ELSEVIER

European Journal of Pharmacology 433 (2001) 101–104

www.elsevier.com/locate/ejphar

Short communication

$GABA_B$ receptor inhibition causes locomotor stimulation in mice

Giancarlo Colombo [a,*], Samuele Melis [b], Giuliana Brunetti [b], Salvatore Serra [b], Giovanni Vacca [b], Mauro A.M. Carai [b], Gian Luigi Gessa [a,b,c]

[a] *C.N.R. Institute of Neurogenetics and Neuropharmacology, c/o "Bernard B. Brodie" Department of Neuroscience, University of Cagliari, S.S. 554, Km. 4.5, I-09042 Monserrato (CA), Italy*

[b] *Neuroscienze S.c.a r.l., Via Palabanda 9, I-09123 Cagliari, Italy*

[c] *Department of Neuroscience, "Bernard B. Brodie", University of Cagliari, S.S. 554, Km. 4.5, I-09042 Monserrato (CA), Italy*

Received 6 August 2001; received in revised form 25 October 2001; accepted 30 October 2001

Abstract

The present study investigated the effect of the administration of the $GABA_B$ receptor antagonists, SCH 50911 [(2*S*)(+)-5,5-dimethyl-2-morpholineacetic acid], CGP 46381 [(3-aminopropyl)(cyclohexylmethyl)phosphinic acid] and CGP 52432 (3-[[(3,4-dichlorophenyl)methyl]amino]propyl]diethoxymethyl)phosphinic acid), on spontaneous locomotor activity in mice. All drugs were acutely administered at the doses of 10 and 30 mg/kg (i.p.). The dose of 30 mg/kg of all drugs resulted in a significant stimulation of locomotor activity. The locomotor stimulation elicited by SCH 50911 was completely blocked by haloperidol (0.1 mg/kg, i.p.), suggesting that hyperactivity induced by blockade of the $GABA_B$ receptor is mediated by enhanced dopamine release. These results suggest the existence of a $GABA_B$ receptor-mediated tonic inhibition of dopamine neurons.

Keywords: $GABA_B$ receptor antagonist; SCH 50911; CGP 46381; CGP 52432; Locomotor activity; Haloperidol; Mesocorticolimbic dopamine system; (Mouse)

1. Introduction

Accumulating evidence suggests that the activity of mesocorticolimbic dopamine neurons is controlled, beside other receptor systems, by $GABA_B$ receptors located somatodendritically. For instance, pharmacological activation of $GABA_B$ receptors in the ventral tegmental area (where mesocorticolimbic dopamine neurons originate) has been reported to inhibit (a) somatodendritic and axonal dopamine release (Yoshida et al., 1994; Westerink et al., 1996) and (b) behavioral events related to enhanced dopamine release, such as reinforcement by heroin and nicotine (Xi and Stein, 1999; Corrigall et al., 2000). In addition, both basal as well as ethanol-, cocaine-, amphetamine- and morphine-stimulated locomotor activity were inhibited by peripheral administration or infusion into the ventral tegmental area of the $GABA_B$ receptor agonist, baclofen, in rats and mice (Cott et al., 1976; Kalivas et al., 1990; Chester and Cunningham, 1999; Phillis et al., 2001; Woo et al., 2001).

The present study was designed to extend to the antagonists the investigation on the pharmacological manipulation of locomotor activity by $GABA_B$ agents. To this aim, the present investigation assessed the effect of the $GABA_B$ receptor antagonists, SCH 50911[(2*S*)(+)-5,5-dimethyl-2-morpholineacetic acid], CGP 46381 [(3-aminopropyl)(cyclohexylmethyl)phosphinic acid] and CGP 52432 (3-[[(3,4-dichlorophenyl)methyl]amino]propyl]diethoxymethyl) phosphinic acid), on spontaneous locomotor activity in mice. We hypothesized that $GABA_B$ receptor antagonists would remove a $GABA_B$ receptor-mediated, inhibitory tone on mesocorticolimbic dopamine neurons, resulting in an increase in locomotor activity.

