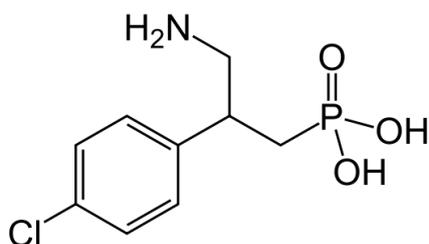


Phaclofen



$C_9H_{13}ClNO_3P$ [3-amino-2-(4-chlorophenyl)propyl] phosphonic acid

In 1987, 25 years after the synthesis of the potent and selective GABA_B agonist baclofen, Kerr et al. described the first GABA_B antagonist phaclofen, the phosphonic acid analogue of baclofen. These compounds showed high receptor selectivity, but their low affinity for GABA_B receptors and weak brain penetration after peripheral administration limited their use in pharmacological studies.

Kerr DI, Ong J, Prager RH, Gynther BD, Curtis DR. Phaclofen: a peripheral and central baclofen antagonist. *Brain Res.* 1987 Mar 3;405(1):150-4. PubMed PMID:3032346.

Bowery NG: GABAB receptors and their significance in mammalian pharmacology. *Trends Pharmacol Sci*, 1989, 10, 401–407

Filip M, Frankowska M. GABA(B) receptors in drug addiction. *Pharmacol Rep.* 2008 Nov-Dec;60(6):755-70. Review. PubMed PMID: 19211967.

Selectivity, Binding and Release

Ki (*IC₅₀) (μM) : 130 (IC₅₀ value, i.e., inhibition of binding of [³H]CGP27492 to GABA_B receptors on rat cerebral cortex membranes)

Bowery NG: GABAB receptors and their significance in mammalian pharmacology. *Trends Pharmacol Sci*, 1989, 10, 401–407

Using the hippocampal slice preparation, it was reported that phaclofen, is a remarkably selective antagonist of both the postsynaptic action of baclofen and the bicuculline-resistant action of GABA, and that it selectively abolishes the slow inhibitory postsynaptic potential in pyramidal cells.

Dutar P, Nicoll RA. A physiological role for GABAB receptors in the central nervous system. *Nature.* 1988 Mar 10;332(6160):156-8. PubMed PMID: 2831457.

Phaclofen antagonises the effects of (-)-baclofen on spinal primary afferent excitatory transmission, as well as preventing the baclofen- or GABA-induced depression of cholinergic twitch responses in the gut.

The major effect of phaclofen was a reversible reduction of the effect of (-)-baclofen on the monosynaptic excitation of interneurons in the cat spinal intermediate nucleus by impulses in muscle group I afferent fibres. The present results in the cat spinal cord suggest that (-)-baclofen may interact with at least two subtypes of bicuculline-insensitive receptor, one antagonized by phaclofen and present on afferent terminals, the other on neurones and insensitive to phaclofen.

Phaclofen (10⁻⁴ to 10⁻³ M) did not influence the resting or transmurally stimulated ileum but reversibly prevented the baclofen-induced depression of the ileal twitch responses, again

with an apparent pA2 approximating 4. Phaclofen also prevented the GABA-induced depression of the ileal twitch, without altering the GABA_A receptor-mediated contraction to GABA.

Microelectroretic administration of phaclofen (75 mM in 75 mM NaCl, pH 3, 120-240 nA) near single interneurons in the dorsal horn of the cat spinal cord generally depressed spontaneous firing, and that generated by ejecting the excitant amino acid DL-homocysteate (0.2 M, pH 7.5, 5-30 nA). The depressant action of phaclofen was not reduced by bicuculline methochloride (10 mM in 150 mM NaCl) ejected with currents (20-40 nA) which abolished the inhibitory action of GABA.

Kerr DI, Ong J, Prager RH, Gynther BD, Curtis DR. Phaclofen: a peripheral and central baclofen antagonist. Brain Res. 1987 Mar 3;405(1):150-4. PubMed PMID:3032346.

The suppression of visually evoked responses (VER) in the cat's striate cortex by baclofen was reversibly antagonized by phaclofen. The antagonistic effect of phaclofen was seen irrespective of whether it enhanced, suppressed or did not change VER on its own. The suppression of VER by GABA was not affected by phaclofen. Phaclofen is an effective baclofen antagonist in the central nervous system of mammals. Preliminary findings indicate that in spite of its possible action on GABA_B receptors, phaclofen does not significantly alter functional properties of striate cortical neurons, in particular direction and orientation sensitivity.

Baumfalk U, Albus K. Phaclofen antagonizes baclofen-induced suppression of visually evoked responses in the cat's striate cortex. Brain Res. 1988 Nov1;463(2):398-402. PubMed PMID: 2848615.

Saturation analysis of [³H]CGP54626A binding to Chinese Hamster Ovary (CHO) cell line stably expressing the GABA_{B1b} and GABA_{B2} receptor subunits revealed the rank order of affinity to inhibit [³H]CGP54626A binding was: CGP62349>CGP54626A>SCH 50911>3-APPA>GABA>baclofen>saclofen>phaclofen. The pK_i rank order of potency at the rat cerebellum membranes was: CGP62349>CGP54626A>3-APPA>SCH 50911>GABA>baclofen>saclofen>phaclofen. Data obtained for both saclofen and phaclofen continued to highlight their much lower potency as antagonists at GABA_B receptors than some previously published observations; both antagonised the GABA attenuation to such a minor extent that no apparent pK_B could be calculated (pK_B<3).

