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Effects of galanin on 8-OH-DPAT induced decrease in body temperature and brain 5-hydroxytryptamine metabolism in the mouse

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Abstract

Central administration of galanin dose-dependently (minimum effective dose, M.E.D. = 1 nmol) blocked the hypothermia induced by the 5-HT_{1A} receptor agonist 8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT, 0.5 mg/kg s.c.), in mice. This inhibitory effect was reversed by pretreatment with the galanin receptor antagonist galantide (0.3 nmol) and also by pretreatment with the ATP-sensitive potassium channel blockers glibenclamide (10 nmol) and gliquidone (10 nmol). The hypothermic response to 8-OH-DPAT was also blocked by the 5-HT_{1A} receptor antagonist (*N*-(2,4(2-methoxyphenyl)-1-piperazinyl)ethyl-*N*-(2-pyridinyl)cyclohexane, (WAY 100,635, M.E.D. = 0.01 mg/kg s.c.), and the centrally acting muscarinic receptor antagonist scopolamine (M.E.D. = 10 mg/kg i.p.) but not the peripheral muscarinic receptor antagonist *N*-methylscopolamine. 8-OH-DPAT (0.5 mg/kg s.c.) also decreased cortical and hypothalamic 5-HT (5-hydroxytryptamine, serotonin) metabolism, an effect which was not blocked by pretreatment with galanin (0.3–3 nmol intracerebroventricular, i.c.v.). Neither did galanin (0.03–3 nmol/5 μl i.c.v.) affect basal 5-HT metabolism in these brain regions. Furthermore, pretreatment in vitro of mouse cortical membranes with galanin (10 or 1000 nM) had no effect on 5-HT_{1A} receptor affinity, B_{max} or pharmacology determined using [^{3}H]8-OH-DPAT. These results suggest that the inhibition of 8-OH-DPAT induced hypothermia by galanin is probably not mediated by an interaction with 5-HT_{1A} receptors but more likely by blocking the indirect activation by 8-OH-DPAT of central cholinergic pathways involved in temperature regulation.

Keywords: 5-HT_{1A} receptor; 8-OH-DPAT (8-hydroxy-2-(di-*n*-propylamino)tetralin); Galanin; Hypothermia; Acetylcholine; Muscarinic receptor

1. Introduction

8-OH-DPAT (8-hydroxy-2-(di-*n*-propylamino)tetralin) has been used extensively to probe the function of the 5-HT_{1A} receptor for which it has high affinity. 5-HT_{1A} receptors labelled with [^{3}H]8-OH-DPAT are heterogenously distributed throughout the brain with particularly high density in the medial and dorsal raphe nuclei (Verge et al., 1986). In these regions they function as inhibitory somatodendritic autoreceptors (Verge et al., 1985) and 5-HT_{1A} receptor agonists such as 8-OH-DPAT act at these receptors to decrease neuronal firing (Sprouse and Aghajanian, 1986), 5-HT (5-hydroxytryptamine, serotonin) synthesis (Hjorth et al., 1982) and release (Hutson et al., 1989). 8-OH-DPAT and other 5-HT_{1A} receptor agonists decrease body temperature in both rats (Hjorth, 1985; Hutson et al., 1987a; Siniscalchi et al., 1990; Bill et al., 1991) and mice (Martin et al., 1992). This effect appears to be mediated by 5-HT_{1A} receptors as the highly selective 5-HT_{1A} receptor antagonist WAY 100,635 completely prevented the hypothermic effects of 8-OH-DPAT (Fletcher et al., 1994). Evidence from brain 5-HT depletion studies (Goodwin et al., 1985; Hutson et al., 1987a) suggests that the hypothermic effects of 8-OH-DPAT are mediated by 5-HT_{1A} receptors located postsynaptically with respect to 5-HT neurones in the rat and presynaptically in the mouse.

Galanin is a 29 amino acid, N-terminal amidated peptide (Tatemoto et al., 1983) which in addition to being colocalised with central cholinergic (Melander et al., 1985) and noradrenergic (Skofitsch and Jacobwitz, 1985) neurones has also been shown, using immunohistochemical studies, to coexist with 5-HT containing cell bodies in the raphe nuclei (Melander et al., 1986a,b). In addition, galanin binding sites (Skofitsch et al., 1986; Melander et al., 1988) and 5-HT_{1A} binding sites (Pazos and Palacios, 1985) appear to be codistributed in several regions such as the limbic system and the hypothalamus, although there is no evidence to suggest that these receptors are located on the same neurones. While it is known that galanin modulates

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the function of cholinergic neurones (Fisone et al., 1987; Palazzi et al., 1988) it is less clear that galanin can affect serotoninergic neuronal function. Thus, central administration of galanin has been shown to increase (Sundstrom and Melander, 1988), or decrease (Fuxe et al., 1988a) 5-HT metabolism in several brain regions including the hippocampus and cortex. Consistent with the former study, Martire et al. (1991) demonstrated that galanin potentiated potassium evoked release of [^{3}H]5-HT from rat hypothalamic slices in vitro indicating the presence of galanin heteroreceptors on 5-HT nerve terminals. Radioligand binding studies in vitro have suggested that galanin may interact directly with the 5-HT$_{1A}$ receptor subtype by decreasing its affinity for 8-OH-DPAT (Fuxe et al., 1988b). Given the importance of 5-HT$_{1A}$ receptors in the regulation of brain 5-HT neuronal function, the present studies attempted to determine the functional consequences of galanin's interaction with the 5-HT$_{1A}$ receptor subtype by determining the effects of centrally administered galanin on basal and 8-OH-DPAT induced changes of brain 5-HT metabolism and thermoregulation.

