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Inverse Agonists and Serotonergic Transmission: From Recombinant, Human Serotonin (5-HT)$_{1B}$ Receptors to G-Protein Coupling and Function in Corticolimbic Structures in vivo

M. J. Millan, Ph.D., A. Gobert, Ph.D., V. Audinot, Ph.D., A. Dekeyne, Ph.D., and A. Newman-Tancredi, Ph.D.

The concept of inverse agonism, whereby "antagonists" exert actions opposite to those of agonists at constitutively active receptors, has been documented both at receptor-modulated ion channels as well as at G-protein-coupled receptors (GPCR) in recombinant expression systems. However, it remains unclear whether physiologically or therapeutically relevant inverse agonists actions at GPCRs occur in the CNS in vivo. The present overview discusses our recent observations concerning 5-HT$_{1B}$ receptors, and focusses on the relationship between actions at heterologous Chinese hamster ovary (CHO) expression systems compared with native CNS populations of receptors. To this end, we have exploited several novel and selective ligands, notably the inverse agonist and neutral antagonist at 5-HT$_{1B}$ receptors, SB224,289 and S18127, respectively. Like 5-HT itself, the agonist, GR46611, markedly increases the binding of [^{35}S]-GTPγS binding to h5-HT$_{1B}$ receptors expressed in CHO cells, while the "antagonist", GR127,935, modestly stimulates binding suggesting partial agonist properties. However, SB224,289 markedly suppresses binding at these sites. S18127, which does not alter [^{35}S]GTPγS binding alone, abolishes the actions of both GR46611 and SB224,289. Nevertheless, in quantitative autoradiographical studies, S18127 and SB224,289 cannot be distinguished as concerns modulation of [^{35}S]-GTPγS binding at substantia nigra and caudate nucleus-localized 5-HT$_{1B}$ receptors, inasmuch as they each block the action of the 5-HT$_{1B}$ agonist, CP93129, yet fail to modify binding alone. Further, S18217 and SB224,289, as well as GR127,935, all abolish the inhibitory influence of GR46611 upon dialysis levels of 5-HT in the frontal cortex of freely moving rats without themselves modifying release. Moreover, they all block the hypothermic actions of GR46611 without themselves modifying core temperature. Thus, differences in intrinsic ativity of S18127, SB224,289 and GR127,935 seen at cloned, h5-HT$_{1B}$ receptors cannot be detected in vivo. Most notably, no evidence for opposite actions of the inverse agonist, SB224,289, as compared to 5-HT$_{1B}$ agonists is apparent. These data suggest that in vitro observations of inverse agonist actions cannot necessarily be extrapolated to intact systems in vivo.
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From the Institut de Recherches Servier, Centre de Recherches de Croissy, Psychopharmacology Department, Croissy-sur-Seine, Paris, France. Tel: 33.01.41.18.24.25; Fax: 33.01.41.18.24.70.

Address correspondence to: M. J. Millan, Institut de Recherches Servier, Centre de Recherches de Croissy, Psychopharmacology Department, 125, Chemin de Ronde, 78290-Croissy-sur-Seine, Paris, France.

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Classical theories of G-protein-coupled receptor (GPCR) activation assume that functional responses are induced by the action of agonists at otherwise quiescent receptors (Milligan et al. 1995). Accordingly, the induction of an active state of the receptor, capable of stimulating signal transduction pathways, is considered to depend upon the presence of an agonist. Antagonists exert no effects themselves on the state of receptor activation, and merely interfere with the actions of agonists. However, over the past two decades, several observations have accumulated to suggest that certain "antagonists" may act in a fashion opposite to agonists. One early observation was that lesions of dopaminergic pathways and dopaminergic antagonists can induce additive effects on striatal levels of acetylcholine (Fibiger and Grewaal 1974). Nevertheless, in this study, it could not be excluded that antagonists might be blocking the actions of residual pools of dopamine. Creese et al. (1975) reported that agonists had higher affinity for [^{3}H]-dopamine-labelled dopamine receptors whereas antagonists had greater affinity for [^{3}H]-haloperidol-labelled sites. Further, at muscarinic receptors labelled with [^{3}H]-quinuclidyl benzylate, Burgisser et al. (1982) proposed that antagonists have higher affinity for receptors uncoupled from G-proteins. Costa and Herz (1989) proposed that certain antagonists act as "inverse agonists" at δ-opioid receptors endogenously expressed by NG 108-15 cells . Indeed, whereas the agonist, DADLE, increased GTPase activity at these δ-opioid receptors, the "antagonist," ICI174864, inhibited basal activity. Both actions were blocked by a neutral antagonist. Although agonists and antagonists are classically considered to down- and up-regulate receptors, respectively, the above observations led to the suggestion (Morris and Millan 1991) that "inverse agonist" rather than "antagonist" properties may favor receptor up-regulation in vitro and/or in vivo (Milligan and Bond 1997; Smit et al. 1996).