Hirst WD, Babbs AJ, Green A, Minton JA, Shaw TE, Wise A, Rice SQ, Pangalos MN, Price GW. Pharmacological characterisation of a cell line expressing GABA B1b and GABA B2 receptor subunits. Biochem Pharmacol. 2003 Apr 1;65(7):1103-13. PubMed PMID: 12663046.

The aim of this study was to characterize the pharmacological profile of the GABA_{B1}/GABA_{B2} heterodimeric receptor expressed in Chinese hamster ovary (CHO) cells. Unlike native receptors, the GABA_{B1}/GABA_{B2}/G_{q15} response was not inhibited by high micromolar concentration of phaclofen, saclofen or CGP 35348. This raises the possibility that the GABA_{B1}/GABA_{B2}/G_{q15} recombinant receptor may represent the previously described GABA_B receptor subtype which is relatively resistant to inhibition by phaclofen.

Wood MD, Murkitt KL, Rice SQ, Testa T, Punia PK, Stammers M, Jenkins O, Elshourbagy NA, Shabon U, Taylor SJ, Gager TL, Minton J, Hirst WD, Price GW, Pangalos M. The human GABA(B1b) and GABA(B2) heterodimeric recombinant receptor shows low sensitivity to phaclofen and saclofen. Br J Pharmacol. 2000 Nov;131(6):1050-4. PubMed PMID: 11082110; PubMed Central PMCID: PMC1572438.

Microdialysis was employed to investigate whether GABA receptor mechanisms are involved in the regulation of noradrenaline (NA) release in the median preoptic nucleus (MnPO) in awake, freely moving rats. Perfusion with the GABA receptor antagonists as well as agonists was performed in the region of the MnPO through a microdialysis probe and dialysate levels of NA were measured. Perfusion with either bicuculline (10 and 50 microM), a GABA_A

receptor antagonist, or phaclofen (10 and 50 microM), a GABA_B receptor antagonist, enhanced the release of NA in the MnPO area.

Sakamaki K, Nomura M, Hatakenaka S, Miyakubo H, Tanaka J. GABAergic modulation of noradrenaline release in the median preoptic nucleus area in the rat. Neurosci Lett. 2003 May 15;342(1-2):77-80. PubMed PMID: 12727322.

The role of GABA_B receptors in the control of serotonergic (5-HT) neurons of the dorsal raphe nucleus (DRN) by using microdialysis *in vivo* and intra- and extracellular recording *in vitro* in the rat were assessed. The GABA_B agonist R(+)-baclofen enhanced the 5-HT output in the DRN (4.7-fold at 15 mg/kg s.c.) and, to a much lesser extent, striatum of unanesthetized rats. Phaclofen (2 mg/kg s.c.) antagonized the effects of 6 mg/kg R(+)-baclofen in dorsal striatum.

Abellán MT, Jolas T, Aghajanian GK, Artigas F. Dual control of dorsal raphe serotonergic neurons by GABA(B) receptors. Electrophysiological and microdialysis studies. Synapse. 2000 Apr;36(1):21-34. PubMed PMID: 10700023.

Using a microdialysis method, the effects of the nipecotic acid-induced increase in content of endogenous GABA on *in vivo* release of histamine from the anterior hypothalamus of urethane-anesthetized rats were investigated. Nipecotic acid (0.5 mM), an inhibitor of GABA uptake, decreased histamine release to approximately 60% of the basal level. This effect was partially antagonized by picrotoxin (0.1 mM), an antagonist of GABA_A receptors, or phaclofen (0.1 mM), an antagonist of GABA_B receptors. These results suggest that histamine release is modulated by endogenous GABA through both GABA_A and GABA_B receptors.

When the tuberomammillary nucleus, where the cell bodies of the histaminergic neurons are localized, was stimulated electrically, the evoked release of histamine from the nerve terminals in the anterior hypothalamus was significantly enhanced by phaclofen, suggesting that GABA_B receptors may be located on the histaminergic nerve terminals and modulate histamine release presynaptically.

Okakura-Mochizuki K, Mochizuki T, Yamamoto Y, Horii A, Yamatodani A. Endogenous GABA modulates histamine release from the anterior hypothalamus of the rat. J Neurochem. 1996 Jul;67(1):171-6. PubMed PMID: 8666988.

Addiction

Ethanol

The ability of the GABA_B receptor antagonist, phaclofen to alter behavioural effects of ethanol was evaluated by loss of righting reflex (sleep time), motor incoordination (bar holding), spontaneous locomotion (open field activity) and hypothermia. Pretreatment with phaclofen significantly decreased the effects of ethanol on motor incoordination, locomotor activity and hypothermia. However, phaclofen had no effect on either pentobarbital or diazepam-induced motor incoordination. Phaclofen slightly increased the ED₅₀ for loss of the righting reflex but did not alter either the duration of reflex loss produced by ethanol or blood ethanol levels at awakening which suggest phaclofen is rapidly inactivated resulting in difficulty in observing antagonism of long duration ethanol effects. These findings suggest that the GABA_B system may play a role in mediating several important actions of ethanol.

Allan AM, Harris RA. A new alcohol antagonist: phaclofen. Life Sci. 1989;45(19):1771-9. PubMed PMID: 2556616.

