have been shown to make synaptic contact with retrogradely-labelled cardiac vagal motoneurons in the nucleus ambiguus of the cat and, additionally, microinjections of 5-HT or 5-HT_{1A} agonists into this nucleus cause a bradycardia (Izzo *et al.*, 1988). In the rat, 5-HT-like immunoreactive fibres have been observed in the nucleus ambiguus and the dorsal vagal motor nucleus (Steinbusch, 1981) and both nuclei contain cardiac vagal motoneurons (Nosaka *et al.*, 1979). In conscious rats, central application of 5-HT causes a profound fall in blood pressure and a bradycardia (Dalton, 1986). This bradycardia was shown to be partly due to an increase in vagal drive and was suggested to be due to activation of 5-HT₁-like receptors. Further, the 5-HT_{1A}-receptor agonist, 8-hydroxy-2-(di-n-propylamino)tetratin (8-OH-DPAT), given i.v. in rats causes a bradycardia which has been shown to be mediated by both an increase in vagal drive and sympatho-inhibition (Gradin *et al.*, 1985; Cherqui *et al.*, 1988). 8-OH-DPAT binding has also been shown in brain areas containing cardiac vagal motoneurons in the rat (Pazos & Palacios, 1985) and microinjections of 8-OH-DPAT or flesinoxan, another 5-HT_{1A}-receptor agonist, into the vicinity of these cardiac vagal motoneurons have been shown to cause an increase in vagal drive (Sporton *et al.*, 1989). These combined observations support the view that there is an excitatory 5-HT-containing pathway which innervates cardiac vagal motoneurons and involves the activation of 5-HT_{1A}-receptors.

Excitatory inputs to cardiac vagal motoneurons arise from cardiopulmonary afferents, arterial baroreceptors, carotid

body afferents, receptors in the airways involved in the diving response (see Daly, 1986) and other areas within the brain (see Loewy & Spyer, 1990). Initially it was decided to determine if the so called 'von Bezold-Jarisch reflex' could be attenuated by the central administration of 5-HT_{1A} antagonists. This reflex can be easily evoked experimentally by giving i.v. injections of either veratridine (Dawes, 1947) or phenylbiguanide (Dawes & Mott, 1950; McQueen & Mir, 1989). In the present experiments the latter substance was used as the reflex can be obtained repeatedly, whereas with veratridine, repeated injections lead to desensitization (Dawes & Mott, 1950).

The present experiments examine the ability of phenylbiguanide to increase cardiac vagal drive, as measured by the bradycardia induced by i.v. administration of phenylbiguanide in atenolol-pretreated, anaesthetized rats. Atenolol was chosen as it is a β-adrenoceptor antagonist which does not bind to 5-HT receptors (Middlemis *et al.*, 1977) and poorly penetrates the central nervous system (Street *et al.*, 1979).

A preliminary account of some of these observations has been made (Bogle & Ramage, 1989).

Methods

Experiments were performed on anaesthetized, spontaneously breathing, male Sprague-Dawley rats (200–300 g). Anaesthesia was induced with halothane and maintained with α-chloralose (70 mg kg⁻¹, i.v.). The femoral artery was cannulated for the measurement of blood pressure and heart rate, and the jugular vein was cannulated for drug administration. Rectal temperature was monitored and maintained between 37–39°C with a homeothermic blanket system (BioScience). The animals were placed in a head holder and a cannula was placed through the atlanto-occipital membrane so that drugs could be administered intracisternally (i.c.). The animals were then pretreated with atenolol (1 mg kg⁻¹). Once blood pressure and heart rate had stabilized a von Bezold-Jarisch reflex was induced with phenylbiguanide (10 µg kg⁻¹) and repeated every 15 min until 3 consistent bradycardias had been obtained. This dose of phenylbiguanide was used as it was found to give a submaximal response and did not show tachyphylaxis over these time intervals. Ten minutes after the third consistent bradycardia, the test drug was administered intracisternally in a volume of 10 µl over a period of 20 s with a Hamilton syringe (100 µl) which was connected to the i.c. cannula. A