2. Materials and methods

2.1. Measurement of hypothermia

Male BKTO mice (20–30 g) were housed individually in Perspex cages at ambient temperature (21 ± 3°C) for at least 60 min prior to experiment. They were placed in a Perspex cylinder and rectal temperature determined using a Sensotek BAT-12 thermometer incorporating a rounded 2.5 mm diameter probe inserted 2.4 cm into the rectum. Probe insertion was lubricated with liquid paraffin. Mice were injected intracerebroventricularly (i.c.v.), under metofane anaesthesia with either galanin (0.1, 0.3, 1, 3, 10 nmol/5 μl) or vehicle [artificial cerebrospinal fluid (CSF, composition, mM: KCl 5, NaCl 120, $MgCl_2$ 1.2, $CaCl_2$ 1.8), 5 μl] followed 5 min later by either 8-OH-DPAT (0.5 mg/kg s.c.), or saline vehicle (4 ml/kg i.p.). In studies investigating the effects of muscarinic receptor antagonists, scopolamine (1, 3, 5, 10 mg/kg i.p.) or *N*-methylscopolamine (1, 3, 5, 10 mg/kg i.p.) were administered 30 min prior to administration of test compound. Similarly, the 5-HT$_{1A}$ receptor antagonist WAY 100,635 (1, 0.1, 0.01, 0.001 mg/kg s.c.) was also administered 30 min prior to administering 8-OH-DPAT. The galanin receptor antagonist galantide (0.3 nmol/5 μl artificial CSF) and the ATP-sensitive potassium (K^+) channel blockers glibenclamide (10 nmol/5 μl) and gliquidone (10 nmol/5 μl) or ethanol vehicle (5 μl) were administered under metofane anaesthesia by intracisternal injection (i.c.m.) 15 min prior to galanin. All animals were used only once. The minimal effective dose (M.E.D.) was defined as the minimal dose of drug required to statistically ($P < 0.05$) affect the parameter being measured.

2.2. Brain 5-HT metabolism

Male BKTO mice (20–30 g) were injected with either saline (10 ml/kg s.c.) or 8-OH-DPAT (0.05–1.0 mg/kg s.c.) and killed 30 min later. Galanin (0.03–10 nmol/5 μl i.c.v.) or vehicle (5 μl i.c.v.) was administered under metofane anaesthesia and mice were killed after 30 min. In the interaction study mice were injected (under metofane anaesthesia) with galanin (0.1–10 nmol/5 μl i.c.v.) or vehicle (5 μl i.c.v.) 5 min prior to 8-OH-DPAT (0.5 mg/kg s.c.; the M.E.D. to decrease brain 5-HT metabolism) or vehicle (10 ml/kg s.c.) and killed 30 min later. Cortex and hypothalamus were rapidly dissected on ice and stored at −70°C until assayed for 5-HT and 5-hydroxyindoleacetic acid (5-HIAA) concentration essentially as described by Hutson et al. (1987b). Briefly, tissue samples were homogenised (1:10 w/v) in 0.4 M perchloric acid containing 0.01% EDTA, 0.1% sodium metabisulphite and 0.1% cysteine. Homogenates were then centrifuged at 3000 × *g* for 15 min. 5-HT and 5-HIAA concentration was then measured in the resulting supernatant by high performance liquid chromatography (HPLC, Ultrasphere incorporating a 3 μM spherical 80 A pore, C18, 4.6 × 75 mm analytical column; flow rate 1.0 ml/min) with electrochemical detection (Waters 460, using a carbon working electrode set at +0.7 V with respect to a silver/silver-chloride reference electrode).

All studies were performed in accordance with the U.K. Animals (Scientific Procedures) Act, 1986.

2.3. 5-HT$_{1A}$ receptor binding

5-HT$_{1A}$ receptor binding was adapted from the method of Gozlan et al. (1983). Mouse cerebral cortices were rapidly dissected on ice and then homogenised in 50 mM Tris-HCl pH 7.7 (1:20 w/v). Following centrifugation (48 000 × *g*, 10 min, 4°C) the pellet was resuspended in ice-cold 50 mM Tris-HCl, pH 7.7 (1:20 w/v) and recentrifuged. The resulting pellet was then lysed by resuspension in 50 mM Tris-HCl pH 7.7 (1:20 w/v) and incubation for 10 min at 37°C. The lysed membranes were then centrifuged as before and the resulting pellet resuspended in assay buffer (50 mM Tris-HCl, pH 7.7 containing 10 μM pargyline, 0.1% ascorbate, 0.1% bovine serum albumin, 1 μM bacitracin and 5.7 mM $CaCl_2$). Binding of [^{3}H]8-OH-DPAT was determined (0.1–15 nM) and non-specific binding determined with 10 μM 5-HT. Subsequent displacement studies were carried out using 0.3 nM [^{3}H]8-OH-DPAT. Displacing drugs were added in a volume of 100 μl to give a final assay volume of 1.0 ml. Incubations were initiated by adding 300 mg of membrane (pretreatment for 10 min at 37°C with either buffer or galanin (10 or 1000 nM) as described by Fuxe et al. (1988b) and allowed to proceed for 20 min at 37°C before being terminated by rapid filtration over GF/B filters presoaked in 0.3% polyethyleneimine/0.5% Triton X-100 using 2 × 10 ml 50 mM Tris-HCl pH 7.7. Radioactivity

was determined using liquid scintillation spectrometry. Protein was assayed by the method of Bradford (1976) with bovine serum albumin as the standard. Binding parameters were determined by non-linear least squares regression analysis using RS1 (BBN Research Systems, Cambridge, MA, USA) and a computerised iterative procedure written by Dr. A. Richardson, NRC Terlings Park.

