Pharmacology Biochemistry & Behavior, Vol. 26, pp. 463–474. Copyright © Pergamon Journals Ltd., 1987. Printed in the U.S.A. 0091-3057/87 $3.00 + .00

GABAergic Mechanisms of Analgesia: An Update

JANA SAWYNOK

Department of Pharmacology, Dalhousie Univerisity, Halifax, Nova Scotia, Canada B3H 4H7

SAWYNOK, J. *GABAergic mechanisms of analgesia: An update.* PHARMACOL BIOCHEM BEHAV **26**(2) 463–474, 1987.—Both directly acting ($GABA_A$ and $GABA_B$ agonists) and indirectly acting GABAergic agents (GABA uptake inhibitors and GABA-transaminase inhibitors) produce analgesia in a variety of animal test systems. Analgesia produced by $GABA_A$ agonists is probably due to a supraspinal action, although spinal sites may also play a role. $GABA_A$ agonist analgesia is insensitive to naloxone, bicuculline, picrotoxin and haloperidol, but is blocked by atropine, scopolamine and yohimbine suggesting a critical role for central cholinergic and noradrenergic pathways in this action. The lack of blockade by the $GABA_A$ antagonist bicuculline is difficult to explain. Both bicuculline and picrotoxin have intrinsic analgesia actions which may not necessarily be mediated by GABA receptors. The $GABA_B$ agonist baclofen produces analgesia by actions at both spinal and supraspinal sites. Baclofen analgesia is insensitive to naloxone, bicuculline and picrotoxin, and blockade by chlorinergic antagonists occurs only under limited conditions. Catecholamines are important mediators of baclofen analgesia because analgesia is potentiated by reserpine, α-methyl-p-tyrosine, phentolamine, ergotamine, haloperidol and chlorpromazine. A role for serotonergic mechanisms is less well defined. Methylxanthines, which produce a clonidine-sensitive increase in noradrenaline (NA) turnover, increase baclofen analgesia by a clonidine-sensitive mechanism. Both ascending and descending NA pathways are implicated in the action of baclofen because dorsal bundle lesions, intrathecal 6-hydroxydopamine and medullary A1 lesions markedly decrease baclofen analgesia. However, simultaneous depletion of NA in ascending and descending pathways by locus coeruleus lesions potentiates baclofen analgesia suggesting a functionally important interaction between the two aspects. Baclofen analgesia within the spinal cord may be mediated by a distinct baclofen receptor because GABA does not mimic the effect of baclofen and the rank order of potency both of close structural analogs of baclofen as well as antagonists differs for analgesia and $GABA_B$ systems. The spinal mechanism may involve an interaction with substance P (SP) because SP blocks baclofen analgesia, and desensitization to SP alters the spinal analgesic effect of baclofen. GABA uptake inhibitors produce analgesia which is similar to that produced by $GABA_A$ agonists because it is blocked by atropine, scopolamine and yohimbine. Analgesia produced by GABA-transaminase inhibitors is similar to that produced by $GABA_A$ agonists because it can be blocked by atropine, but it is potentiated by haloperidol while THIP analgesia is not. Analgesia by GABA-transaminase inhibitors also is similar to baclofen analgesia in that it is increased by haloperidol and chlorpromazine, but it is inhibited by theophylline while baclofen analgesia is potentiated. The possibility that indirectly acting GABAergic agents can produce analgesia by mechanisms unrelated to GABA should be considered.