More recent studies employing homogeneous populations of cloned (wild-type) receptors in heterologous expression systems free of endogenous agonist have underpinned the concept of "negative intrinsic efficacy" and "inverse agonism" (see Costa et al. 1992; Kenakin 1996; Milligan et al. 1995; Schütz and Freissmuth 1992). Thus, many types of wild-type GPCR have been observed to display an agonist-independent constitutive activation of intracellular transduction mechanisms inhibited by inverse agonists. In contrast, genuinely neutral antagonists block the actions of both agonists and inverse agonists, without themselves altering activity.

EXPERIMENTAL STRATEGY: CHARACTERIZATION OF POTENTIAL INVERSE AGONIST ACTIONS AT 5-HT$_{1B}$ RECEPTORS

Several types of recombinant, heterologously-expressed 5-HT receptor (5-HT$_{1A}$, 5-HT$_{1B}$, 5-HT$_{1D}$, 5-HT$_{2A}$, 5-HT$_{2C}$, and 5-HT$_7$) display constitutive activity and, correspondingly, reveal inverse agonist properties of certain antagonists (Barker et al. 1994; Egan et al. 1998; Newman-Tancredi et al. 1997b; Thomas et al. 1995, 1998). The 5-HT$_{1A}$, 5-HT$_{1B}$, and 5-HT$_{1D}$ receptors are of particular interest inasmuch as they are localized both postsynaptically to serotonergic neurones as well as presynaptically, as inhibitory autoreceptors on their dendrites (5-HT$_{1A}$ and a minor population of 5-HT$_{1B/1D}$ sites) and terminals (5-HT$_{1B}$ sites) (Piñeyro et al. 1995; Starkey and Skingle 1994). It might, thus, be hypothesized that inverse agonist actions at 5-HT$_{1A}$, 5-HT$_{1B}$, and/or 5-HT$_{1D}$ autoreceptors may facilitate serotonergic transmission (Gaster et al. 1998; Moret and Briley 1993; Roberts et al. 1997). In addition, postsynaptic structures enriched in these receptor types, such as the substantia nigra and striatum (5-HT$_{1B}$ receptors), are also potential targets for the detection of inverse agonist actions (Boess and Martin 1994).

However, while constitutive activity may be readibly detectable in recombinant systems in vitro, it remains to be determined whether CNS populations of 5-HT receptors (and other classes of GPCR) display constitutive activity in situ under normal and/or pathological conditions. It is also unclear whether drugs express physiologically and therapeutically relevant inverse agonist properties at CNS populations of receptors in vivo. The present commentary summarizes the findings from several complementary approaches which we have utilized to examine the potential existence of inverse agonist actions at 5-HT$_{1B}$ receptors in vitro and in vivo. First, we examine binding of the GTP analogue, [^{35}S]-GTPγS, to G-proteins acti-

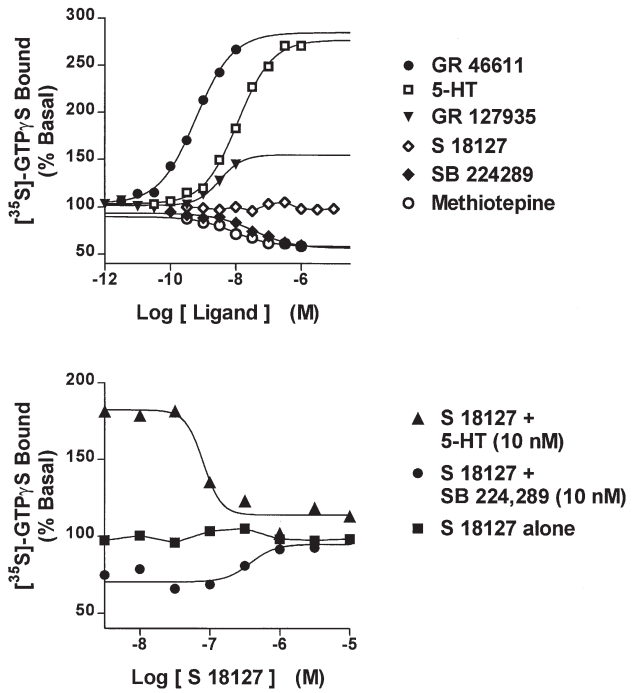

Figure 1. Influence of 5-HT$_{1B}$ ligands upon [^{35}S]GTPγS binding to CHO-h5-HT$_{1B}$ receptors.

vated by recombinant h5-HT_{1B} receptors expressed in CHO cells. Whereas agonists increase [^{35}S]-GTPγS binding, inverse agonists inhibit the basal [^{35}S]-GTPγS binding observed in the absence of ligand(s) (Newman-Tancredi et al. 1997b; Thomas et al. 1995). Second, we discuss the binding of [^{35}S]-GTPγS to native rat brain h5-HT_{1B} receptors visualized by quantitative autoradiography (Sim et al. 1995). Third, we consider the modulation of extracellular 5-HT levels as determined by dialysis of the FCX of freely moving rats (Gobert et al. 1998). Fourth, we evaluate the induction of hypothermia in the guinea pig (Hagan et al. 1997; Skingle et al. 1996).