2.4. Materials

Compounds and reagents for these studies were obtained from the following sources: porcine galanin, galantide (Bachem, UK); 8-hydroxy-2-(di-*n*-propylamino)tetralin HBr, buspirone and glibenclamide (Research Biochemicals International, Semat, UK); scopolamine, *N*-methylscopolamine (Sigma); WAY 100,135 (*N*-tert-butyl 3-4-(2-methoxyphenyl) piperazin-1-yl-2-phenylpropanamide dihydrochloride), and WAY 100,635 (*N*-(2,4(2-methoxyphenyl)-1-piperazinyl)ethyl-*N*-(2-pyridinyl)cyclohexane) were synthesized in the Medicinal Chemistry Department at Terlings Park; gliquidone (Boehringer-Mannheim, UK); [^{3}H]8-OH-DPAT (210–240 Ci mmol^{-1}) was purchased through Amersham International.

2.5. Statistical analysis

Statistical analysis was carried out using analysis of variance (ANOVA) followed, where appropriate, by Tukey's standardised range test (BMDP statistical software, California, USA).

3. Results

3.1. Effect of galanin, WAY 100,635 and scopolamine on 8-OH-DPAT induced hypothermia

Systemic administration of 8-OH-DPAT (0.5 mg/kg s.c) significantly reduced rectal temperature with a typical

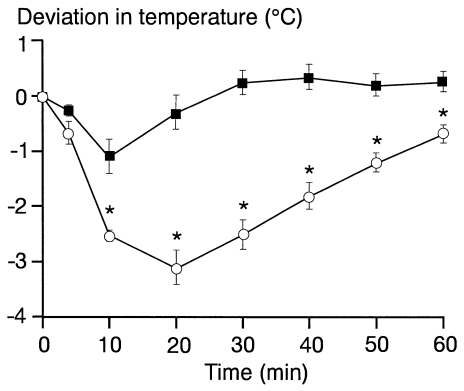

Fig. 1. Time course of the hypothermic effect of 8-OH-DPAT (0.5 mg/kg s.c.) in mice (○) versus saline (4 ml/kg s.c.) controls (■). Results are expressed as mean ± S.E.M. of the deviation in temperature (°C) from the temperature at $t = 0$; $n = 6/7$ per group. Significant differences were determined by Tukey tests following ANOVA (* $P < 0.05$ compared to mice given only 8-OH-DPAT). Initial baseline temperatures were comparable in all groups: vehicle/vehicle, 37.0 ± 0.4°C; vehicle/8-OH-DPAT, 36.8 ± 0.3°C.

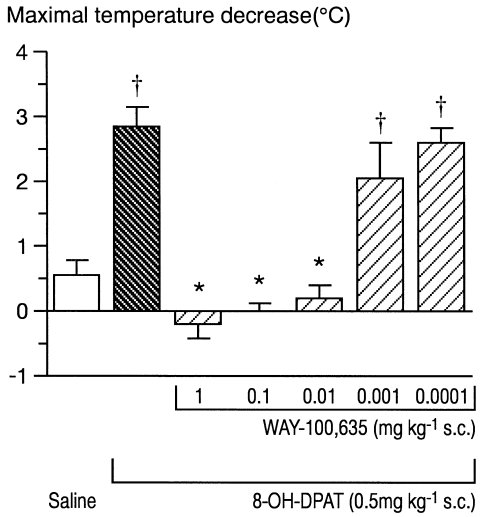

Fig. 2. Effect of WAY 100,635 (0.0001, 0.001, 0.01, 0.1, 1 mg/kg s.c.) on 8-OH-DPAT (0.5 mg/kg s.c.) induced hypothermia in mice. Results are expressed as means ± S.E.M. of the maximal change in temperature (°C) from the temperature at $t = 0$; $n = 6/8$ per group. Significant differences were determined by Tukey tests following ANOVA (* $P < 0.01$ compared to mice given only 8-OH-DPAT; † $P < 0.05$ compared to saline controls). Initial baseline temperatures were comparable in all groups: vehicle/vehicle, 37.1 ± 0.3°C; vehicle/8-OH-DPAT, 37.1 ± 0.15°C; 1 mg/kg WAY 100,635/8-OH-DPAT, 36.8 ± 0.3°C; 0.1 mg/kg WAY 100,635/vehicle, 37.3 ± 0.3°C; 0.01 mg/kg WAY 100,635/8-OH-DPAT, 37.2 ± 0.4°C; 0.001 mg/kg WAY 100,635/8-OH-DPAT 36.9 ± 0.3°C; 0.0001 mg/kg WAY 100,635/8-OH-DPAT, 37.4 ± 0.2°C.