Phaclofen and baclofen dose-dependently decreased ethanol-induced locomotor activity in mice (1.75 g/kg, i.p.), and, of these, baclofen did so at doses which did not attenuate locomotor activity when administered alone. However phaclofen failed to reverse the effects of baclofen. These results suggest that the GABA_B receptor may modulate locomotor stimulation induced by low doses of ethanol, and furthermore, that agonist, rather than antagonist activity at the GABA_B receptor is responsible for this reduction. The GABA_B receptor subtype responsible for modulating the effects of ethanol may have properties different from those GABA_B receptors characterised to date.

Humeniuk RE, White JM, Ong J. The role of GABAB receptors in mediating the stimulatory effects of ethanol in mice. Psychopharmacology (Berl). 1993;111(2):219-24. PubMed PMID: 7870956.

The dorsal raphe nucleus (DRN) has been implicated in the neural control of escalated aggression. Baclofen (0.06 nmol), microinfused into the DRN, increased aggressive behaviour like self-administered ethanol (1.0 g/kg). DRN phaclofen (0.3 nmol) did not suppress heightened-aggressive behaviour as induced by ethanol self-administration.

Takahashi A, Kwa C, Debold JF, Miczek KA. GABA(A) receptors in the dorsal raphe nucleus of mice: escalation of aggression after alcohol consumption. Psychopharmacology (Berl). 2010 Sep;211(4):467-77. doi: 10.1007/s00213-010-1920-x. Epub 2010 Jun 30. PubMed PMID: 20589493; PubMed Central PMCID: PMC2992972.

Direct cerebellar microinfusion of GABAB agonist, baclofen, and antagonist, phaclofen, into the permanently cannulated mice, produced a dose-dependent accentuation and attenuation, respectively of ethanol-induced motor impairment, investigated in the mice using rotorod performance as the test response.

Dar MS. Mouse cerebellar GABAB participation in the expression of acute ethanol-induced ataxia and in its modulation by the cerebellar adenosinergic A1 system. Brain Res Bull. 1996;41(1):53-9. PubMed PMID: 8883916.

Ethanol, 1 g/kg administered i.v. or 25-200 nmol microinjected bilaterally into the dorsal vagal complex, inhibited the reflex bradycardic response to bolus i.v. doses of phenylephrine both in spontaneously breathing and in paralysed, artificially ventilated animals. Phaclofen (2 mg/kg s.c.) prevented the baroreflex inhibitory action of ethanol and also prevented ethanol potentiation of the pressor.

Varga K, Kunos G. Inhibition of baroreflex bradycardia by ethanol involves both GABAA and GABAB receptors in the brainstem of the rat. Eur J Pharmacol. 1992 Apr 22;214(2-3):223-32. PubMed PMID: 1325357.

Low concentrations of ethanol (10-30 mM) in the presence of a GABA_B receptor agonist, baclofen, promoted ³⁶Cl⁻ uptake into membrane vesicles (microsacs) prepared from mouse cortex. Neither ethanol nor baclofen alone altered chloride influx. The GABA_B antagonists, phaclofen completely blocked the increase in chloride flux produced by ethanol in the presence of either baclofen or GABA. Ethanol increased the chloride conductance produced by the different GABA_A agonists muscimol, isoguvacine, imidazolacetic acid and amino-propane sulfonic acid and this action of ethanol was blocked by phaclofen.

Allan AM, Burnett D, Harris RA. Ethanol-induced changes in chloride flux are mediated by both GABA(A) and GABA(B) receptors. Alcohol Clin Exp Res. 1991 Mar;15(2):233-7. PubMed PMID: 1647705.

The effects of ethanol on the expression levels of GABA_B receptor mRNA and protein in the cortex and hippocampus from adult rat brain were studied. The results showed that ethanol significantly increased GABA_{B1} and GABA_{B2} receptor protein expression in the cortex, whereas only GABA_{B2} was increased in the hippocampus. GABA_B receptor agonist baclofen could partially reverse the effect of ethanol. Finally, GABA_B agonist

baclofen and antagonist phaclofen showed significant decreasing effects on GABA_{B1} receptor mRNA levels in the cortex, but not in the hippocampus.

Li SP, Park MS, Jin GZ, Kim JH, Lee HL, Lee YL, Kim JH, Bahk JY, Park TJ, Koh PO, Chung BC, Kim MO. Ethanol modulates GABA(B) receptor expression in cortex and hippocampus of the adult rat brain. Brain Res. 2005;1061:27–35.

GABA_{B1}R and GABA_{B2}R showed different age-dependent expressions in *in vivo* fetal rat forebrain from gestational days (GD) 15.5 to 21.5 upon 10% ethanol treatment to mother, with and without baclofen at a dose of 10 mg/kg body weight/day.

Using *in vitro* cultivated cortical neurons from GD 17.5 fetuses, the modulatory effects of ethanol on protein kinase A (PKA) and DA_{D1}R through GABA_BRs, under 50 microM baclofen and 100 microM phaclofen administration, with or without 100 mM of ethanol treatment in the culture media were also explored. The results showed that 20 min ethanol treatment without baclofen or phaclofen had increasing effects on both the GABA_BRs. Further, baclofen and phaclofen administration significantly affected PKA and GABA_BRs levels upon 20 min and 1 h ethanol treatment.

