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REGIONAL FOS-EXPRESSION INDUCED BY γ-HYDROXYBUTYRATE (GHB): COMPARISON WITH γ-BUTYROLACTONE (GBL) AND EFFECTS OF CO-ADMINISTRATION OF THE $GABA_B$ ANTAGONIST SCH 50911 AND PUTATIVE GHB ANTAGONIST NCS-382

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Abstract—γ-Hydroxybutyrate (GHB) has a complex array of neural actions that include effects on its own high-affinity GHB receptor, the release of neuroactive steroids, and agonist actions at $GABA_A$ and $GABA_B$ receptors. We previously reported partial overlap in the c-Fos expression patterns produced by GHB and the $GABA_B$ agonist, baclofen in rats. The present study extends these earlier findings by examining the extent to which GHB Fos expression and behavioral sedation are prevented by (2*S*)-(+)-5,5-dimethyl-2-morpholineacetic acid (SCH 50911), a $GABA_B$ antagonist, and NCS-382, a putative antagonist at the high-affinity GHB receptor. We also compare Fos expression caused by GHB and its precursor γ-butyrolactone (GBL), which is a pro-drug for GHB but lacks the high sodium content of the parent GHB molecule. Both GHB (1000 mg/kg) and GBL (600 mg/kg) induced rapid sedation in rats that lasted over 90 min and caused similar Fos expression patterns, albeit with GBL causing greater activation of the nucleus accumbens (core and shell) and dentate gyrus (granular layer). Pretreatment with SCH 50911 (100 mg/kg) partly reversed the sedative effects of GHB and significantly reduced GHB-induced Fos expression in only four regions: the tenia tecta, lateral habenula, dorsal raphe and laterodorsal tegmental nucleus. NCS-382 (50 mg/kg) had no effect on GHB-induced sedation or Fos expression. When given alone, both NCS-382 and SCH 50911 increased Fos expression in the bed nucleus of the stria terminalis, central amygdala, parasubthalamic nucleus and nucleus of the solitary tract. SCH 50911 alone affected the Islands of Calleja and the medial, central and paraventricular thalamic nuclei. Overall, this study shows a surprising lack of reversal of GHB-induced Fos expression by two relevant antagonists, both of which have marked intrinsic actions. This may reflect the limited doses tested but also suggests that GHB Fos expression reflects mechanisms independent of GHB and $GABA_B$ receptors.

Key words: c-Fos, GHB, gamma-butyrolactone, SCH 50911, NCS-382, $GABA_B$.

INTRODUCTION

Gamma-hydroxybutyrate (GHB) is a popular recreational drug that acts as a central nervous system depressant. It is approved in the treatment of narcolepsy with cataplexy in both the USA as well as Europe (Fuller and Hornfeldt, 2003; Bosch et al., 2012). In Italy, GHB is also approved in the treatment of alcohol addiction and withdrawal (Caputo et al., 2009). The closely related drug γ-butyrolactone (GBL) is a pro-drug for GHB and is rapidly converted to GHB in the body by peripheral lactonases or by non-enzymatic hydrolysis (Snead and Gibson, 2005; Goodwin et al., 2009). GBL has no approved therapeutic application and is widely used as an industrial solvent. The scheduling of GHB led to increased popularity of GBL as a recreational drug due to its greater availability (Wong et al., 2004b; Andresen et al., 2011).

GHB is also an endogenous neurotransmitter that is thought to act through its own high-affinity GHB receptor. However, it also binds with lower affinity to $GABA_B$ receptors to produce a wide range of behavioral and physiological effects (Kaupmann et al., 2003; Queva et al., 2003; Vienne et al., 2010). GHB also causes increased release of neuroactive steroids, perhaps through an action on $GABA_B$ receptors (Barbaccia et al., 2002). GHB may also have an action on specifically configured $GABA_A$ receptor subtypes, particularly those containing delta subunits (Absalom et al., 2012).

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Abbreviations: ANOVA, analysis of variance; BNST, bed nucleus of stria terminalis; DHEAS, dehydroepiandrosterone sulfate; GBL, gamma-butyrolactone; GHB, gamma-hydroxybutyrate; GrDG, granular layer of the dentate gyrus; LDTg, laterodorsal tegmental nucleus; LHb, lateral habenula; NAC, nucleus accumbens; NCS-382, 6,7,8,9-tetrahydro-5-hydroxy-5H-benzocyclohept-6-ylideneacetic acid; PAG, periaqueductal gray; PB, phosphate buffer; PBH, phosphate-buffered horse serum; PBS, phosphate-buffered saline; PFA, paraformaldehyde; PoDG, polymorph layer of the dentate gyrus; PVN, paraventricular nucleus of the hypothalamus; SAL, saline; SCH 50911, (2*S*)-(+)-5,5-dimethyl-2-morpholineacetic acid; SON, supraoptic nucleus.

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However, the relative importance of these different actions in producing GHB effects is currently uncertain.