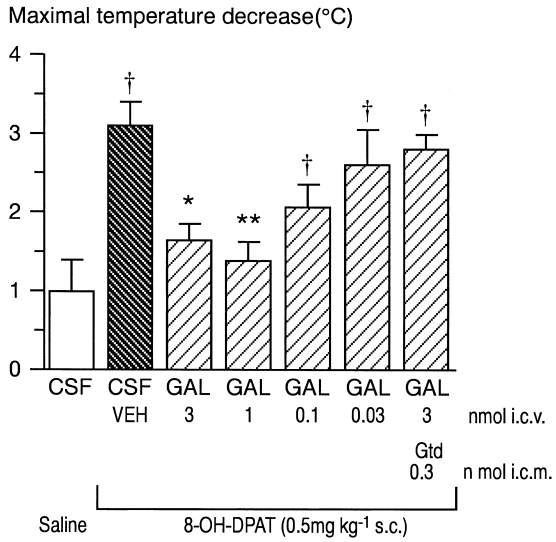

Fig. 3. Effect of galanin (GAL, 0.03, 0.1, 1, 3 nmol i.c.v.) on 8-OH-DPAT (0.5 mg/kg s.c.) induced hypothermia in mice, and effect of galantide (Gtd, 0.3 nmol i.c.m.) on galanin (3 nmol i.c.v.) inhibition of 8-OH-DPAT (0.5 mg/kg s.c.) induced hypothermia in mice. Results are expressed as mean ± S.E.M. of the deviation in temperature (°C) from the temperature at $t = 0$; $n = 6/7$ per group. Significant differences were determined by Tukey tests following ANOVA (* $P < 0.05$ versus vehicle/8-OH-DPAT, ** $P < 0.01$; † $P < 0.05$ compared to saline controls). Initial baseline temperatures were comparable in all groups: vehicle/vehicle, 36.9 ± 0.3°C; vehicle/8-OH-DPAT, 36.8 ± 0.4°C; 0.03 nmol galanin/8-OH-DPAT, 37.0 ± 0.04°C; 0.1 nmol galanin/vehicle, 37.3 ± 0.21°C; 1 nmol galanin/8-OH-DPAT, 37.2 ± 0.18°C; 3 nmol galanin/8-OH-DPAT, 37.1 ± 0.12°C; galantide/galanin/vehicle 37.0 ± 0.4°C; galantide/galanin/8-OH-DPAT 36.8 ± 0.35°C.

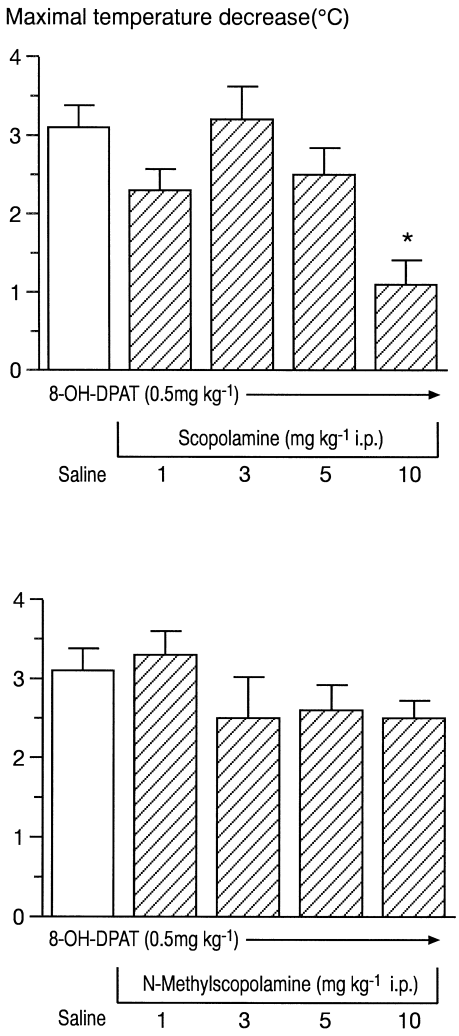

Fig. 4. (a) Effect of scopolamine (1, 3, 5, 10 mg/kg i.p.) on 8-OH-DPAT (0.5 mg/kg s.c.) induced hypothermia in mice, and (b) effect of *N*-methylscopolamine (NMS, 1, 3, 5, 10 mg/kg i.p.) on 8-OH-DPAT induced hypothermia in mice. Results are expressed as means ± S.E.M. of the maximal change in temperature (°C) from the temperature at $t = 0$; $n = 6/8$ per group. Significant differences were determined by Tukey tests following ANOVA ($^{*}P < 0.05$ versus vehicle/8-OH-DPAT). Initial baseline temperatures were comparable in all groups: vehicle/vehicle, 37.3 ± 0.45°C; vehicle/8-OH-DPAT, 37.1 ± 0.25°C; 1 mg/kg scopolamine/8-OH-DPAT, 37.0 ± 0.4°C; 3 mg/kg scopolamine/vehicle, 37.1 ± 0.2°C; 5 mg/kg scopolamine/8-OH-DPAT, 36.8 ± 0.3°C; 10 mg/kg scopolamine/8-OH-DPAT, 37.2 ± 0.35°C; 1 mg/kg NMS/8-OH-DPAT, 36.8 ± 0.2°C; 3 mg/kg NMS/vehicle, 36.9 ± 0.4°C; 5 mg/kg NMS/8-OH-DPAT, 36.7 ± 0.3°C; 10 mg/kg NMS/8-OH-DPAT, 36.9 ± 0.5°C.

duration of 60 min and a maximum change from the temperature at time (t) = 0 min of 3.1 ± 0.3°C ($P < 0.05$, $n = 6$, mean ± S.E.M.) 15–30 min post administration (Fig. 1). This response was significantly attenuated by the 5-HT_{1A} receptor antagonist WAY 100,635 (M.E.D. = 0.01 mg/kg s.c., Fig. 2). Administration of galanin (0.03–3 nmol/5 μl i.c.v.) under metofane anaesthesia did not affect rectal temperature at any dose tested (data not shown) but dose dependently blocked the hypothermia induced by 8-OH-DPAT with a M.E.D. of 1 nmol (Fig. 3).