2. Materials and methods

2.1. Animals

The present study employed male DBA/2JIco mice (Charles River, Calco, LC, Italy), weighing 20–25 g and approximately 8 weeks old. Mice were housed 20 per cage under a 12-h artificial light–dark cycle (lights on at 7:00 a.m.), at a constant temperature of 22 ± 2 °C and relative

* Corresponding author. Tel.: +39-070-675-4325; fax: +39-070-675-4320.

E-mail address: colomb@unica.it (G. Colombo).

0014-2999/01/$ - see front matter
PII: S0014-2999(01)01494-7

humidity of approximately 60%. Water and rodent chow were always available. Before inclusion in the experimental procedure, mice were left undisturbed for at least 1 week to adapt to the new housing conditions.

2.2. Apparatus and experimental procedure

Horizontal locomotor activity was measured in Plexiglas test cages [425 × 266 × 150 (h) mm] by a computer-operated eight-photocell apparatus (Motil, TSE, Bad Homburg, Germany). Photocell counts were recorded in 5-min bins.

On the test days, mice were divided in groups of $n = 8–12$. Each mouse was adapted to the motility cage for 45 min (this time period resulted in a virtually complete habituation of mice to the test cage, as shown by levels of activity close to zero in the last 5–10 min in all mice), removed and injected with either SCH 50911, CGP 46381, or CGP 52432 (0, 10 or 30 mg/kg), and returned to the test cage where locomotor activity was monitored for 60 min. In a separate experiment, the nonselective dopamine receptor antagonist, haloperidol (0 or 0.1 mg/kg), was administered 15 min before the injection of SCH 50911 (0 or 30