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Figure 1 Atenolol-pretreated, anaesthetized rat. A trace showing the effects of spiperone given intracisternally (i.c.) on the reflex bradycardia evoked by i.v. injections of phenylbiguanide (PBG; $10 \mu\text{g kg}^{-1}$). The time interval is that measured from intracisternal (i.c.) injection of spiperone

further 5 min was then allowed before the reflex was evoked by another i.v. injection of phenylbiguanide. The phenylbiguanide injection was repeated at 15 min intervals in order to monitor any changes in the amplitude of the reflex bradycardia. In experiments where the test drugs were administered i.v. the same protocol was applied.

Analysis of results

The control bradycardia was taken as the mean of three reflex bradycardias elicited by phenylbiguanide before the adminis-

tration of the test drugs. The changes in the size of this reflex bradycardia after administration of the test drug were measured from this control value. The background resting heart rate and mean blood pressure were measured 10 min before administration of the test drug. Changes in resting heart rate and blood pressure were measured at 5, 20 and 35 min after administration of the test drug and just before the i.v. injection of phenylbiguanide occurring at that time period. Comparisons of the changes in the reflex bradycardia, resting heart rate and mean blood pressure caused by the test drugs with the appropriate controls were carried out by use of Student's

Table 1 Decreases in heart rate (beats min^{-1}) evoked by i.v. phenylbiguanide ($10 \mu\text{g kg}^{-1}$) before (control value) and after administration of the test drugs in atenolol-pretreated, anaesthetized rats

Drug (dose, route)	n	Control	5 min	20 min	35 min
Spiperone $100 \mu\text{g kg}^{-1}$, i.c.	6	149 ± 21	$30 \pm 10^{**\S\S}$	$48 \pm 12^{**\S\S}$	$84 \pm 25^{*\S}$
Spiperone $100 \mu\text{g kg}^{-1}$, i.v.	6	112 ± 9	130 ± 22	150 ± 23	150 ± 20
Lactic acid $10 \mu\text{l}$, i.c.	6	112 ± 6	78 ± 7	96 ± 12	102 ± 12
Alfuzosin $100 \mu\text{g kg}^{-1}$, i.c.	5	121 ± 15	118 ± 24	118 ± 16	153 ± 15
BW 501C67 $100 \mu\text{g kg}^{-1}$, i.c.	6	146 ± 13	144 ± 18	152 ± 17	163 ± 11
(-)-Sulpiride $100 \mu\text{g kg}^{-1}$, i.c.	5	115 ± 9	123 ± 12	130 ± 9	129 ± 3
Saline $10 \mu\text{l}$, i.c.	5	148 ± 17	164 ± 12	140 ± 21	151 ± 11
Methiothepin $200 \mu\text{g kg}^{-1}$, i.c.	5	131 ± 16	$45 \pm 10^{***}$	$65 \pm 20^{*}$	94 ± 30
(±)-Pindolol $100 \mu\text{g kg}^{-1}$, i.c.	5	125 ± 9	$39 \pm 10^{***}$	82 ± 22	117 ± 26
Buspirone $200 \mu\text{g kg}^{-1}$, i.c.	5	160 ± 15	$126 \pm 23^{*}$	$103 \pm 23^{**}$	126 ± 26
Acidic saline $10 \mu\text{l}$, i.c.	5	150 ± 15	150 ± 18	150 ± 15	148 ± 23

Results are shown as the mean $\pm$ s.e.mean. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ compared with appropriate vehicle; § $P < 0.05$; §§ $P < 0.01$ compared with i.v. spiperone.