Cloned h5-HT_{1B} Receptors: G-Protein Activation Quantified by [^{35}S]-GTPγS Binding

The influence of ligands upon [^{35}S]-GTPγS binding at h5-HT_{1B} receptors was evaluated using essentially the same procedure as described for h5-HT_{1A} receptors (Newman-Tancredi et al. 1997a). 5-HT and GR46611 behaved as agonists while GR127,935, originally described as an antagonist (Skingle et al. 1996), displayed partial agonist actions (Price et al. 1996; Watson et al. 1996). The non-selective 5-HT_1 receptor antagonist, methiothepin and the selective antagonist, SB224,289 (Gaster et al. 1998), inhibited basal activation, suggestive of inverse agonist actions at 5-HT_{1B} receptors. Importantly, the novel and selective 5-HT_{1B} ligand, S18127 (Millan et al. 1998), behaved as a neutral antagonist inasmuch as it was inactive alone and it blocked the actions of both GR46611 and SB224,289 (Figure 1).

Native Rat 5-HT_{1B} Receptors: Functional Autoradiography of G-Protein Activation by [^{35}S]-GTPγS Binding to Cerebral Tissue Sections

In the substantia nigra, employing a procedure based on that detailed by Sim et al. (1995), a pronounced stimula-

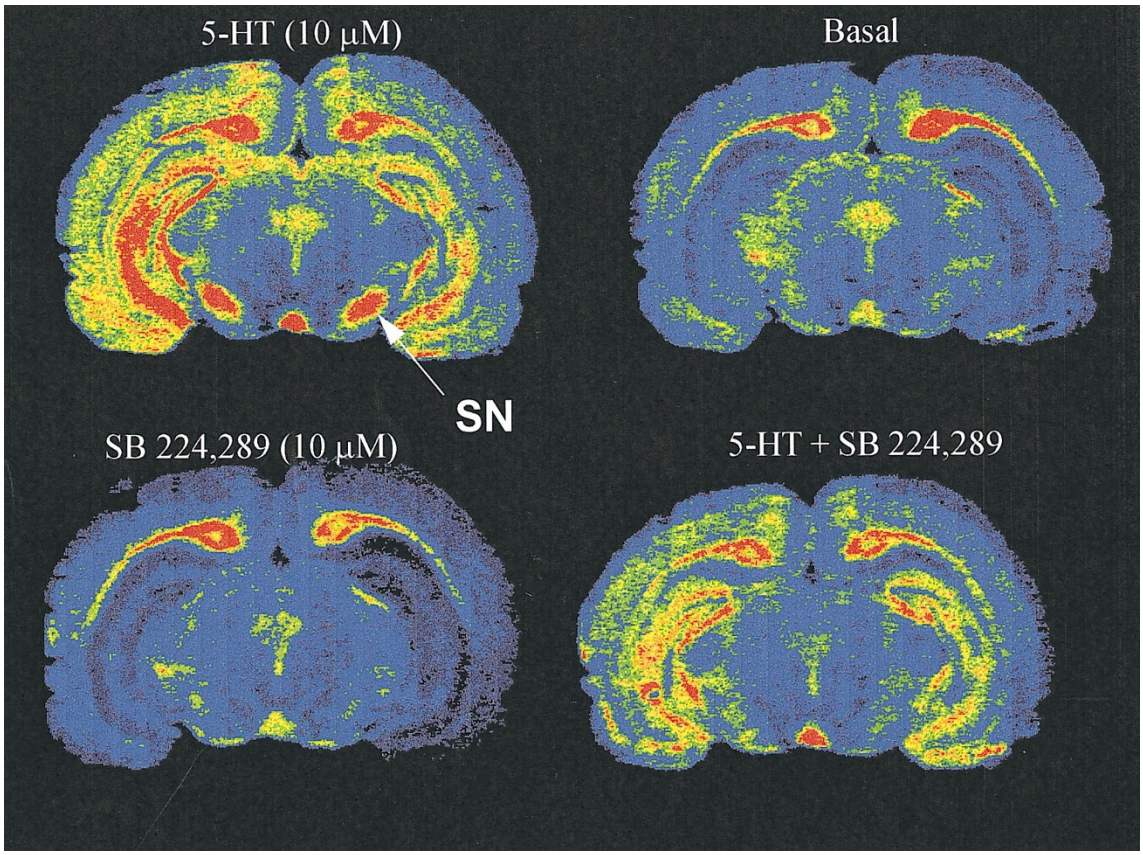

Figure 2. Influence of 5-HT_{1B} ligands upon [^{35}S]GTPγS binding to substantia nigra in situ. Upper left panel: Binding of [^{35}S]GTPγS in the presence of 5-HT (10 μM) which activates 5-HT_{1B} receptor-coupled G-proteins. Upper right panel: Basal binding observed in the absence of ligand. Lower left panel: Binding of [^{35}S]GTPγS in the presence of SB224,289 (10 μM). No difference from basal values was observed. Lower right panel: Binding of [^{35}S]GTPγS in the presence of 5-HT (10 μM) and SB224,289 (10 μM). SB224,289 blocked the activation induced by 5-HT. Images are pseudo-color representations of [^{35}S]GTPγS labelling observed under the following conditions. Sections were incubated for 60 minutes at 37°C in a buffer containing HEPES 50 mm (pH 7.5); NaCl 150 mM, EGTA 0.2 mM, DTT 0.2 mM, GDP 2.5 mM, $MgCl_2$ 10 mM, and [^{35}S]GTPγS 0.05 nM. Sections were then rinsed and exposed to Hyperfilm. Calibration was according to commercially available C^{14} standards.