We previously compared c-Fos expression in rats given GHB versus the $GABA_B$ agonist, baclofen, and found that many of the areas that expressed Fos after GHB were also activated by baclofen (van Nieuwenhuijzen et al., 2009). This supports the notion that many behavioral and physiological effects of GHB reflect its partial agonist effect at $GABA_B$ receptors. Indeed, several studies indicate that $GABA_B$ receptor antagonists can prevent GHB effects including hypothermia, sedation, catalepsy and lethality (Maitre, 1997; Carai et al., 2001, 2008; Wong et al., 2004a; Carter et al., 2009). For example, (2*S*)-(+)-5,5-dimethyl-2-morpholineacetic acid (SCH 50911), a potent $GABA_B$ receptor antagonist (Bolser et al., 1995), attenuated GHB hypothermia and sedation (van Nieuwenhuijzen and McGregor, 2009; Morse et al., 2012) and prevented GHB-induced lethality in mice (Carai et al., 2005). Here, to provide additional evidence of the role of $GABA_B$ receptors in GHB effects, we determined whether SCH 50911 would prevent regional GHB-induced Fos expression.

NCS-382 is a structural analog of GHB that is devoid of activity at $GABA_B$ receptors. From binding studies it is clear that NCS-382 binds at the high-affinity GHB binding site (Mehta et al., 2001; Gould et al., 2003). In some studies, NCS-382 reversed GHB's effects, including sedation and catalepsy (Schmidt et al., 1991; Colombo et al., 1995) and discriminative stimulus effects (Schmidt et al., 1991; Colombo et al., 1995). However, in other studies NCS-382 was ineffective (Castelli et al., 2004; Carai et al., 2005).

GHB is often administered at relatively high doses (e.g. 1000 mg/kg) in the form of a sodium salt (sodium oxybate). This imposes a considerable osmotic burden and raises the possibility that some of the Fos expression previously observed with GHB reflects this high sodium content affecting osmolality (van Nieuwenhuijzen et al., 2009). For example, GHB strongly activates regions such as the supraoptic nucleus (SON) and parabrachial nucleus that have well described roles in water balance (van Nieuwenhuijzen et al., 2009). In contrast, GBL comes as a pure liquid form, without sodium content, but is rapidly converted to GHB *in vivo*. By comparing GBL and GHB-induced Fos expression in the present study we therefore hoped to delineate Fos expression that was a result of salt loading (Roth and Giarman, 1966).

To summarize, the present study sought to gain further insight into the brain mechanisms engaged by GHB, by comparing the regional patterns of neural activation produced by GHB and GBL, and by the combination of GHB with NCS-382 or SCH 50911. In addition, NCS-382 or SCH 50911 were given alone to elucidate their intrinsic effects on Fos expression.

EXPERIMENTAL PROCEDURES

Subjects

The subjects were 42 male Wistar rats (obtained from ARC Perth, WA, Australia) aged 33 days (early adolescence) at the start of the experiments. Adolescent rats were used to reduce the costs associated with purchasing expensive ligands such as SCH 50911. The rats weighed between 115 g and 152 g (mean 130 g, SD = 9.0 g) and were housed in groups of five in large white plastic tubs with wire mesh lid and lined with corncob bedding in a temperature controlled environment (22 ± 2 °C). A 12-h reversed light–dark cycle was in operation (lights on from 19:00 h to 7:00 h). All testing took place in the dark cycle. With exception of the test sessions, food and water were available *ad libitum*. All experimental procedures were conducted in accordance with the *Australian Code of Practice for the Care and Use of Animals for Scientific Purposes*. Ethical approval for experiments was obtained from the Sydney University Animal Ethics Committee. The experiments were designed to minimize the number of animals used and their suffering.

Drugs

Gamma-hydroxubutyrate (GHB) (as a sodium salt) and gamma-butyrolactone (GBL) were obtained from Sigma (Castle Hill, NSW, Australia). NCS-382 (6,7,8,9-tetrahydro-5-hydroxy-5*H*-benzocyclohept-6-ylideneacetic acid), a putative GHB antagonist (Maitre et al., 1990; Schmidt et al., 1991; Castelli et al., 2004) and SCH 50911, a selective $GABA_B$ antagonist (Bolser et al., 1995) were purchased from Tocris Bioscience (Gymea, NSW, Australia).

All drugs were dissolved in saline (SAL, 0.9%) and administered in a volume of 2 ml/kg. Doses were chosen based on previous experiments and freshly made on the morning of each test session. A 1000-mg/kg GHB is a high dose that induces rapid sedation and sleep in rats (van Nieuwenhuijzen and McGregor, 2009; van Nieuwenhuijzen et al., 2009). A 600 mg/kg of GBL was chosen as being approximately equivalent to 1000-mg/kg GHB based on results from prior studies involving schedule-controlled responding (Carter et al., 2006), body temperature (Snead, 1990), drug discrimination (Baker et al., 2005) and pharmacokinetics (Roth and Giarman, 1966).

For SCH 50911, we previously showed that 50 mg/kg prevented hyperthermia and produced a faster recovery from sedation following 1000 mg/kg of GHB (van Nieuwenhuijzen and McGregor, 2009). In mice, 100-mg/kg SCH 50911 reversed sedative effects of 1000 mg/kg of GHB (Carai et al., 2001). Higher doses of SCH 50911 induce convulsions (de Groote et al., 1999), and we therefore selected a dose of 100 mg/kg of SCH 50911 for the present study.

For NCS-382, 50 mg/kg was chosen based on previous rat studies showing this dose blocked GHB appropriate responding for 300- and 700-mg/kg GHB discriminative stimulus effects (Colombo et al., 1995). In mice, 50 mg/kg of NCS-382 reduced sleep time induced by 1000-mg/kg GHB, but this effect was not present using higher doses of NCS-382 (Carai et al., 2001).

Procedure

Rats were randomly assigned to one of seven groups (n = 6 per group) and were injected on the test day with