In contrast, DA_{D1}R showed increasing effects upon ethanol treatment, which was modulated by GABA_BR's agonist baclofen and antagonist phaclofen. Therefore the present study suggested that the GABA_BRs activity could modulate ethanol's cellular effects, which possibly including PKA and DA_{D1}R activities, and may be an underlying cause of ethanol's effects.

Lee HY, Naha N, Li SP, Jo MJ, Naseer MI, Park MS, Park TJ, Chung BC, Kim MO. In vivo and in vitro ethanol exposure in prenatal rat brain: GABA(B) receptor modulation on dopamine D(1) receptor and protein kinase A. Synapse. 2008 Jul;62(7):534-43. doi: 10.1002/syn.20522. Erratum in: Synapse. 2010 Jul;64(7):578. Naseer, M L [corrected to Naseer, M I]. PubMed PMID: 18435423.

Baclofen and/or phaclofen could decrease ethanol's up-regulation effects on protein kinase A (PKA) alpha subunit expression in primary cultured cortical neurons in which the GABA_{B1} receptor was specifically knocked down using GABA_{B1} receptor RNA interference.

Furthermore, these effects could lead to changes of phospho (p)- cAMP-response element binding (p-CREB) protein expression, which showed the same expression pattern as protein kinase A. Finally, it was observed changes of GABA_{B1}, PKA, and p-CREB distribution within the same neuronal cells. These results showed that the GABA_BRs are critical to ethanol's cellular effects, which occur via modulating the PKA and CREB transcription pathway, and may be an underlying cause of ethanol's effects.

Lee HY, Li SP, Park MS, Bahk YH, Chung BC, Kim MO. Ethanol's effect on intracellular signal pathways in prenatal rat cortical neurons is GABAB1 dependent. Synapse. 2007 Aug;61(8):622-8. PubMed PMID: 17476683.

Ethanol treatment increased GABA_{B1}R protein levels, but decreased protein kinase A- α (PKA- α), calcium/calmodulin-dependent protein kinase II (CaMKII) and phosphorylation of cAMP-response element binding protein (p-CREB), in cultured hippocampal neurons harvested at gestation day 17.5. Baclofen increased GABA_BR, CaMKII and p-CREB levels, whereas phaclofen decreased GABA_BR, CaMKII and p-CREB levels except PKA- α . Because PKA- α levels were unchanged, it was speculated that CaMKII might instead regulate p-CREB.

To further confirm the modulatory effects of ethanol treatment on GABA_BR, GABA_{B1}R expression were knocked down by RNAi and the hippocampal responses to ethanol *in vitro* were examined. The same effects were observed with baclofen, phaclofen, and baclofen plus phaclofen after RNAi against GABA_{B1}R. GABA_B suppresses neurotransmitter release via inhibition of Ca²⁺ channels.

Fetal alcohol syndrome is a developmental neuropathology resulting from *in utero* exposure to ethanol; many of ethanol's effects are likely to be mediated by GABA. The results showed that stimulation of GABA_{B1}R activity by ethanol treatment can modulate CaMKII and p-CREB signalling to detrimental effect on fetal brain development.

Lee HY, Yang BC, Lee ES, Chung JI, Koh PO, Park MS, Kim MO. Modulation by the GABA(B) receptor siRNA of ethanol-mediated PKA- α , CaMKII, and p-CREB intracellular signaling in prenatal rat hippocampal neurons. *Anat Cell Biol.* 2011 Sep;44(3):210-7. doi: 10.5115/acb.2011.44.3.210. Epub 2011 Sep 29. PubMed PMID: 22025973; PubMed Central PMCID: PMC3195825.

Ethanol caused deficits in working memory at 2.0 g/kg and higher. Phaclofen increased performance accuracy at 10 and 30 mg/kg but had no effect on the total number of trials completed. When combined with ethanol (2.5 g/kg), phaclofen did not significantly alter ethanol-induced deficits in performance.

Escher T, Mittleman G. Effects of ethanol and GABAB drugs on working memory in C57BL/6J and DBA/2J mice. *Psychopharmacology (Berl).* 2004 Nov;176(2):166-74. Epub 2004 Apr 3. PubMed PMID: 15064920.

Recent research suggests that the GABA_B receptor may mediate some of the acute effects of alcohol, but little is known of its involvement in alcohol withdrawal. Mice made dependent on alcohol exhibited tremor and tail arch when consumption ceased. Diazepam dose-dependently attenuated both tremor and tail arch, whereas baclofen had no effect on either of these two withdrawal symptoms. However, baclofen dose-dependently induced convulsant behaviour in the withdrawing mice, and this was significantly attenuated by the GABA_B antagonists phaclofen (50 mg/kg) and CGP 35348 (300 mg/kg), but not BPBA (50 mg/kg). Phaclofen, BPBA, and CGP 35348, when administered alone and in combination with a single dose of baclofen, did have an effect on tremor, although the magnitude was small in comparison to that seen with diazepam. It appears that the GABA_B receptor may play a role in mediating convulsions during alcohol withdrawal, and that in this system baclofen is proconvulsant.

Humeniuk RE, White JM, Ong J. The effects of GABAB ligands on alcohol withdrawal in mice. *Pharmacol Biochem Behav.* 1994 Nov;49(3):561-6. PubMed PMID: 7862708.