tion of [^{35}S]GTPγS was seen with the agonist at rat 5-HT$_{1B}$ receptors, CP93129 (10 μM, 70.5 ± 7.1%). The action of CP93129 was inhibited by S18127 and SB224,289 (10 μM, 12 ± 13% and 11.4 ± 5.9%, respectively). However, neither of these ligands modifed basal [^{35}S]-GTPγS binding alone. S18127 and SB224,289 alone: 4.4 ± 6.7% and 4.3 ± 4.3%, respectively. In a further experiment, shown in Figure 2, SB224,289 (10 μM) also abolished stimulation of [^{35}S]-GTPγS binding by 5-HT (10 μM) in substantia nigra. In contrast to the present study, in which the sections were extensively washed to ensure removal of endogenous 5-HT, Stanton et al. (1997) reported that, the non-selective ligand, methiothepin lowered basal [^{35}S]-GTPγS binding to rat brain sections. However, the present data concur with results on guinea pig brain sections where, similarly, no action of SB224,289 was detected on basal [^{35}S]-GTPγS binding to hippocampus (Dupuis et al. 1998).

Modulation of Serotonergic Transmission by 5-HT$_{1B}$ Autoreceptors

As reported in Gobert et al. (1998), GR46611 markedly suppresses dialysate levels of 5-HT in the FCX. Notwithstanding their contrasting actions at cloned h5-HT$_{1B}$ receptors (vide supra), GR127,935, S18127 and SB224,289 abolished the actions of GR46611 without themselves modifying 5-HT release (Figure 3). Because blockade of 5-HT$_{1B}$ autoreceptors may result in compensatory activation of 5-HT$_{1A}$ autoreceptors by 5-HT, the actions of SB224,289 and GR127,935 were re-determined in the presence of the 5-HT$_{1A}$ antagonist, WAY100,635, but they still failed to enhance 5-HT levels (Figure 3). GR127,935, S18127, and SB224,289 likewise all facilitated the increase in FCX levels of 5-HT elicited by fluoxetine. These actions of WAY100,635 and SB224,289 were exerted synergistically.

Modulation of Core Temperature

It has been shown that activation of 5-HT$_{1B}$ receptors elicits hypothermia in guinea pigs (Hagan et al. 1997). Accordingly, GR46611 decreased core temperature in guinea pigs, and this action was inhibited by GR127,935, S18127 and SB224,289 (Figure 4). However, administered in the absence of GR 46611, none of these drugs modified core temperature (Hagan et al. 1997) (Figure 4).