Table 2 Changes in resting heart rate and mean blood pressure caused by the test drugs from pre-drug baseline values in atenolol-pretreated, anaesthetized rats

Drug (dose, route)	n	Baseline HR	Change in HR (beats min^{-1})			Baseline BP	Change in BP (mmHg)		
			5 min	20 min	35 min		5 min	20 min	35 min
Spiperone $100 \mu\text{g kg}^{-1}$, i.c.	6	383 ± 7	-13 ± 5	-5 ± 4	-3 ± 3	91 ± 2	-15 ± 5	-7 ± 7	2 ± 5
Spiperone $100 \mu\text{g kg}^{-1}$, i.v.	6	358 ± 10	1 ± 11	9 ± 12	9 ± 11	99 ± 7	-5 ± 3	4 ± 1	7 ± 1
Lactic acid $10 \mu\text{l}$, i.c.	6	385 ± 7	8 ± 8	5 ± 9	8 ± 10	89 ± 4	0 ± 7	-1 ± 4	-1 ± 4
Alfuzosin $100 \mu\text{g kg}^{-1}$, i.c.	5	371 ± 18	1 ± 2	4 ± 4	12 ± 7	95 ± 6	0 ± 3	2 ± 3	4 ± 3
BW 501C67 $100 \mu\text{g kg}^{-1}$, i.c.	6	301 ± 25	4 ± 2	6 ± 2	6 ± 7	94 ± 7	3 ± 1	3 ± 3	5 ± 4
(-)-Sulpiride $100 \mu\text{g kg}^{-1}$, i.c.	5	378 ± 9	1 ± 5	2 ± 5	7 ± 6	91 ± 3	3 ± 2	6 ± 3	8 ± 3
Saline $10 \mu\text{l}$, i.c.	5	353 ± 8	-1 ± 1	1 ± 4	4 ± 2	87 ± 6	-1 ± 1	3 ± 3	7 ± 3
Methiothepin $200 \mu\text{g kg}^{-1}$, i.c.	5	363 ± 10	-3 ± 2	-1 ± 2	2 ± 3	94 ± 8	$-10 \pm 2^{**}$	$-10 \pm 3^{**}$	$5 \pm 1^{**}$
(±)-Pindolol $100 \mu\text{g kg}^{-1}$, i.c.	5	371 ± 13	4 ± 3	$17 \pm 2^{**}$	$18 \pm 3^{**}$	89 ± 4	$-14 \pm 3^{**}$	$-9 \pm 3^{*}$	3 ± 3
Buspirone $200 \mu\text{g kg}^{-1}$, i.c.	5	357 ± 6	$-18 \pm 8^{*}$	-8 ± 5	-5 ± 5	82 ± 4	-5 ± 6	2 ± 2	11 ± 3
Acidic saline $10 \mu\text{l}$, i.c.	5	379 ± 13	0 ± 1	3 ± 1	6 ± 1	81 ± 3	0 ± 1	7 ± 2	17 ± 4

Results are shown as the mean $\pm$ s.e.mean. * $P < 0.05$; ** $P < 0.01$ compared with appropriate vehicle.

unpaired *t* test. All values given are the mean $\pm$ s.e.mean, differences were considered significant at $P < 0.05$.

Drugs

Drugs and suppliers were as follows: alfuzosin HCl (Synthelabo, France); atenolol (ICI, U.K.); BW 501C67, (2-anilino-N-(2-(3-chlorophenoxy)propyl) acetamidine HCl) (Wellcome, U.K.); buspirone HCl (Bristol-Myers Company, U.S.A.); α -chloralose (Sigma); halothane (ICI, U.K.); ICS 205-930, (3-tropanylindole-3-carboxylate methiodide) (Sandoz, Switzerland); methiothepin monomethanesulphonate (see Jilek *et al.*, 1980); phenylbiguanide (K + K Labs., U.S.A.); ($\pm$)-pindolol (Sigma); spiperone (Janssen, Belgium); (–)-sulpiride (Semat Tech., U.K.). All drugs were freshly prepared and dissolved in saline, with the exception of spiperone which was dissolved in 0.4 M lactic acid, ($\pm$)-pindolol which was dissolved in 0.1 N HCl and saline and buffered to pH (7.4) of normal saline, and (–)-sulpiride which was dissolved in a few drops of 1 N HCl and then diluted in saline (pH 4). Buspirone, when dissolved in saline produced an acidic solution (pH 4). Saline as a buspirone control was acidified to this pH with 0.4 M lactic acid. In control experiments the appropriate vehicle was administered.