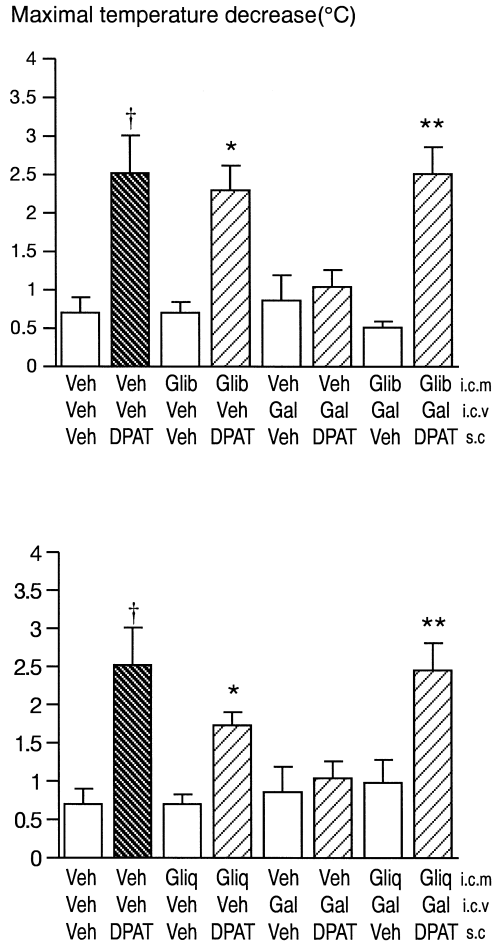

Fig. 5. (a) Effect of glibenclamide (Glib, 10 nmol/5 μl i.c.m.) on galanin (Gal, 3 nmol/5 μl i.c.v.) inhibition of 8-OH-DPAT induced hypothermia in mice. Results are expressed as mean ± S.E.M. of the maximal change in temperature (°C) from the temperature at $t = 0$; minimum $n = 6$ per group. Significant differences were determined by Tukey tests following ANOVA ($^{\dagger}P < 0.05$ versus vehicle/vehicle/vehicle; $^{*}P < 0.05$ versus glibenclamide/vehicle/vehicle; $^{**}P < 0.05$ versus glibenclamide/galanin/vehicle. Initial baseline temperatures were comparable in all groups: vehicle/vehicle/vehicle, 37.0 ± 0.1°C; vehicle/vehicle/8-OH-DPAT, 36.9 ± 0.3°C; glibenclamide/vehicle/vehicle, 37.3 ± 0.2°C; glibenclamide/vehicle/8-OH-DPAT, 36.7 ± 0.4°C; vehicle/galanin/vehicle, 37.0 ± 0.1°C; vehicle/galanin/8-OH-DPAT, 37.4 ± 0.3°C; glibenclamide/galanin/vehicle, 37.0 ± 0.2°C; glibenclamide/galanin/8-OH-DPAT, 37.2 ± 0.3°C. (b) Effect of gliquidone (Gliq, 10 nmol/5 μl i.c.m.) on galanin (Gal, 3 nmol/5 μl i.c.v.) inhibition of 8-OH-DPAT induced hypothermia in mice. Results are expressed as mean ± S.E.M. of the maximal change in temperature (°C) from the temperature at $t = 0$; minimum $n = 6$ per group. Significant differences were determined by Tukey tests following ANOVA ($^{\dagger}P < 0.05$ versus vehicle/vehicle/vehicle; $^{*}P < 0.05$ versus gliquidone/vehicle/vehicle; $^{**}P < 0.05$ versus gliquidone/galanin/vehicle. Initial baseline temperatures were comparable in all groups: vehicle/vehicle/vehicle, 37.0 ± 0.1°C; vehicle/vehicle/8-OH-DPAT, 36.9 ± 0.3°C; gliquidone/vehicle/vehicle, 36.8 ± 0.4°C; gliquidone/vehicle/8-OH-DPAT, 37.5 ± 0.6°C; vehicle/galanin/vehicle, 37.0 ± 0.1°C; vehicle/galanin/8-OH-DPAT, 37.4 ± 0.3°C; gliquidone/galanin/vehicle, 36.8 ± 0.1°C; gliquidone/galanin/8-OH-DPAT, 37.0 ± 0.5°C.

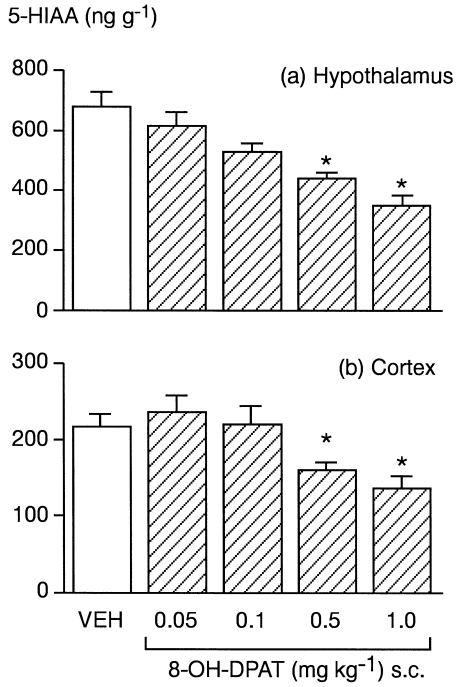

Fig. 6. Effect of 8-OH-DPAT (0.05, 0.1, 0.5, 1 mg/kg s.c.) on (a) hypothalamic and (b) cortical 5-HIAA concentration 30 min after administration. Values are mean ± S.E.M. $n = 5/6$ per group. * $P < 0.05$ compared with saline treated animals by ANOVA followed by Tukey's test.