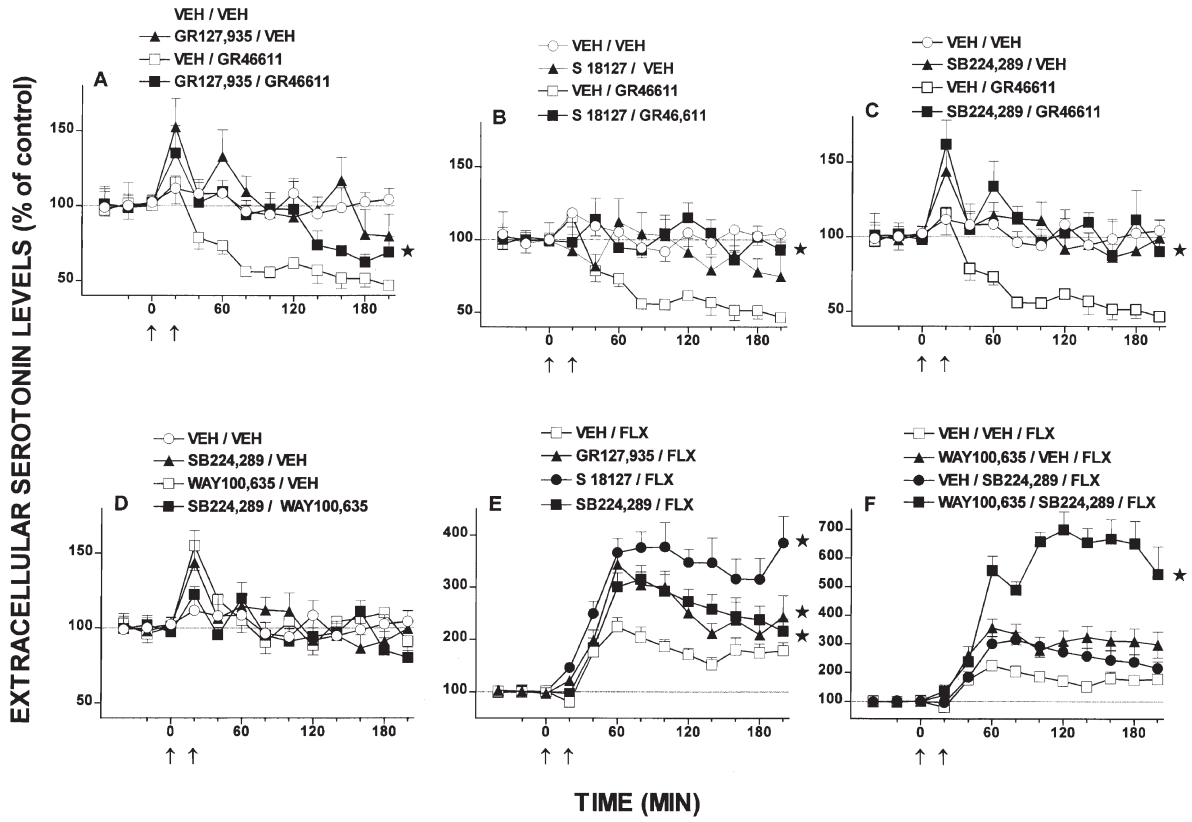

Figure 3. Influence of 5-HT$_{1B}$ ligands upon extracellular, dialysate 5-HT levels in frontal cortex of freely moving rats. n = 5–8 per group. Data are means ± SEM. Asterisks ($p < .05$) indicate significance in ANOVA of following differences; **(A)** GR127,935/GR46611 vs. vehicle/GR46611; **(B)** S18127/GR46611 vs. vehicle/GR46611; **(C)** SB224,289/GR46611 vs. vehicle/GR46611; **(D)** no statistical differences; **(E)** GR127,935/ FLX, S18127/FLX and SB224,289/FLX vs. vehicle/FLX; and **(F)** WAY100,635/ SB224,289/FLX vs. vehicle/vehicle/FLX. 5-HT levels were determined by HPLC followed by colormetric detection. Drugs were given SC. In F, vehicle/vehicle, WAY100,635/vehicle, vehicle/SB224,289 and WAY100,635/ SB224,289 combinations were injected at arrow 1, followed by fluoxetine at arrow 2.

GENERAL DISCUSSION: ABSENCE OF INVERSE AGONIST ACTIONS IN VIVO

In agreement with previous reports (Gaster et al. 1998; Pauwels et al. 1998; Thomas et al. 1995), the present study detected negative intrinsic activity actions of both methiothepin and the selective inverse agonist, SB224,289, at h5-HT_{1B} receptors in vitro. Further, the discovery of a selective neutral antagonist, S18127 (Millan et al. 1998), permits an examination of this issue both in vitro and in vivo. However, although S18127 abolished the actions of SB224,289 in CHO cells, no evidence was obtained for inverse agonist actions of SB224,289 at native 5-HT_{1B} receptors in vivo. Indeed, although SB224,289 slightly increased resting 5-HT levels in the guinea pig dentate gyrus in vitro (Gaster et al. 1998), this effect could not unambiguously be attributed to inverse agonism rather than interruption of the actions of spontaneously-released 5-HT. Further, no evidence that SB224,289 increases dialysate levels of 5-HT was obtained herein. There are several possible explanations for the absence of evidence for inverse agonist actions in vivo.

First, SB224,289 may be metabolized in vivo to structurally related neutral antagonists. However, this is unlikely by the SC route and does not account for the lack of suppression of [^{35}S]-GTPγS binding in autoradiographic studies. Second, methiothepin and SB224,289 may act as inverse agonists at recombinant human 5-$HT_{1B/1D}$ receptors but not at their native rat and guinea pig counterparts. Indeed, marked species differences have been documented for the pharmacological profiles of 5-HT_{1B} receptors, reflecting changes as minimal as a single amino acid substitution (Boess and Martin 1994; Price et al. 1996). However, in a direct comparison of recombinant guinea pig and human 5-HT_{1B} receptors, Pauwels et al. (1998) showed that SB224,289 and methiothepin acted as inverse agonists in each case. Third, the intracellular and extracellular enviroment plays an important role in determining ligand-receptor–G-protein interrelationships (Kenakin 1996). For example, the precise complement of G-protein subtypes, protein kinases and other substrates differs between transfected cell lines and native neurones. Thus, conditions used to demonstrate inverse agonist activity in vitro by [^{35}S]-GTPγS binding may not be present in the CNS. Fourth, the efficacy of agonists and inverse agonists is a function of receptor and G-protein expression levels and we have shown that, in CHO-h5-HT_{1A} cell membranes, inverse agonist activity can be enhanced by an augmentation of receptor: G-protein ratios (Newman-Tancredi et al. 1997a). Thus, the receptor and/or G-protein level may be too low at native 5-HT_{1B} receptors to permit inverse agonist actions to be detected. However, modulation of 5-HT release reflects actions at autoreceptors which, in analogy to 5-HT_{1A} sites, likely show a high receptor reserve/density (Boess and Martin 1994). Fifth, the techniques employed may not be sufficiently sensitive to detect modest inverse agonist actions in vivo. This may be the case for the [^{35}S]-GTPγS autoradiography procedure, in which actions of SB224,289 upon 5-HT_{1B}-