Analgesia THIP Muscimol Baclofen Noradrenergic pathways Baclofen antagonists

THE role of GABA (γ-aminobutyric acid), a major inhibitory neurotransmitter in the mammalian central nervous system, in analgesic mechanisms has recently emerged as an area of considerable interest. Firstly, GABA may play a role in opiate analgesia. Acute administration of morphine alters the GABA content of discrete brain and spinal cord regions which are important in processing nociceptive information [49], while chronic exposure to morphine alters GABA receptor binding [90]. In addition, systemic administration of GABAergic agents enhances opiate analgesia (reviewed [77,92]), although central administration (intracerebroventricular (ICV) [104] or directly into the periaqueductal gray (PAG) [103] or nucleus raphe dorsalis [73]) paradoxically reduces morphine analgesia. Secondly, GABAergic agents themselves produce analgesia (reviewed [19, 77, 92]) and this is of interest because of the possibility of developing novel non-opioid analgesics. THIP, a $GABA_A$ agonist (see below) has been shown to produce analgesia in human trials [48,54]. Baclofen, a $GABA_B$ agonist whose major clinical use is as an antispastic agent [102], reduces pain associated with spasticity [68], perhaps reflecting a direct as well as an indirect action, and is useful in the treatment of trigeminal neuralgia [27].

There are a variety of GABAergic agents available both with respect to chemical structures and to sites at which the activity of GABA in the synapse can be altered. GABA agonists can be divided into $GABA_A$ and $GABA_B$ subtypes. The prototype $GABA_A$ agonists muscimol and the bicyclic analog THIP (4,5,6,7-tetrahydroisoxazdo (5,4-c) pyrindin-3-ol), activate receptors at which bicuculline is an antagonist [8]. Baclofen, the prototype $GABA_B$ agonist, activates a distinct receptor which is stereoselective for the L-isomer, is insensitive to bicuculline and can be clearly distinguished from $GABA_A$ sites in binding studies [8]. $GABA_A$ receptors are linked to chloride channels [8] while activation of $GABA_B$ receptors reduces calcium currents [20,23], increases potassium conductance [59] and may interact with the cyclic AMP second messenger system [39,45]. Progabide (SL 76002) and its active metabolite SL 75102 bind to

$GABA_A$ and $GABA_B$ receptors with a greater potency for $GABA_A$ sites [55]. Inhibitors of GABA-transaminase such a aminooxyacetic acid (AOAA), γ-acetylenic GABA (GAG) and γ-vinyl GABA (GVG) decrease the degradation of GABA and increase brain GABA levels, while inhibitors of GABA uptake such as nipecotic acid derivatives, SKF 89976A and SKF-100330A increase the levels of GABA in the synapse. Agents which decrease the activity of GABA include the $GABA_A$ receptor antagonists bicuculline and picrotoxin ($GABA_B$ antagonists are presently being established, see below) and thiosemicarbazide, a glutamic acid decarboxylase inhibitor which inhibits the synthesis of GABA.

GABAergic agents produce analgesia in a wide variety of animal test systems. The purpose of the present review is to provide an updated understanding of the mechanisms by which $GABA_A$ and $GABA_B$ agonists, GABA uptake inhibitors and GABA-transaminase inhibitors produce analgesia. Both receptor types activated by these agents and endogenous substrates mediating their actions will be the focus of attention.

$GABA_A$ AGONISTS: ANALGESIA AND SITES OF ACTION

Systemic injection of muscimol or THIP produces analgesia in the tail flick, tail immersion, hot plate, paw pressure, arthritis pain, writhing, grid shock and shock titration tests [3, 11, 40, 46, 83, 91, 97]. With both muscimol [83] and THIP [97], the doses required to produce analgesia and sedation, as indicated by an impairment of rotorod performance, do not appear to differ. However, sedation per se is unlikely to account for analgesic properties of these drugs because systemic injection of profoundly sedating drugs, such as diazepam and phenobarbital, lack analgesic activity [50,83]. In addition, the two effects can be separated pharmacologically because atropine blocks THIP analgesia but does not alter rotorod performance [97].

The predominant site of action of $GABA_A$ agonists appears to be in brain rather than at spinal sites because spinal transection markedly reduces analgesia by THIP in the tail immersion test [105]. In support of this conclusion is the observation that the microinjection of THIP into the ventrolateral PAG, an area important in processing nociceptive information, produced analgesia in the hot plate test [72]. However, analgesia was only observed with a 2 μg dose; higher and lower doses were without effect. Intracerebroventricular administration of muscimol (0.1–0.5 μg) produced analgesia in the pinch vocalization test [52]. Bilateral microinjection of muscimol (0.6 μg) into the substantia nigra produced analgesia in both the tail flick and hot plate tests [6], which in the tail flick test was blocked by lesions to the midbrain reticular formation suggesting a mediation by descending pathways [6]. Finally, unilateral injection of muscimol (0.05–0.1 μg) into the lateral preoptic area increased hot plate latencies on the side contralateral to injection [53]. It should be noted that analgesia in the tail flick test was not observed following injection of muscimol [103] or THIP [72] into the PAG, or of muscimol ICV [52,104] or into the lateral preoptic area [53].