Opiate

The effects exerted by GABA_B receptor agonists and antagonists on the acute opiate withdrawal induced by mu and k opiate receptor agonists were investigated *in vitro*. Following a 4 min *in vitro* exposure to morphine (less selective mu agonist), DAGO (highly selective mu agonist) and U50-488H (highly selective k agonist) the guinea-pig isolated ileum exhibited a strong contracture after the addition of naloxone. The selective GABA_B receptor agonist, baclofen was able to reduce dose-dependently the naloxone-induced contracture after exposure to opiate agonists. Pretreatment with phaclofen inhibited dose dependently baclofen antagonism on responses to both mu and k agonists. The results of these experiments indicate that GABA_B receptors are involved in the control of opiate withdrawal *in vitro*, confirming an important functional interaction between the GABAergic system and opioid withdrawal.

Capasso A. GABAB receptors are involved in the control of acute opiate withdrawal in isolated tissue. *Prog Neuropsychopharmacol Biol Psychiatry.* 1999 Feb;23(2):289-99. PubMed PMID: 10368870.

Subcutaneous administration of morphine sulphate (0.5 – 6 mg/kg) produced a dose-dependent conditioned place preference (CPP) in male Wistar rats, a model widely used to study the rewarding effects of morphine. Intra-hippocampal CA1 administration of baclofen

(1 and 2 ug/rat) decreased this morphine-induced (3 mg/kg; s.c.) CPP acquisition, though baclofen (0.5 – 2 ug/rat; intra-CA1) was ineffective *per se*. Similarly, intra-hippocampal phaclofen (1 – 3 ug/rat; intraCA1) did not produce a significant place preference or place aversion but in combination with a lower dose of morphine (1 mg/kg) elicited a significant CPP. Moreover, the response of baclofen (2 ug/rat; intra-CA1) was reversed by phaclofen (4 and 6 ug/rat; intra-CA1). Furthermore, intra-CA1 administration of baclofen but not phaclofen before testing significantly decreased the expression of morphine (3 mg/kg; s.c.)-induced place preference. Baclofen or phaclofen injections had no effects on locomotor activity on the testing sessions. It is concluded that the GABA_B receptors in dorsal hippocampus may play an active role in morphine reward.

GABA_B receptor stimulation could interfere with both acquisition (contextual learning) and expression (retrieval of contextual information) of CPP. Pretreatment with phaclofen during conditioning, attenuated the decrease of morphine reward by baclofen. Furthermore, CPP is a reward-related learning paradigm and it seems possible that co-administration of phaclofen and morphine may increase learning. In support, it was reported that GABA_B receptor antagonists CGP 35348 and phaclofen enhanced paradigm learnings in mice and rat and facilitated the induction of long-term potentiation in rat hippocampal slices.

Zarrindast MR, Massoudi R, Sepehri H, Rezayof A. Involvement of GABA(B) receptors of the dorsal hippocampus on the acquisition and expression of morphine-induced place preference in rats. Physiol Behav. 2006 Jan 30;87(1):31-8. Epub 2005 Nov 7. PubMed PMID: 16274709.

Morphine significantly increased the number of active lever pressing dose dependently in self-administration session in comparative with saline group in rats. Administration of baclofen, 20 min before morphine self-administration produced significant decrease in the initiation of morphine self-administration during all session. Conversely, pre-treatment of phaclofen increased the number of active lever pressing and self-infusion in this test. The results indicated a short-term treatment by baclofen, reduced morphine-maintenance response in a dose-dependent manner, suggesting that GABAB receptor agonists could be useful for reversing the neuroadaptations related to opiates.

RAMSHINI, E., ALAEI, H., REISI, P., ALAEI, S., SHAHIDANI, S.. The Role of GABAB Receptors in Morphine Self-Administration. International Journal of Preventive Medicine, North America, 4, aug. 2012. Available at: <<http://ijpm.mui.ac.ir/index.php/ijpm/article/view/881>>. Date accessed: 25 Feb. 2013.

Opioid system may regulate prolactin secretion at the end of pregnancy. On day 19 of pregnancy, intracerebroventricular administration of the mu-opioid receptor agonist (D-Ala², NMe-Phe⁴, Gly-ol⁵)-enkephalin (DAMGO) or beta-endorphin (beta-END) induced a dose-related increase in serum prolactin levels 30 min later. The intracerebroventricular administration of the GABA_BR antagonist phaclofen had no effect on the serum prolactin levels either in naive or DAMGO-treated rats.

Soaje M, Bregonzio C, Carón RW, Deis RP. Neurotransmitters involved in the opioid regulation of prolactin secretion at the end of pregnancy in rats. Neuroendocrinology. 2004;80(1):11-20. Epub 2004 Aug 15. PubMed PMID: 15340248.

Cocaine

The convulsant effects of cocaine can be modulated by compounds that increase levels of endogenous GABA or that directly stimulate GABA_A receptors. Baclofen dose-dependently inhibited acute (ED₅₀=4.1 mg/kg) and kindled (6.4 mg/kg) seizures induced by cocaine at doses somewhat lower than those producing behavioral side effects (11.5 mg/kg). Phaclofen dose-dependently enhanced the convulsant effects of a threshold dose of cocaine (60 mg/kg).

Gasior M, Kaminski R, Witkin JM. Pharmacological modulation of GABA(B) receptors affects cocaine-induced seizures in mice. *Psychopharmacology (Berl)*. 2004 Jul;174(2):211-9. PubMed PMID: 14985936.