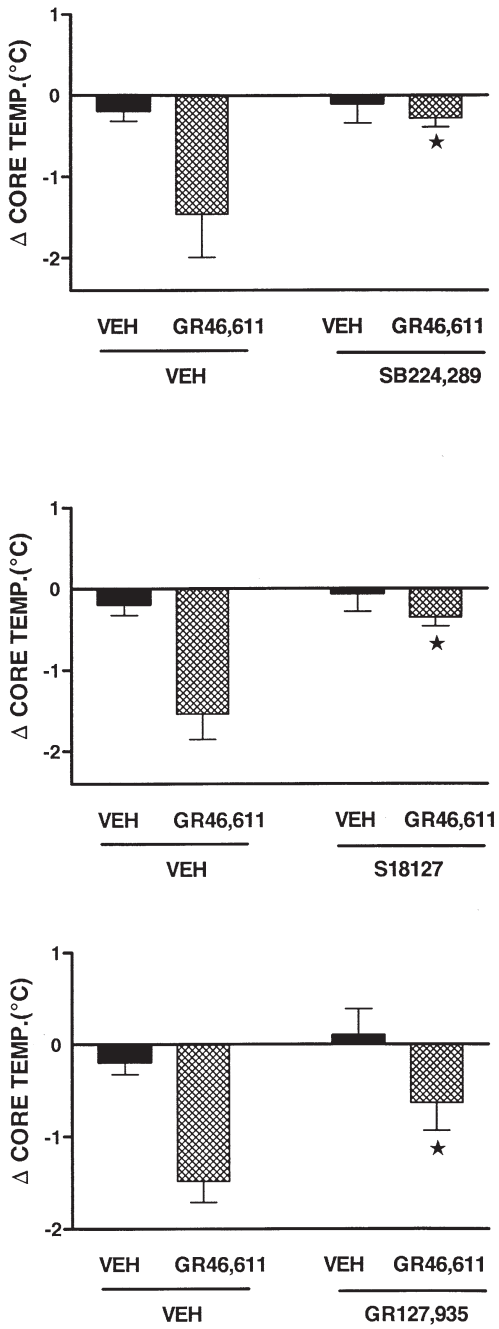

Figure 4. Influence of 5-HT_{1B} ligands upon core temperature in guinea pigs. Data are means ± SEM. n = 5–7 per group. *$p < .05$ to vehicle/GR46611 values in Newman-Keuls test. Note lack of significance of vehicle/SB224,289, vehicle/S18127, and vehicle/GR127,935 versus vehicle/vehicle values. Core temperature was determined via a digital thermistoprobe placed into the ear for 30 seconds. Vehicle or antagonist were given (SC) 60 minutes, and vehicle or GR46,611 (5.0 mg/kg, IP), 30 minutes prior to testing.

coupled G-proteins might be diluted by other pools of G-proteins. Sixth, compensatory mechanisms in vivo may mask inverse agonist actions. For example, core temperature is homeostatically controlled by multiple factors. Further, blockade of dendritic 5-HT_{1D} (or 5-HT_{1B}) sites may increase concentrations of 5-HT at colocalized 5-HT_{1A} autoreceptors, thereby masking this action (Starkey and Skingle 1994). However, combined administration of WAY100,635 with GR127,935 or SB224,289 for simultaneous blockade of 5-HT_{1A} and 5-$HT_{1B/1D}$ autoreceptors also failed to enhance dialysate 5-HT levels. Seventh, for inverse agonist actions to be detected, the receptor must be present in a constitutively active state and, ideally; endogenous agonist(s) should not be present. However, in vivo, 5-HT is spontaneously released both at the dendritic and terminal level (Piñeyro et al. 1995). Indeed, basal dialysate levels of 5-HT are reliably measurable, suggesting that 5-HT is available to pre- and postsynaptic receptors (Gobert et al. 1998). Thus, spontaneous release of 5-HT may impede the detection of inverse agonist actions. Indeed, as alluded to above, it is difficult to distinguish a neutral antagonist blockade of the actions of spontaneously released 5-HT from an inverse agonist action opposite to that of 5-HT. For this purpose, studies showing that neutral antagonists block inverse agonist actions are essential (Kenakin 1996; Newman-Tancredi et al. 1997a).

In the light of these comments, an evaluation of the potential inverse agonist actions of SB224,289 upon 5-HT_{1B} receptor would be of interest to perform in rats deprived of 5-HT. Indeed, such 5-HT-depleted conditions might resemble depressive states in which serotonergic transmission has been proposed to be deficient (Maes and Meltzer 1995). Finally, support for constitutively active receptors would be provided by the demonstration that inverse agonists elicit actions which are not evoked by antisense probes, and which are not mimicked by effects observed in gene knock-out mice.

CONCLUSIONS

The above comments illustrate the difficulties faced in demonstrating inverse agonist actions at 5-HT_{1A} and 5-$HT_{1B/1D}$ receptors in vivo and little evidence has been forthcoming from other CNS-localized receptor types that inverse agonist effects of antagonists underlie their functional profiles of activity. For example, although the extrapyramidal actions of neuroleptics might reflect negative intrinsic activity at D_2 receptors, both clozapine (non cataleptogenic) and haloperidol (cataleptogenic) display inverse agonist properties at cloned hD_2 sites (Nilsson et al. 1996; Hall and Strange 1997). Nevertheless, the potential relevance of constitutive activity at GPRCs and of inverse agonist actions in vivo cannot be dismissed, as illustrated by a transgenic system of β_2-adrenoceptor overexpression (Bond et al. 1995). As emphasized above, the availability of a highly selective neutral antagonist and inverse agonist is imperative for the rigorous demonstration of inverse agonist actions in vitro and in vivo. The present article describes the availability of such ligands for 5-HT_{1B} sites and further studies will be required for a more precise evaluation of the concept of inverse agonism in vivo at diverse GPCRs. Irrespective of such considerations, the present study strongly suggests that extrapolation of data from in vitro expression models of cloned receptors should be made cautiously to intact physiological systems.