Metofane anaesthesia itself did not significantly alter rectal temperature (data not shown). The inhibitory effect of galanin on 8-OH-DPAT induced hypothermia was blocked by the galanin receptor antagonist galantide (0.3 nmol i.c.m., Fig. 3) which also had no effect on body temperature (data not shown). The hypothermic response to 8-OH-DPAT was blocked by the centrally acting muscarinic receptor antagonist scopolamine (10 mg/kg i.p., Fig. 4a) but not by pretreatment with the poorly brain penetrant muscarinic receptor antagonist *N*-methylscopolamine (10 mg/kg i.p., Fig. 4b).

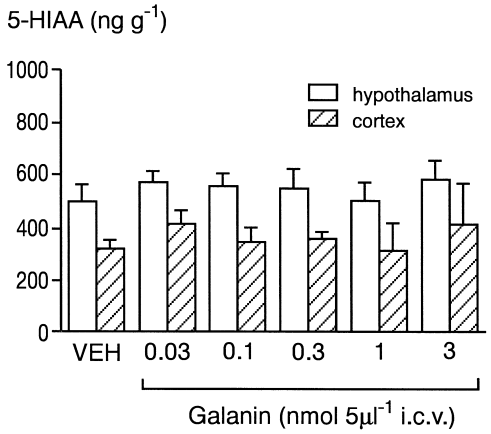

Fig. 7. Effect of galanin (0.03, 0.1, 0.3, 1, 3 nmol/5 µl i.c.v.) on cortical and hypothalamic 5-HIAA concentration 30 min after administration. Values are mean ± S.E.M. $n = 6$ per group.

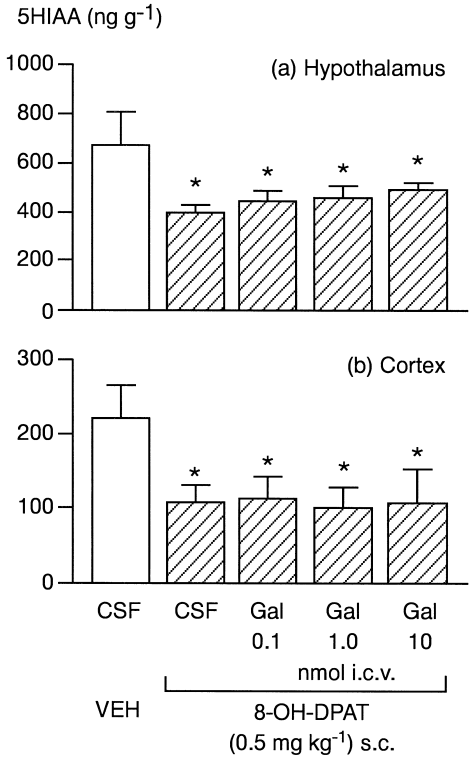

Fig. 8. Effect of galanin (0.1, 1, 10 nmol/5 µl i.c.v.) pretreatment on the reduction of 5-HIAA concentration by 8-OH-DPAT (0.5 mg/kg s.c.). Values are mean ± S.E.M. $n = 6$ per group. (* $P < 0.05$ compared with vehicle controls by ANOVA followed by Tukey's test).

3.2. The effect of glibenclamide and gliquidone on the inhibition of 8-OH-DPAT induced hypothermia by galanin)

The ATP-sensitive K^+ channel blockers glibenclamide (10 nmol) and gliquidone (10 nmol) attenuated the inhibition of 8-OH-DPAT (0.5 mg/kg s.c.) induced hypothermia by galanin (3 nmol i.c.v., Fig. 5a and 5b respectively) without affecting rectal temperature per se (data not shown).

Table 1
Saturation analysis of [^{3}H]8-OH-DPAT binding to mouse cerebral cortical membranes

Treatment (10 min, 37°C)	K_d (nM)	B_{max} (fmol/mg)
Control [a]	1.3 (1.3; 1.4)	190 ± 18
+ 10 nM galanin	1.5 (1.3; 1.7)	200 ± 37
+ 1000 nM galanin	1.5 (1.3; 1.7)	200 ± 37

Affinity constants (K_d) are expressed as geometric means. Numbers in parentheses indicate low and high error values of this mean. Maximum binding capacities (B_{max}) are expressed as arithmetic means ± S.E.M. Means are obtained from four separate experiments carried out in triplicate.

[a] Control membranes were pretreated with 50 mM Tris-HCl, pH 7.7 containing 10 µM pargyline, 0.1% ascorbate, 0.1% bovine serum albumin, 1 µM bacitracin and 5.7 mM $CaCl_2$, for 10 min at 37°C.