The mesopallidal dopamine system, which originates from the ventral tegmental area and projects to the ventral pallidum (VP), has been recently shown to play an important role in self-stimulation reward and cocaine reward. Ventral pallidum also receives a GABAergic projection from nucleus accumbens (NAS) which raises the possibility of involvement of this GABAergic projection in the modulation of VP dopamine release. Both the GABA_A antagonist picrotoxin (2-200 microM) and the GABA_B antagonist phaclofen (20-2,000 microM), perfused locally, dose-responsively increased VP extracellular dopamine 2-2.5-fold. Cocaine (10 microM) produced a 6.5-fold increase of VP dopamine. Neither picrotoxin (200 microM), phaclofen (2,000 microM), nor GABA (20-2,000 microM) altered the response of VP dopamine to locally applied cocaine.

Gong W, Neill DB, Justice JB Jr. GABAergic modulation of ventral pallidal dopamine release studied by in vivo microdialysis in the freely moving rat. *Synapse*. 1998 Aug;29(4):406-12. PubMed PMID: 9661258.

Memory and Learning

Phaclofen, injected into the left- or right-side of Walker's area 46 in freely moving infant rhesus monkeys, produced deficits in the performance of a 5-s delayed response task, regardless of which side of the brain was injected.

Kubota K. Delayed response deficits induced by local injection of GABA antagonists (bicuculline and phaclofen) into area 46 in infant rhesus monkeys. *Neurosci Res*. 1996 Feb;24(3):245-63. PubMed PMID: 8815445.

Ethanol caused deficits in working memory at 2.0 g/kg and higher. Phaclofen increased performance accuracy at 10 and 30 mg/kg but had no effect on the total number of trials completed. When combined with ethanol (2.5 g/kg), phaclofen did not significantly alter ethanol-induced deficits in performance.

Escher T, Mittleman G. Effects of ethanol and GABAB drugs on working memory in C57BL/6J and DBA/2J mice. *Psychopharmacology (Berl)*. 2004 Nov;176(2):166-74. Epub 2004 Apr 3. PubMed PMID: 15064920.

The effect of GABA receptor agonists and antagonists on acquisition of a step-down passive avoidance learning in mice was measured in the presence and absence of physostigmine (0.1-0.3 mg/kg), which increased acquisition in mice dose dependently. Pretreatment with the higher doses of phaclofen did not impair learning but reduced the learning improvement induced by physostigmine. Phaclofen increased the impairment of learning induced by GABA_A receptor agonist muscimol. Also, phaclofen decreased the baclofen-induced inhibition of physostigmine effect.

Zarrindast MR, Lahiji P, Shafaghi B, Sadegh M. Effects of GABAergic drugs on physostigmine-induced improvement in memory acquisition of passive avoidance learning in mice. *Gen Pharmacol*. 1998 Jul;31(1):81-6. PubMed PMID: 9595284.

The study examined changes in GABA_B receptor function using the GABA_B agonist baclofen (2 mg/mL) or the GABA_B antagonist phaclofen (0.3 mg/mL) on trace cued and contextual fear conditioning and extinction in male rats. The compounds were either administered during training, 24 h after training and throughout extinction via intraperitoneal injection. Considerably fewer studies have investigated phaclofen in behaviour and the dose was selected based on preliminary testing identifying the minimal dose that induced behavioural or tissue differences (unpublished data). Phaclofen-treated animals showed no behavioural differences compared to the saline control group. The current study found that using a GABA_B agonist did not impair learning, but that it did prevent extinction.

Heaney CF, Bolton MM, Murtishaw AS, Sabbagh JJ, Magcalas CM, Kinney JW. Baclofen administration alters fear extinction and GABAergic protein levels. *Neurobiol Learn Mem.* 2012 Oct;98(3):261-71. doi: 10.1016/j.nlm.2012.09.005. Epub 2012 Sep 23. PubMed PMID: 23010137; PubMed Central PMCID: PMC3491140.

The activation of GABA_B receptors by baclofen accelerated extinction of fear memory at mice with depressive-like state. The blockade of GABA_A receptors by bicuculline was ineffective in modification of extinction. The blockade of GABA_B receptors by phaclofen promoted retention of fear expression at intact mice and facilitation of extinction at "depressive" mice.

Dubrovina NI, Zinov'ev DR. [Contribution of GABA receptors in extinction of memory trace in norm and depressive-like state]. *Russ Fiziol Zh Im I M Sechenova.* 2007 Nov;93(11):1285-91. Russian. PubMed PMID: 18198596.

The influence of agonists and antagonists of GABA_B receptors on the development of amnesia has been studied using the passive avoidance test in C57Bl/6J mice with previously generated aggressive and submissive behavioural stereotypes. It is established that baclofen in a dose of 1 mg/kg produces amnesia in aggressive mice and imparts stability with respect to the amnestic influence in submissive mice. Phaclofen in a dose of 5 mg/kg prevents the development of amnesia that is most strongly pronounced in submissive mice.

Dubrovina NI, Loskutova LV. [GABA(B) receptors and amnesia in mice with alternative stereotypes of behavior]. *Eksp Klin Farmakol.* 2007 May-Jun;70(3):10-2. Russian. PubMed PMID: 17650625.