Results

Effect of spiperone, methiothepin, ($\pm$)-pindolol and buspirone on the bradycardia induced by i.v. phenylbiguanide (10 μ g kg⁻¹)

Spiperone (100 μ g kg⁻¹) given i.c. (Figure 1) caused a significant ($P < 0.05$) attenuation of this bradycardia at 5, 20 and 35 min compared with the same dose given i.v. or the vehicle (0.4 M lactic acid) given i.c. (see Table 1). Methiothepin (200 μ g kg⁻¹, i.c.) also caused a significant attenuation of this bradycardia at 5 and 20 min as did buspirone (200 μ g kg⁻¹, i.c.), while ($\pm$)-pindolol (100 μ g kg⁻¹, i.c.) only caused a significant attenuation of this reflex after 5 min compared to the appropriate vehicle (Table 1).

Effect of alfuzosin, BW 501C67, (–)-sulpiride and ICS 205-930 on the bradycardia induced by i.v. phenylbiguanide (10 μ g kg⁻¹)

Alfuzosin, BW 501C67 and (–)-sulpiride given i.c. (100 μ g kg⁻¹) failed to have any effect on the phenylbiguanide-induced bradycardia (Table 1). ICS 205-930 in two experiments at a dose of 0.5 μ g kg⁻¹, which when given i.v. did not affect this bradycardia, also failed to have any effect when given i.c.

Effect of spiperone, methiothepin, ($\pm$)-pindolol, buspirone, alfuzosin, BW 501C67, and (–)-sulpiride on resting blood pressure and heart rate

The effects of these drugs on baseline heart rate and mean blood pressure are shown in Table 2. Spiperone had no significant effect on these parameters compared with the vehicle control. However, methiothepin and ($\pm$)-pindolol caused a significant fall in blood pressure but differed in that methiothepin had no effect on heart rate whereas ($\pm$)-pindolol caused a significant increase in heart rate after 20 min. Buspirone caused only a small transient bradycardia after 5 min.

Discussion

The present experiments in atenolol-pretreated, anaesthetized rats demonstrate that intracisternal administration of the 5-HT_{1A}-receptor antagonists, spiperone, methiothepin, ($\pm$)-pindolol and the partial agonist, buspirone (Schoeffter & Hoyer, 1988; Hoyer, 1988) attenuate the bradycardia elicited

by i.v. phenylbiguanide. It is possible that these drugs may be leaking out of the brain and blocking this bradycardia by action on muscarinic receptors. However, this may be considered extremely unlikely, particularly in the case of spiperone and methiothepin, as these drugs have an extremely low affinity for the muscarinic receptor (Leysen, 1985). This is further confirmed by the observation that the same dose of spiperone, as that given i.c., when given i.v. had no effect on the reflex bradycardia. This latter experiment also demonstrates that it is blockade of central not peripheral 5-HT_{1A}-receptors that is responsible for the attenuation of this reflex bradycardia. These drugs could attenuate this reflex by activation of other pathways involved in central cardiovascular control and in this respect some of these drugs did cause changes in the levels of resting heart rate and blood pressure. However, the drugs did not have a consistent effect on these variables, a fact which would give little support to such an interpretation. As the reflex bradycardia occurs in atenolol-pretreated rats it can only be attributed to an increase in vagal drive. Therefore, these observations, taken together with the evidence presented in the Introduction, strongly indicate an involvement of 5-HT_{1A}-receptors in the activation of cardiac vagal motoneurons by the von Bezold-Jarisch reflex.