Table 2
5-HT ligand displacement of 0.3 nM [^{3}H]8-OH-DPAT from mouse cerebral cortical membranes pretreated with galanin

5-HT receptor ligand	K_i (nM)		
	Control [a]	+10 nM galanin	+1000 nM galanin
8-OH-DPAT	0.37 (0.18; 0.75)	0.41 (0.10; 1.6)	0.26 (0.13; 0.50)
Buspirone	3.6 (2.3; 5.7)	2.1 (1.3; 3.4)	2.4 (1.3; 4.4)
WAY 100,135	7.9 (7.7; 8.2)	19 (8.0; 30)	6.8 (5.4; 8.4)
WAY 100,635	1.0 (0.35; 3.5)	0.71 (0.49; 1.0)	0.50 (0.30; 0.81)

Results are expressed as apparent inhibition constants (K_i, nM) corrected for ligand occupancy using the Cheng-Prusoff equation (Cheng and Prusoff, 1973). Each value is a geometric mean from four separate experiments, and each curve obtained from 7–8 concentrations performed in triplicate. Numbers in parentheses indicate low and high errors of the geometric mean.

[a] Control membranes were pretreated with 50 mM Tris-HCl, pH 7.7 containing 10 μM pargyline, 0.1% ascorbate, 0.1% bovine serum albumin, 1 μM bacitracin and 5.7 mM $CaCl_2$, for 10 min at 37°C.

3.3. Effect of galanin on 8-OH-DPAT induced decrease of brain 5-HT metabolism

8-OH-DPAT (0.05–1 mg/kg s.c.) dose dependently decreased both hypothalamic and cortical 5-HIAA concentration (M.E.D. = 0.5 mg/kg s.c., Fig. 6) without affecting 5-HT concentration (data not shown). Administration of galanin (0.03–3 nmol i.c.v.) did not significantly affect cortical or hypothalamic 5-HIAA concentration (Fig. 7). Furthermore, pretreatment of animals with galanin (0.1–10 nmol i.c.v.) did not prevent the decrease of cortical or hypothalamic 5-HIAA concentration by 8-OH-DPAT (0.5 mg/kg s.c., Fig. 8).

3.4. Effect of galanin on 5-HT$_{1A}$ receptor binding in mouse cortex

[^{3}H]8-OH-DPAT bound to control and galanin-pretreated mouse cerebral cortical membranes with high affinity and in a saturable manner over the range 0.1–15 nM (data not shown). Scatchard analysis gave a dissociation constant (K_D) of 1.3 (1.3; 1.4) nM (geometric mean ± low and high errors, $n = 4$), and a maximum binding capacity (B_{max}) of 190 ± 18 fmol/mg protein (mean ± S.E.M., $n = 4$). Pretreatment of brain membranes (as described by Fuxe et al., 1988b) with galanin (10 or 1000 nM) in vitro did not affect either the K_D or B_{max} of [^{3}H]8-OH-DPAT binding to mouse cortex (Table 1). This pretreatment also did not affect the affinity of the 5-HT receptor agonists 8-OH-DPAT and buspirone, or the 5-HT$_{1A}$ receptor antagonists WAY 100,135 and WAY 100,635 for the mouse cortical 5-HT$_{1A}$ receptor (Table 2). Furthermore, galanin did not displace [^{3}H]8-OH-DPAT binding to mouse cortical membranes (0% inhibition at 10 μM, $n = 3$).

4. Discussion

Results in the present study demonstrate that central administration of galanin dose dependently attenuated the hypothermic response of the 5-HT$_{1A}$ receptor agonist 8-OH-DPAT in mice. Pretreatment of mice with galantide, a selective galanin receptor antagonist (Bartfai et al., 1991), prevented the inhibitory effect of galanin on 8-OH-DPAT induced hypothermia providing evidence for the involvement of central galanin receptors in this response. In confirmation of previous findings (Patel and Hutson, 1994) neither galanin nor galantide significantly affected body temperature per se suggesting that galanin is not tonically involved in thermoregulation in the mouse.

The hypothermic effect of 8-OH-DPAT and other 5-HT$_{1A}$ receptor agonists has been described in both rat (Hjorth, 1985; Hutson et al., 1987a; Bill et al., 1991) and mouse (Goodwin et al., 1985; Martin et al., 1992) and results in the present study with WAY 100635, the potent and selective 5-HT$_{1A}$ receptor antagonist (Fletcher et al., 1994), confirm previous suggestions that this effect is mediated via 5-HT$_{1A}$ receptors, although there may be an involvement of central α_2-adrenoceptors (Durcan et al., 1991). 8-OH-DPAT has recently been shown to have appreciable affinity for cloned rat, human and to a lesser extent mouse 5-HT$_7$ receptors (see Boess and Martin, 1994 for review). The central physiological function(s) of this 5-HT receptor subtype are not fully understood at present and it could account for some of the responses to 8-OH-DPAT. However, it is unlikely to be involved in the hypothermic effect of 8-OH-DPAT since this was completely blocked by WAY 100,635 which has no appreciable affinity (30% inhibition at 1 μM) at the cloned human 5-HT$_7$ receptor (G. McAllister, personal communication). Whether 8-OH-DPAT induced hypothermia is mediated at pre- or postsynaptically located 5-HT$_{1A}$ receptors is controversial and may involve species differences (Bill et al., 1991; Martin et al., 1992).