Data obtained following intrathecal (IT) injection suggests an additional spinal site of action. In one study, THIP 5 μg transiently increased hot plate but not tail flick latencies while muscimol 0.25–0.5 μg increased tail flick but not hot plate latencies [34]. The effect of muscimol but not THIP could be separated from an impairment of motor function [34]. An earlier study had demonstrated IT injection of both THIP and muscimol could produce hot plate analgesia without altering motor function [11] and concluded their site of action was in the spinal cord. Thus, there appears to be some evidence for spinal as well as supraspinal sites of action for $GABA_A$ agonists.

Chronic administration of THIP produces tolerance to THIP analgesia [3] as well as cross tolerance to both morphine [3,97] and baclofen analgesia [97]. Although tolerance to morphine reduces the analgesia effect of THIP in the hot plate test [3], the effect of muscimol in the writhing test is not altered [91]. Whether this reflects a difference in nociceptive tests, or a difference in the action of THIP and muscimol is unclear. (THIP and muscimol displayed differential effects in the tail flick and hot plate tests following IT injection [34].) The mutual cross-tolerance between THIP and morphine is thought to reflect a common pathway in the mechanism by which these agents produce analgesia [3]. However, in view of the observation that THIP and baclofen produce a mutual cross tolerance [97] but appear to activate quite dissimilar central mechanisms (Table 1), this interpretation may require some refinement.

$GABA_A$ ANALGESIA: MODIFICATION BY RECEPTOR ANTAGONISTS

Opiate Antagonists

Despite the mutual cross-tolerance between morphine and THIP, naloxone does not block analgesia produced by THIP or muscimol in the hot plate test [40]. A higher dose of naloxone (15 mg/kg) reduced the effect of muscimol in the tail immersion but not the writhing test [91] (Table 1). In higher doses, the specificity of naloxone as an opiate antagonist is open to question [85]. It appears that opioids are not directly involved in analgesia produced by $GABA_A$ agonists, and a relationship between the two systems may be functional in nature.

$GABA_A$ Antagonists

Although both THIP and muscimol are clearly defined as $GABA_A$ agonists and their effects in other systems can be blocked by bicuculline [8], analgesia is generally reported to be insensitive to bicuculline [40,97]. There is one report of antagonism of the effect of muscimol by bicuculline in the hot plate test [83] and of potentiation of muscimol in the writhing test [91]. Bicuculline has an intrinisic analgesic activity in the writhing [91,93], tail flick [93], tail immersion [91] and hot plate tests [79] and this could account for potentiation of the action of muscimol. The reason for the inability of bicuculline to block analgesia by $GABA_A$ agonists is not clear. Invoking intrinsic activity to explain the lack of effect would require an exact counterbalance between the two actions over the range of studies, doses and tests examined. Although subconvulsive doses of bicuculline are necessarily used, other behavioural effects of THIP [40] are bicuculline-sensitive at these doses, suggesting inadequate dosage is not the explanation. In addition, the ability of muscimol to *inhibit* the analgesic effect of morphine following ICV administration [104] or microinjection into the PAG [103] is blocked by bicuculline administered centrally, indicating an involvement of bicuculline-sensitive receptors in this modulatory effect of muscimol. THIP and muscimol do have some affinity for $GABA_B$ receptor [8], but this is unlikely to account for analgesia because such activity is only seen with high doses, while $GABA_A$ and $GABA_B$ agonists can be differentially al-