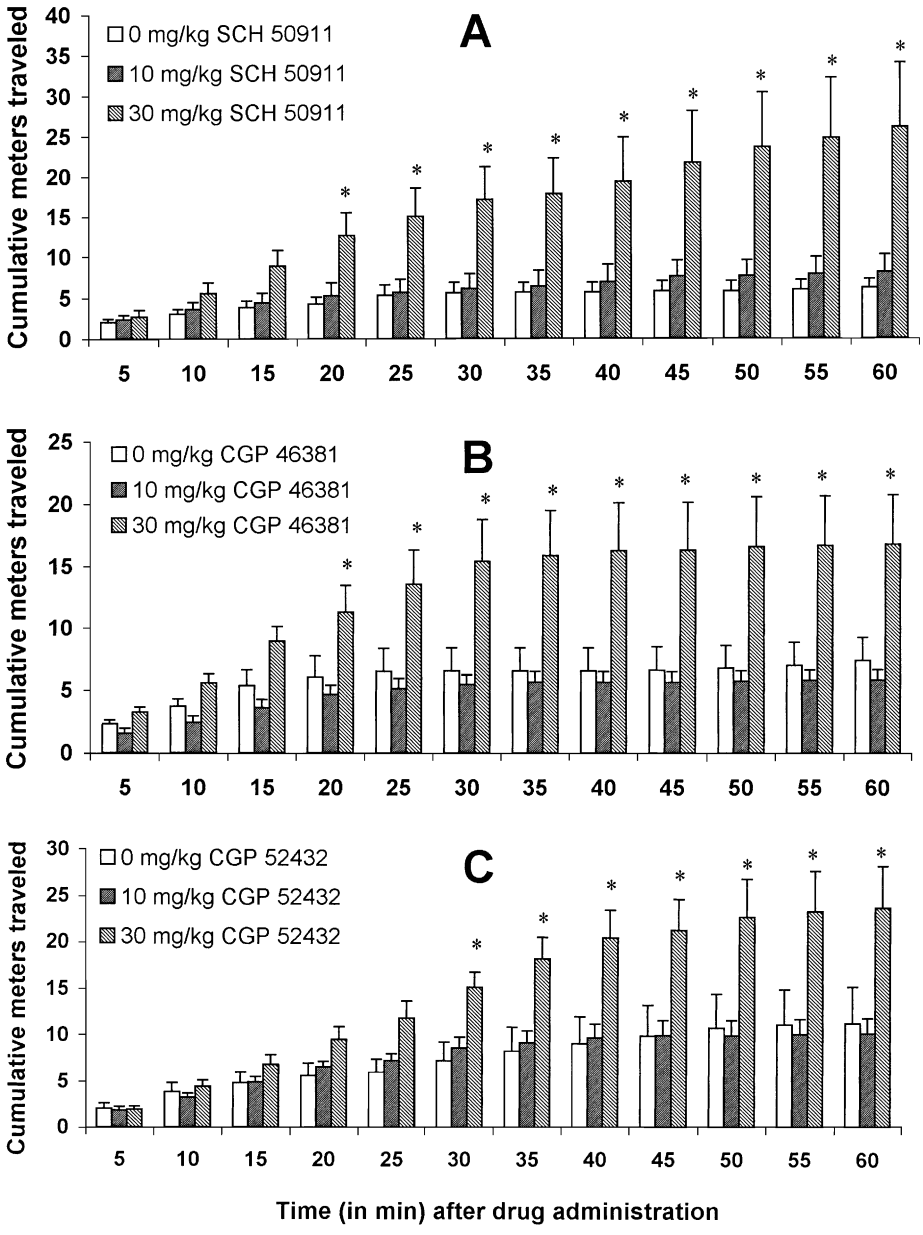

Fig. 1. Stimulation of spontaneous locomotor activity by the $GABA_B$ receptor antagonists, SCH 50911 (panel A), CGP 46381 (panel B), and CGP 52432 (panel C) in DBA mice. Recording of cumulative photocell counts, in 5-min bins, started immediately after administration of the $GABA_B$ receptor antagonists and lasted for 60 min. SCH 50911, CGP 46381, and CGP 52432 were administered i.p. at the doses of 0, 10 and 30 mg/kg. Each bar is the mean ± S.E.M. of $n = 8–12$ subjects. * $P < 0.05$ with respect to saline-treated mice at the corresponding observation time (Newman–Keuls test).

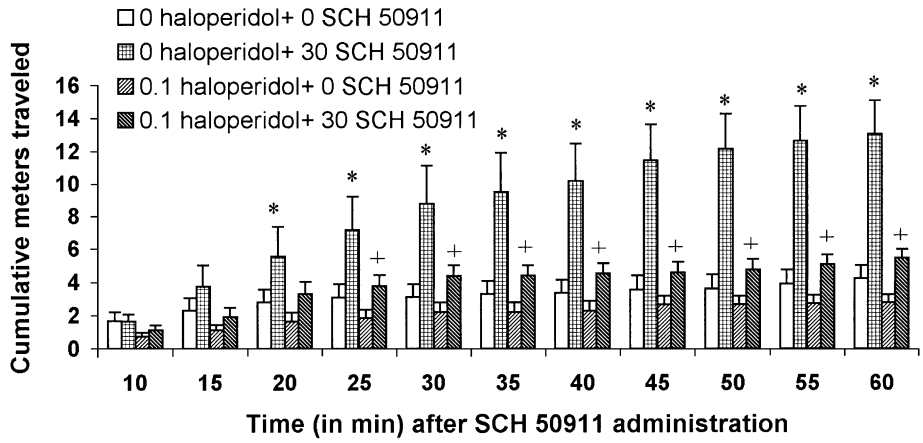

Fig. 2. Prevention of SCH 50911-induced hyperactivity by the dopamine receptor antagonist, haloperidol, in DBA mice. Recording of cumulative photocell counts, in 5-min bins, started immediately after administration of SCH 50911 and lasted for 60 min. SCH 50911 was administered i.p. at the doses of 0 and 30 mg/kg. Haloperidol (0 and 0.1 mg/kg, i.p.) was administered 15 min prior to SCH 50911. Each bar is the mean ± S.E.M. of $n = 10$ subjects. * $P < 0.05$ with respect to 0 mg/kg haloperidol plus 0 mg/kg SCH 50911-treated mice at the corresponding observation time (Newman–Keuls test), and + $P < 0.05$ with respect to 0 mg/kg haloperidol plus 30 mg/kg SCH 50911-treated mice at the corresponding observation time (Newman–Keuls test).

mg/kg). All experiments were conducted between 8:00 a.m. and noon.

SCH 50911 (synthesized as previously described by Blythin et al., 1996), CGP 46381 (Tocris, Ballwin, MO, USA), and CGP 52432 (Tocris) were dissolved in saline. Haloperidol (Tocris) was dissolved in distilled water with acetic acid and sodium bicarbonate. All drugs were injected i.p. in a 12.5-ml/kg volume.