Prepulse Inhibition (PPI)

The acoustic startle reflex is strongly inhibited by a moderate-intensity acoustic stimulus that precedes the startling stimulus by roughly 10-1000 ms (prepulse inhibition, PPI). At long interstimulus intervals (ISIs) of 100-1000 ms, PPI in rats is reduced by the muscarinic receptor antagonist scopolamine. The GABA_B antagonist phaclofen (10 or 30 mg/kg i.p. in rats or mice, respectively) reduced PPI only at long ISIs, similar to the effects scopolamine (1 mg/kg i.p.). The effects of phaclofen and scopolamine were additive in rats, suggesting independent effects of GABA_B and muscarinic receptors.

Yeomans JS, Bosch D, Alves N, Daros A, Ure RJ, Schmid S. GABA receptors and prepulse inhibition of acoustic startle in mice and rats. *Eur J Neurosci.* 2010 Jun;31(11):2053-61. doi: 10.1111/j.1460-9568.2010.07236.x. Epub 2010 May 24. PubMed PMID: 20497471.

Microinjections of phaclofen into the pedunculo-pontine tegmental nucleus significantly impaired prepulse inhibition of the startle reflex in mice.

Takahashi K, Nagai T, Kamei H, Maeda K, Matsuya T, Arai S, Mizoguchi H, Yoneda Y, Nabeshima T, Takuma K, Yamada K. Neural circuits containing pallidotegmental GABAergic neurons are involved in the prepulse inhibition of the startle reflex in mice. *Biol Psychiatry.* 2007 Jul 15;62(2):148-57. Epub 2006 Oct 6. PubMed PMID:17027927.

To test the hypothesis that the substantia nigra pars reticulata (SNR) mediates prepulse inhibition (PPI) via a GABAergic inhibition of the startle pathway, the effects of SNR lesions on PPI of the acoustic startle response, stereotypy and locomotion in drug-free rats were assessed. Infusion of the GABA_B antagonist phaclofen but not the GABA_A antagonist picrotoxin into the caudal pontine reticular nucleus reduced PPI. Hence, lesion of the SNR reduces sensorimotor gating possibly by elimination of a nigroreticular GABAergic projection interacting with GABA_B receptors.

Koch M, Fendt M, Kretschmer BD. Role of the substantia nigra pars reticulata in sensorimotor gating, measured by prepulse inhibition of startle in rats. *Behav Brain Res.* 2000 Dec 20;117(1-2):153-62. PubMed PMID: 11099769.

Stress

Results show that corticosterone secretion induced by a stressful stimulus was increased by blocking GABA receptors (of either the A or B subtype) in the paraventricular nucleus (PVN) of the hypothalamus with bicuculine or phaclofen, respectively, when microinjected (0.5, 5 and 50 pmol) in the PVN. Moreover, corticosterone levels were higher with phaclofen pretreatment than with bicuculine indicating that inhibitory action of GABA in the PVN on HPA axis activity particularly depends on GABA_B receptors, but both receptors may be involved.

GABAergic transmission in the PVN does not mediate stress-induced prolactin secretion as both bicuculine or phaclofen (0.5, 5 and 50 pmol, microinjected) did not alter prolactin secretion.

Moreover, unlike bicuculine, oxytocin secretion induced by a stressful stimulus was inhibited by phaclofen in the PVN. The oxytocin secretion induced by stress was lower at 10 min, in rats given phaclofen at doses of 0.5 and 5 pmol and integrally blocked during whole time of experiment with dose 50 pmol of phaclofen.

Marques de Souza L, Franci CR. GABAergic mediation of stress-induced secretion of corticosterone and oxytocin, but not prolactin, by the hypothalamic paraventricular nucleus. Life Sci. 2008 Nov 7;83(19-20):686-92. doi: 10.1016/j.lfs.2008.09.007. Epub 2008 Sep 23. PubMed PMID: 18840449.

Using voltammetry to examine the effects of intra-VTA (ventral tegmental area) administration of GABA_A and GABA_B agonists and antagonists on restraint stress-induced increases in nucleus accumbens (NAcc) DA. The results show that local VTA GABA_B receptor activation with baclofen (0.01, 0.1 and 1.0 nmol) dose-dependently inhibited the NAcc DA stress response whereas GABA_B receptor blockade with phaclofen had the opposite effect, resulting in a dose-dependent potentiation of the stress response. These results indicate that the NAcc DA stress response is regulated by GABA afferents to VTA DA cells and the relevant GABA_B receptors are located on DA neurons.

Doherty M, Gratton A. Differential involvement of ventral tegmental GABA(A) and GABA(B) receptors in the regulation of the nucleus accumbens dopamine response to stress. Brain Res. 2007 May 30;1150:62-8. Epub 2007 Mar 6. PubMed PMID: 17395162.

Using voltammetry to investigate the role of prefrontal cortex GABA in regulating the nucleus accumbens (NAcc) DA response to stress showed that the NAcc stress response was inhibited following bilateral cortical microinjections of baclofen. While phaclofen blocked the effect of baclofen, it had no significant effect of its own.

Locally applied phaclofen enhanced the DA stress response in prefrontal cortex raised the possibility that GABA influences the NAcc DA stress response indirectly by modulating stress-induced DA release in prefrontal cortex.

Doherty MD, Gratton A. Effects of medial prefrontal cortical injections of GABA receptor agonists and antagonists on the local and nucleus accumbens dopamine responses to stress. Synapse. 1999 Jun 15;32(4):288-300. PubMed PMID: 10332804.