Brain areas in the rat that contain cardiac vagal motoneurons have also been shown to have binding sites for α_1 -adrenoceptors (Unnerstall, 1987), 5-HT₂-receptors (Pazos *et al.*, 1985) as well as 5-HT_{1A}-receptors (Pazos & Palacios, 1985). Although spiperone, methiothepin, ($\pm$)-pindolol and buspirone all have in common the ability to act at the putative 5-HT_{1A}-receptor (Schoeffter & Hoyer, 1988; Hoyer, 1988), both spiperone and methiothepin are antagonists at α_1 -adrenoceptors and 5-HT₂-receptors (Feniuk *et al.*, 1985; Leysen, 1985). It is therefore possible that blockade of these receptors by these antagonists is contributing to their ability to attenuate the reflex bradycardia. However, this is unlikely as alfuzosin, a highly selective α_1 -adrenoceptor antagonist (Cavero *et al.*, 1984) with a similar affinity for α_1 -adrenoceptors to spiperone (alfuzosin has a pA₂ of 7.95 on rabbit aorta (Cavero *et al.*, 1984) while spiperone has a pA₂ of 7.76 on rabbit aorta (Feniuk *et al.*, 1985)), had no effect on this reflex bradycardia when given i.c. at the same dose as spiperone. Further, BW 501C67, a highly selective 5-HT₂-receptor antagonist (Mawson & Whittington, 1970; Fozard, 1982) again with a similar affinity for 5-HT₂-receptors to spiperone (pA₂ 9.3 rabbit aorta (G.R. Martin, personal communication) compared with that of spiperone 8.64 (Feniuk *et al.*, 1985)), also failed to attenuate this reflex bradycardia when given i.c. at the same dose as spiperone. In addition, both spiperone and methiothepin bind to the dopamine D₂-receptor (Leysen, 1985). However (–)-sulpiride, a selective dopamine D₂-receptor antagonist (Stoof & Kebabian, 1984) which was given at a dose known to block the central hypotensive action of the dopamine agonist B-HT 958 (Brown & Harland, 1986), had no effect on this reflex.

Further evidence for the involvement of 5-HT in the activation of cardiac vagal motoneurons by stimulation of vagal afferents is that the cell bodies of these afferents, which are found in the nodose ganglia, have been shown to contain 5-HT-like immunoreactivity (Gaudin-Chazel *et al.*, 1982). In addition 5-HT_{1A} (Pazos & Palacios, 1985) and 5-HT₃-binding sites have been demonstrated in the nucleus tractus solitarius of the rat (Pratt & Bowery, 1989) which is a major site of termination of these afferents (Kalia & Sullivan, 1982). These 5-HT₃-binding sites are thought to be presynaptic on vagal afferents, as nodose ganglionectomy has been shown to reduce this binding by 50% (Pratt & Bowery, 1989). In the present experiments an attempt was made to study the role of 5-HT₃-receptors. However, a low dose of the 5-HT₃ antagonist, ICS 205-930 had to be used in order to ensure that if this antagonist leaked out of the brain it would not block the ability of phenylbiguanide to activate the reflex. The dose chosen was the lowest dose of ICS 205-930 that could be given i.v. which did not affect the ability of phenylbiguanide to

activate the reflex. Although the results proved negative, they cannot be considered conclusive.

In conclusion, these results indicate that activation of cardiac vagal motoneurons by the von Bezold-Jarisch reflex involves the activation of 5-HT_{1A}-receptors. Furthermore, when taken together with other evidence (see Introduction) these receptors would seem to be excitatory, which indicates that they differ from 5-HT_{1A}-receptors found in other brain regions e.g. the raphe (Sprouse & Aghajanian, 1987) and the hippocampus (Colino & Halliwell, 1987), which cause inhibi-

tion. The possible involvement of these receptors in the apnoea caused by activation of pulmonary chemoreceptor reflexes and the sympathoinhibition caused by activation of the von Bezold-Jarisch reflex, along with their involvement in other types of reflex and central activation of cardiac vagal motoneurons, remains to be determined.

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