REFERENCES

Barker EL, Westphal RS, Schmidt D, Sanders-Bush E (1994): Constitutively active 5-hydroxytryptamine$_{2C}$ receptors reveal novel inverse agonist activity of receptor ligands. J Biol Chem 269:11687–11690

Bond RA, Leff P, Johnson TD, Milano CA, Rockman HA, McMinn TR, Apparsundaram S, Hyek MF, Kenakin TP, Allen LF, Lefkowitz RJ (1995): Physiological effects of inverse agonists in transgenic mice with myocardial overexpression of the β_2-adrenoceptor. Nature 374:272–274

Boess FG, Martin IL (1994): Molecular biology of 5-HT receptors. Neuropharmacology 33:275–317

Burgisser E, DeLean A, Lefkowitz RJ (1982): Reciprocal modulation of agonist and antagonist binding to muscarinic cholinergic receptors by guanine nucleotides. Proc Natl Acad Sci USA 79:1732–1736

Costa T, Herz A (1989): Antagonists with negative intrinsic activity at δ-opioid receptors coupled to GTP-binding proteins. Proc Natl Acad Sci USA 86:7321–7325

Costa T, Ogino Y, Munson PJ, Onaran HO, Rodbard D (1992): Drug efficacy at guanine nucleotide-binding regulatory protein-linked receptors: Thermodynamic interpretation of negative antagonism of receptor activity in the absence of ligands. Mol Pharmacol 41:549–560

Creese I, Burt DR, Snyder SH (1975): Dopamine receptor binding: Differentiation of agonist and antagonist states with [^{3}H]-dopamine and [^{3}H]-haloperidol. Life Sci 17: 993–1003

Dupuis DS, Palmier C, Colpaert FC, Pauwels PJ (1998): Autoradiography of serotonin 5-HT_{1A} receptor-activated G proteins in guinea pig sections by agonist-stimulated [^{35}S]-GTPγS binding. J Neurochem 70:1258–1268

Egan CT, Herrick-Davis K, Teitler M (1998): Creation of a constitutively activated state of the 5-hydroxytryptamine$_{2A}$ receptor by site-directed mutagenesis: Inverse agonist activity of antipsychotic drugs. J Pharmacol Exp Ther 286:85–90

Fibiger HC, Grewaal DS (1974): Neurochemical evidence for denervation supersensitivity: The effect of unilateral substantia nigra lesions on apomorphine-induced increases in neostriatal acetylcholine levels. Life Sci 15: 57–63

Gaster LM, Blaney FE, Davies S, Duckworth M, Ham P, Jenkins S, Jennings AJ, Joiner GF, King FD, Mulholland KR, Wyman PA, Hagan JJ, Hatcher J, Jones BJ, Middlemiss

DN, Price GW, Riley G, Roberts C, Routledge C, Selkirk J, Slade PD (1998): The selective 5-HT_{1B} receptor inverse agonist 1′-methyl-5-[[2′-methyl-4′-(5-methyl-1,2,4-oxadiazol-3-yl)biphenyl-4-yl]-2,3,6,7-tetrahydro-spiro[furo [2,3-f]indole-3,4′-piperidine] (SB-224289) potently blocks terminal 5-HT autoreceptor function both in vitro and in vivo. J Med Chem 41:1218–1235

Gobert A, Rivet J-M, Audinot V, Newman-Tancredi A, Cistarelli L, Millan MJ (1998): Simultaneous quantification of serotonin, dopamine and noradrenaline levels in single frontal cortex dialysates of freely-moving rats reveals a complex pattern of reciprocal auto- and hetero receptor-mediated control of release. Neuroscience 84:413–429

Hagan JJ, Slade PD, Gaster L, Jeffrey P, Hatcher JP, Middlemiss DN (1997): Stimulation of 5-HT_{1B} receptors causes hypothermia in the guinea pig. Eur J Pharmacol 331:169–174

Hall DA, Strange PG (1997): Evidence that antipsychotic drugs are inverse agonists at D_2 dopamine receptors. Br J Pharmacol 121:731–736

Kenakin T (1996): The classification of seven transmembrane receptors in recombinant expression systems. Pharmacol Rev 48:413–463

Maes M, Meltzer HY (1995): The serotonin hypothesis of major depression. In Bloom FE, Kupfer DJ (eds), Psychopharmacology: The Fourth Generation in Progress. pp 933–944. Raven Press, New York