The antinociceptive effects of GABAergic agents in presence or absence of swim-stress were investigated in mice, using the tail-flick test. The antinociception, induced by an interaction of stress and GABA agonists, was higher than that of stress or a GABA agonist alone. The GABA_B antagonist phaclofen decreased the antinociceptive action induced by

stress plus baclofen, whereas, phaclofen itself did not decrease the stress-induced antinociception.

Zarrindast MR, Sabetkasai M. Stress-induced antinociception and GABAergic mechanism. *Arch Int Pharmacodyn Ther.* 1992 Jul-Aug;318:5-12. PubMed PMID:1463371.

Sleep

To explore the role of brainstem GABAergic processes in the control of the behavioural states of sleep and wakefulness, GABA_A and GABA_B agonists and antagonists were microinjected into the nucleus pontis oralis (NPO) in chronic, unanesthetized cats. Microinjections of either muscimol or baclofen immediately induced wakefulness; a significant increase in the duration and the percentage of time spent in wakefulness as well as an increase in the latency to active (REM) sleep. These changes were accompanied by a decrease in the percentage of time spent in active and quiet sleep. In contrast, injections of bicuculline or phaclofen produced active sleep. The percentage of time spent in active sleep and the frequency of active sleep increased while the percentage of time spent in wakefulness and the latency to active sleep was significantly reduced.

Xi MC, Morales FR, Chase MH. Induction of wakefulness and inhibition of active (REM) sleep by GABAergic processes in the nucleus pontis oralis. *Arch Ital Biol.* 2001 Feb;139(1-2):125-45. PubMed PMID: 11256181.

Subcutaneous injection of modafinil (30-300 mg/kg) dose dependently increased dopamine release from the intermediate level of the nucleus accumbens along the rostral-caudal axis of the halothane anaesthetized rat. The effect of modafinil (100 mg/kg) was counteracted by the local perfusion in the nucleus accumbens with the GABA_B receptor antagonist phaclofen (50 µM), whereas it was increased by the GABA_B receptor agonist (-)-baclofen (10 µM). In addition, the modafinil-induced increase of dopamine release was associated with a significant reduction of accumbens GABA release. These results suggest that the dopamine releasing action of modafinil in the rat nucleus accumbens is secondary to its ability to reduce local GABAergic transmission.

Ferraro L, Tanganelli S, O'Connor WT, Antonelli T, Rambert F, Fuxe K. The vigilance promoting drug modafinil increases dopamine release in the rat nucleus accumbens via the involvement of a local GABAergic mechanism. *Eur J Pharmacol.* 1996 Jun 13;306(1-3):33-9. PubMed PMID: 8813612.

In freely moving unanaesthetized rats, microinjection of the GABA_A antagonist bicuculline into the dorsal subcoeruleus nucleus significantly increased the amount and reduced the latency to rapid eye movement sleep during a 2-h recording in the mid-light period. However, injection of the GABA_B antagonist, phaclofen, were unsuccessful.

Pollock MS, Mistlberger RE. Rapid eye movement sleep induction by microinjection of the GABA-A antagonist bicuculline into the dorsal subcoeruleus area of the rat. *Brain Res.* 2003 Feb 7;962(1-2):68-77. PubMed PMID: 12543457.

Feeding

Intracerebroventricular Baclofen (25-100 nmol) produced a dose-related increase in food intake in satiated pigs previously trained to make operant responses for ad libitum food and water. Baclofen (50 nmol) increased feeding during the first 15 min after administration ($P < 0.01$), while the 100 nmol dose increased feeding during the first 30 min ($P < 0.01$). None of these doses of baclofen had any effect on the daily (24 h) food intake. The effect of baclofen (50 nmol) on feeding was prevented by pretreating the animals with the GABA_B antagonist phaclofen (500 nmol, ICV).

Ebenezer IS, Baldwin BA. Effect of intracerebroventricular administration of the GABAB-receptor agonist baclofen on operant feeding in satiated pigs. Br J Pharmacol. 1990 Nov;101(3):559-62. PubMed PMID: 1963798; PubMed Central PMCID: PMC1917733.

The effects of intracerebroventricular (ICV) administration of baclofen (0.1-5.0 nmol) was investigated on food intake in non-fasted rats. Baclofen (1.0, 2.5 and 5.0 nmol) increased food consumption in a dose-related manner during the first 15-30 min after administration. The effects of baclofen (5.0 nmol) on feeding were prevented by pretreating the rats with the specific GABA_B receptor antagonist phaclofen (40 nmol, ICV). These results suggest that baclofen increases food intake in rats by an action at central GABA_B receptors.

Ebenezer IS. The effect of intracerebroventricular administration of baclofen on food intake in rats. Neuroreport. 1990 Sep;1(1):73-6. PubMed PMID: 1966600.

The effects of GABA_A and GABA_B receptors in the anterior piriform cortex on intake of an amino acid imbalanced diet and a basal diet were evaluated in rats. The GABA_B receptor antagonist phaclofen decreased consumption of the basal diet but did not affect consumption of the amino acid imbalanced diet.

Truong BG, Magrum LJ, Gietzen DW. GABA(A) and GABA(B) receptors in the anterior piriform cortex modulate feeding in rats. Brain Res. 2002 Jan4;924(1):1-9. PubMed PMID: 11743989.