The experimental procedure employed in the present study was approved by the Ethical Committee of the University of Cagliari.

2.3. Statistical analysis

The effect of drug treatments on spontaneous locomotor was statistically evaluated by two-way analyses of variance (drug treatment × time), followed by the Newman–Keuls test for post hoc comparisons.

3. Results

At the dose of 30 mg/kg, the three $GABA_B$ receptor antagonists tested in the present study significantly stimulated locomotor activity [SCH 50911: $F_{treatment}(2,231) = 5.273$, $P = 0.0139$ (Fig. 1A); CGP 46381: $F_{treatment}(2,319) = 5.349$, $P = 0.0105$ (Fig. 1B); CGP 52432: $F_{treatment}(2,319) = 4.823$, $P = 0.0156$ (Fig. 1C)]. At the end of 60-min test, the mean distance traveled by mice treated with 30 mg/kg of the $GABA_B$ receptor antagonists was two to four-fold greater than that recorded in saline-treated mice. In contrast, 10 mg/kg of all drugs failed to alter the locomotor activity.

In the antagonism test, pretreatment with 0.1 mg/kg haloperidol (i.e., a dose that failed to significantly affect locomotor activity per se) resulted in a complete blockade of the locomotor stimulant effect exerted by 30 mg/kg SCH 50911 [$F_{treatment}(3,385) = 6.970$, $P = 0.0008$] (Fig. 2).

4. Discussion

The results of the present study indicate that three structurally different $GABA_B$ receptor antagonists, namely SCH 50911, CGP 46381 and CGP 52432, share the ability to induce hyperactivity in mice. These results are suggestive of the existence of a tonic $GABA_B$ receptor-mediated inhibitory control on locomotion. Consistently, it has been recently reported that deletion of the gene encoding the $GABA_{B(1)}$ receptor subunit resulted in spontaneous hyperlocomotion in mice (Schuler et al., 2001).

In spite of the limited size of the dose–response curves determined, SCH 50911, CGP 46381 and CGP 52432 displayed comparable potency in stimulating locomotion. This appears to be in contrast with the differential affinity for the $GABA_B$ receptor, reported to be remarkably higher for CGP 52432 [$IC_{50} = 55$ nM, Froestl et al., 1996] than SCH 50911 [$IC_{50} = 1.1$ μM, Bolser et al., 1995] and CGP 46381 [$IC_{50} = 4.0$ μM, Froestl et al., 1996]. Such discrepancy between in vitro and in vivo studies may be due to different factors, including possible differences in the bioavailability of the drugs, and the poor penetrability of CGP 52432 into the brain (Froestl et al., 1996), which might compensate its higher affinity.

Suppression of the stimulating effect of SCH 50911 by the dopamine receptor antagonist, haloperidol, suggests that hyperactivity produced by blockade of the $GABA_B$ receptor is eventually mediated by the release of dopamine. Accordingly, it has been found that administration of SCH 50911 produced an activation of nigral dopamine neurons in rats by increasing firing rate and burst firing activity (Erhardt et al.,

1999). Further, a recent in vivo microdialysis study demonstrated that the acute, intraperitoneal administration of doses of SCH 50911 in the 10–30 mg/kg range significantly increased the extracellular concentration of dopamine in the nucleus accumbens of DBA mice (Gessa et al., in preparation).

In agreement with the guiding hypothesis of the present study, the results of the present study lead to hypothesize the existence of a $GABA_B$ receptor-mediated, inhibitory tone on mesocorticolimbic dopamine neurons, the removal of which results in excitation of these neurons and, in turn, augmentation of locomotion. These results are also suggestive of the potential efficacy of $GABA_B$ receptor antagonists in those conditions where enhancement of dopamine mesocorticolimbic neurotransmission may be advisable.

Acknowledgements

The authors are grateful to Professor Giovanna Minardi, University of Sassari, Italy, for the gift of SCH 50911, and Mrs. Anne Farmer for the language editing of the manuscript. The present study was partially supported by the European Community, the Italian Government and the *Regione Autonoma della Sardegna* through "Programma Operativo Plurifondo–Sardegna".