8-OH-DPAT inhibits 5-HT neuronal firing (Sprouse and Aghajanian, 1986) causing a decrease of 5-HT synthesis and metabolism (Hjorth et al., 1982; Hutson et al., 1987b,1989) and a comparable reduction of 5-HT release in forebrain regions (Hutson et al., 1989). This is probably due to the activation of somatodendritic 5-HT$_{1A}$ autoreceptors in the raphe nuclei as there is little evidence to involve postsynaptically located 5-HT$_{1A}$ receptors in the regulation of 5-HT metabolism. It is conceivable therefore that if the hypothermic effect of 8-OH-DPAT is mediated presynaptically by activating somatodendritic autoreceptors and thereby reducing 5-HT availability then galanin may act to attenuate 8-OH-DPAT hypothermia by blocking its inhibitory action on 5-HT release and metabolism. The reported effects of galanin on brain 5-HT metabolism are inconsistent, with studies showing both excitatory (Sundstrom and Melander, 1988) and inhibitory effects (Fuxe et al., 1988a). In the present study, central administration of galanin at doses which blocked 8-OH-DPAT induced hypothermia had no effect on mouse brain 5-HT metabolism, as indicated by the concentration of 5-HIAA in either the cortex or hypothalamus. Neither did galanin

significantly affect the decrease of cortical or hypothalamic 5-HT metabolism by 8-OH-DPAT. Furthermore, we were unable to demonstrate that pretreatment of mouse cortical membranes with galanin (10 or 1000 nM) in vitro significantly affected the affinity, receptor density (B_{max}) or the pharmacology of the mouse 5-HT_{1A} receptor as labelled with [^{3}H]8-OH-DPAT. Moreover, galanin did not displace [^{3}H]8-OH-DPAT binding to the mouse cortical 5-HT_{1A} receptor to any great extent indicating that it has negligible affinity for this site. However, it must be remembered that 5-HT_{1A} receptors in the cortex are probably not involved in the hypothermic effects of 8-OH-DPAT and may behave differently, in the presence of galanin, to 5-HT_{1A} receptors in more appropriate brain regions. It is unlikely that the lack of effect in the present study is due to the degradation of galanin as it is known to be stable for up to 2 h at 37°C in the cerebrospinal fluid and in membrane preparations from the spinal cord of rodents (Bedecs et al., 1995).

There is a lack of concordance between results not only in the present study where we were unable to observe any effects of either galanin on 5-HT_{1A} receptor affinity and pharmacology, but also between those of Fuxe et al. (1988b) who showed that galanin decreased 5-HT_{1A} receptor affinity in rat ventral limbic cortex and a recent study by Hedlund et al. (1994) where 5-HT_{1A} receptor affinity in dorsal hippocampus was increased by a galanin fragment, galanin-(1–15) yet unaffected by galanin-(1–29). Thus it is clear that a number of variables including species, brain regions and potential galanin receptor subtypes as defined by sensitivity to galanin-(1–29) or galanin-(1–15) (Hedlund et al., 1992) may account for some of these discrepancies. Nevertheless, all aspects of the present study were conducted in mice and the effects of 8-OH-DPAT on brain 5-HT metabolism have been shown to be comparable in different brain regions (Hjorth et al., 1982; Hutson et al., 1987b). Consequently, the mechanism by which galanin attenuates 8-OH-DPAT mediated hypothermia in the mouse does not appear to involve modulating the decrease of forebrain 5-HT metabolism by 8-OH-DPAT. Neither is there evidence in the present study to indicate a direct interaction with or modification of cortical 5-HT_{1A} receptor affinity, density or pharmacology by galanin. It is also unlikely that galanin's effects are mediated by affecting the coupling of 5-HT_{1A} receptors with adenylyl cyclase (Billecocq et al., 1994). Taken together, these results suggest that the inhibitory effect of galanin on 8-OH-DPAT induced hypothermia is probably mediated subsequent to the activation of 5-HT_{1A} receptors and inhibition of serotonin release.

It is conceivable that central cholinergic mechanisms are involved in this response. Thus facilitation of cholinergic transmission by the muscarinic receptor agonist RS86 (2-ethyl 8-methyl-2,8 diazospiro (4,5-decan-1,3-dion hydrobromide) and the acetylcholinesterase inhibitors physostigmine and tetrahydroaminoacridine causes hypothermia which is blocked by centrally acting muscarinic receptor antagonists (Freedman et al., 1989; Patel and Hutson, 1994). 8-OH-DPAT has been shown to increase acetylcholine release in the cortex (Siniscalchi et al., 1990) and hippocampus (Izumi et al., 1994; Wilkinson et al., 1994) of rats and guinea-pigs although this has not been demonstrated in the mouse. Consistent with this suggestion, results in the present study show that 8-OH-DPAT induced hypothermia was attenuated by pretreatment with the muscarinic receptor antagonist scopolamine, but not the poorly brain penetrant analogue *N*-methylscopolamine, at a dose similar to that previously shown to block RS86 induced hypothermia (Patel and Hutson, 1994), suggesting that this effect of 8-OH-DPAT is mediated, at least in part, by muscarinic receptors. Several studies have shown that galanin inhibits pre- and postsynaptic cholinergic function. Thus, galanin blocks scopolamine and potassium evoked acetylcholine release in vitro and in vivo (Fisone et al., 1987, 1991; Consolo et al., 1991), inhibits muscarinic agonist induced phosphoinositide turnover (Consolo et al., 1991; Fisone et al., 1991; Palazzi et al., 1988, 1991) and attenuates muscarinic receptor agonist and acetylcholinesterase inhibitor induced hypothermia and phosphoinositol turnover via ATP-sensitive K^+ channels (Patel and Hutson, 1994). Interestingly, the inhibitory effects of galanin on 8-OH-DPAT induced hypothermia were also sensitive to ATP-sensitive K^+ channel blockers. Therefore, it is conceivable that 8-OH-DPAT induced hypothermia in the mouse is mediated by enhanced central cholinergic transmission which is attenuated by galanin acting presynaptically to inhibit acetylcholine release and/or postsynaptically by decreasing the interaction of acetylcholine with muscarinic receptors.

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