Millan MJ, Audinot V, Newman-Tancredi A, Dekeyne A, Gobert A, Lochon S, Rivet J-M, Goument B, Peglion J-L (1998): S18127: A novel and selective, aminopiperidine antagonist at serotonin $(5\text{-HT})_{1B/1D}$ receptors. Am Soc Neurosci 24:1364

Milligan G, Bond RA, Lee M (1995): Inverse agonism: Pharmacological curiosity or potential therapeutic strategy? Trends Pharmacol Sci 16:10–13

Milligan G, Bond RA (1997): Inverse agonism and the regulation of receptor number. Trends Pharmacol Sci 18:468–474

Moret C, Briley M (1993): The unique effect of methiothepin on the terminal serotonin autoreceptor in the rat hypothalamus could be an example of inverse agonism. J Psychopharmacol 7:331–337

Morris BJ, Millan MJ (1991): Inability of an opioid antagonist lacking negative intrinsic activity to induce opioid receptor up-regulation in vivo. Br J Pharmacol 102:883–886

Newman-Tancredi A, Conte C, Chaput C, Spedding M, Millan MJ (1997a): Inhibition of the constitutive activity of human 5-HT_{1A} receptors by the inverse agonist, spiperone but not the neutral antagonist, WAY 100,635. Br J Pharmacol 120:737–739

Newman-Tancredi A, Conte C, Chaput C, Verriéle L, Millan MJ (1997b): Agonist and inverse agonist efficacy at human recombinant serotonin 5-HT_{1A} receptors as a function of receptor: G-protein stoichiometry. Neuropharmacology 36:451–459

Nilsson CL, Ekman A, Hellstrand M, Eriksson E (1996): Inverse agonism at dopamine D_2 receptors. Haloperidol-induced prolactin release from GH4C1 cells transfected with the human D2 receptor is antagonized by R(-)-n-propylnorapomorphine, raclopride and phenoxybenzamine. Neuropsychopharmacology 15:54–61

Pauwels PJ, Wurch T, Palmier C, Colpaert FC (1998): Pharmacological analysis of G-protein activation mediated by guinea-pig recombinant 5-HT_{1B} receptors in C6-glial cells: Similarities with the human 5-HT_{1B} receptor. Br J Pharmacol 123:51–62

Piñeyro G, de Montigny C, Blier P (1995): 5-HT_{1D} receptors regulate 5-HT release in the rat raphe nuclei: in vivo voltametry and in vitro superfusion studies. Neuropsychopharmacology 13:249–260

Price GW, Roberts C, Watson J, Burton MJ, Mulholland K, Middlemiss DN, Jones B (1996): Species differences in 5-HT autoreceptors. Behav Brain Res 73:79–82

Roberts C, Price GW, Gaster L, Jones BJ, Middlemiss DN, Routledge C (1997): Importance of h5-HT_{1B} receptor selectivity for 5-HT terminal autoreceptor activity: An in vivo microdialysis study in the freely-moving guinea-pig. Neuropharmacology 36:549–557

Schütz W, Freissmuth M (1992): Reverse intrinsic activity of antagonists on G protein-coupled receptors. Trends Pharmacol Sci 13:376–379

Sim LJ, Selley DE, Childers SR (1995): In vitro autoradiography of receptor-activated G-proteins in rat brain by agonist-stimulated guanylyl 5′-[-[^{35}S]thio]-triphosphate binding. Proc Natl Acad Sci USA 92:7242–7246

Skingle M, Beattie DT, Scopes DIC, Starkey SJ, Connor HE, Feniuk W, Tyers MB (1996): GR127935: A potent and selective 5-HT_{1D} receptor antagonist. Behav Brain Res 73:157–161

Smit MJ, Leurs R, Alewijnse AE, Blauw J, Van Nieuw Amerongen GP, Van de Vrede Y, Roovers E, Timmerman H (1996): Inverse agonism of histamine H2 antagonists accounts for upregulation of spontaneously active histamine H2 receptors. Proc Natl Acad Sci USA 93:6802–6807

Stanton JA, Handford EJ, Beer MS (1997): In vitro visualisation of 5-HT receptors in rat brain using [^{35}S]-GTPγS binding. Br J Pharmacol 120(suppl):121P

Starkey SJ, Skingle M (1994): 5-HT_{1D} as well as 5-HT_{1A} autoreceptors modulate 5-HT release in the guinea pig dorsal raphe nucleus. Neuropharmacology 33:393–402

Thomas DR, Faruq SA, Balcarek JM, Brown AM (1995): Pharmacological characterisation of [^{35}S]-GTPγS binding to Chinese hamster ovary cell membranes stably expressing cloned human 5-HT_{1D} receptor subtypes. J Recept Signal Transduct Res 15:199–211

Thomas DR, Gittins S, Collin LL, Middlemiss DN, Riley G, Hagan J, Gloger I, Ellis CE, Forbes IT, Brown AM (1998): Functional characterization of the human cloned 5-HT_7 receptor (long form): Antagonist profile of SB-258719. Br J Pharmacol 124:1300–1306

Watson JM, Burton MJ, Price GW, Jones BJ, Middlemiss DN (1996): GR127935 acts as a partial agonist at recombinant human 5-HT_{1D} and 5-HT_{1D} receptors. Eur J Pharmacol 314:365–372