References

Blythin, D.J., Kuo, S.-C., Shue, H.-J., McPhail, A.T., Chapman, R.W., Kreutner, W., Rizzo, C., She, H.S., West, R., 1996. Substituted morpholine-2*S*-acetic acid derivatives: Sch 50911 and related compounds as novel $GABA_B$ antagonists. Bioorg. Med. Chem. Lett. 6, 1529–1534.

Bolser, D.C., Blythin, D.J., Chapman, R.W., Egan, R.W., Hey, J.A., Rizzo, C., Kuo, S.-C., Kreutner, W., 1995. The pharmacology of SCH 50911: a novel, orally-active GABA-B receptor antagonist. J. Pharmacol. Exp. Ther. 274, 1393–1398.

Chester, J.A., Cunningham, C.L., 1999. Baclofen alters ethanol-stimulated activity but not conditioned place preference or taste aversion in mice. Pharmacol. Biochem. Behav. 63, 325–331.

Corrigall, W.A., Coen, K.M., Adamson, K.L., Chow, B.L.C., Zhang, J., 2000. Response of nicotine self-administration in the rat to manipulation of mu-opioid and γ-aminobutyric acid receptors in the ventral tegmental area. Psychopharmacology 149, 107–114.

Cott, J., Carlsson, A., Engel, J., Lindqvist, M., 1976. Suppression of ethanol-induced locomotor stimulation by GABA-like drugs. Naunyn-Schmiedeberg's Arch. Pharmacol. 295, 203–209.

Erhardt, S., Nissbrandt, H., Engberg, G., 1999. Activation of nigral dopamine neurons by the selective $GABA_B$-receptor antagonist SCH 50911. J. Neural Transm. 106, 383–394.

Froestl, W., Mickel, S.J., Schmutz, M., Bittiger, H., 1996. Potent, orally active $GABA_B$ receptor antagonists. Pharmacol. Rev. Commun. 8, 127–133.

Kalivas, P.W., Duffy, P., Eberhardt, H., 1990. Modulation of A10 dopamine neurons by γ-aminobutyric acid agonists. J. Pharmacol. Exp. Ther. 253, 858–866.

Phillis, B.D., Ong, J., White, J.M., Bonnielle, C., 2001. Modification of *d*-amphetamine-induced responses by baclofen in rats. Psychopharmacology 153, 277–284.

Schuler, V., Lüscher, C., Blanchet, C., Klix, N., Sansig, G., Klebs, K., Schmutz, M., Heid, J., Gentry, C., Urban, L., Fox, A., Spooren, W., Jaton, A.-L., Vigouret, J.-M., Pozza, M., Kelly, P.H., Mosbacher, J., Froestl, W., Käslin, E., Korn, R., Bischoff, S., Kaupmann, K., van der Putten, H., Bettler, B., 2001. Epilepsy, hyperalgesia, impaired memory, and loss of pre- and postsynaptic $GABA_B$ responses in mice lacking $GABA_{B(1)}$. Neuron 31, 47–58.

Westerink, B.H., Kwint, H.F., De Vries, J.B., 1996. The pharmacology of mesolimbic dopamine neurons: a dual-probe microdialysis study in the ventral tegmental area and nucleus accumbens of the rat brain. J. Neurosci. 16, 2605–2611.

Woo, S.-H., Kim, H.-S., Yun, J.-S., Lee, M.-K., Oh, K.-W., Seong, Y.-H., Oh, S.-K., Jang, C.-G., 2001. Inhibition of baclofen on morphine-induced hyperactivity, reverse tolerance and postsynaptic dopamine receptor supersensitivity. Pharmacol. Res. 43, 335–340.

Xi, Z.-X., Stein, E.A., 1999. Baclofen inhibits heroin self-administration behavior and mesolimbic dopamine release. J. Pharmacol. Exp. Ther. 290, 1369–1374.

Yoshida, M., Yokoo, H., Tanaka, T., Emoto, H., Tanaka, M., 1994. Opposite changes in the mesolimbic metabolism in the nerve terminal and cell body sites induced by locally infused baclofen in the rat. Brain Res. 636, 111–114.