TABLE 1

EFFECT OF VARIOUS RECEPTOR ANTAGONISTS ON THIP/MUSCIMOL OR BACLOFEN ANALGESIA

Treatment	Dose (mg/kg)	THIP/mus	Baclofen	Reference
naloxone	1.8, 5.6	←→* MHP† THIP,mus		[40]
	2		←→ MHP	[50]
	10,20		←→ MHP	[83]
	15	↓ MTI,mus ←→ MW,mus	←→ MW	[91]
	10,50		←→ MHP	[5]
bicuculline	0.56–0.7	←→ MHP THIP,mus		[40]
	1	↓ MHP mus	←→ MHP	[83]
	1	←→ MHP THIP	←→ MHP	[97]
	1.5	↑ MTF, MW mus	↑ MW	[91]
	2		←→ MHP	[5]
picrotoxin	1	←→ MHP mus	←→ MHP	[83]
	1	←→ MHP THIP	←→ MHP	[97]
atropine	1		←→ MHP	[5]
	5,10	↓ MHP 48+55°C THIP	↓ MHP 55°C only	[97]
scopolamine	2.5	↓ MHP THIP	↓ MHP 55°C only	[97]
haloperidol	0.5		↑ RTF,RHP	[75]
	0.5	←→ MHP THIP	↑ MHP	[97]
chlorpromazine	5		↑ RTF,RHP	[75]
phentolamine	5		↑ RTF,RHP	[75]
ergotamine	0.5		↑ MHP	[5]
yohimbine	5	↓ MTI THIP		[12]
methysergide	1		↑ MHP	[5]
	5,10		←→ RTF,RHP	[75]
cianserin	10		↑ RTF,RHP	Fig. 1
theophylline	50		↑ RTF,RHP	[75]
caffeine	50		↑ RTF,RHP	[75]

*←→ no effect, ↑ increase, ↓ decrease in analgesia.

†Denotes test used. First letter indicates species, M=mouse, R=rat. Subsequent letters indicate test, HP=hot plate, TF=tail flick, TI=tail immersion, VT=vocalization test, W=writhing test.

tered by a number of agents indicating distinct mechanisms of action (Table 1).

Picrotoxin does not influence muscimol or THIP analgesia [83,97]. As with bicuculline, picrotoxin has intrinsic analgesic activity in the tail flick [93], hot plate [83] and writhing tests [91,93]. It is unclear whether actions at GABA receptors are involved in this intrinsic activity of $GABA_A$ antagonists, because agonists for such receptors produce analgesia. With respect to the mechanism of antagonist analgesia, spinal transection blocks the analgesic effect of picrotoxin in the tail flick test [93] suggesting a supraspinal action, but tolerance to morphine does not alter the activity of either bicuculline or picrotoxin in the writhing test [91] suggesting an independence from opioid systems.

Cholinergic Antagonists

Analgesia produce by THIP appears to involve a central cholinergic link. Thus, analgesia is blocked by atropine and scopolamine but not by cholinergic antagonists which do not penetrate the central nervous system [97]. Atropine produces a parallel shift in the dose-response curve to THIP, but does not alter the effect of THIP or rotorod performance suggesting a dissociation of neural mechanisms involved in

analgesia and motor impairment [97]. Oxotremorine, a muscarinic agonist, also produces analgesia [46] which is blocked by spinal transection [105], but the anatomical location of cholinergic pathways involved in THIP analgesia remains to be identified.

Catecholamine Antagonists

Central catecholaminergic pathways are involved in opiate analgesia (see [43] for review), but a potential role for these mediators in THIP or muscimol analgesia has received little attention to date. Yohimbine, an alpha-2 antagonist, inhibits THIP analgesia [12] suggesting such a role should be explored. THIP [95], progabide and SL 75102 [89] increase NA turnover in a number of brain regions supporting a role for NA in the action of $GABA_A$ agonists, while chronic treatment with THIP alters alpha-2 [12] and β-adrenergic receptor binding [96]. Progabide decreases dopamine turnover in striatum [89], but haloperidol does not influence THIP analgesia [97].

BACLOFEN ANALGESIA: SITES OF ACTION

The systemic injection of baclofen produces analgesia in the tail flick, hot plate, writhing, arthritis pain and shock titration tests [5, 13, 40, 50, 75, 83, 97]. In contrast to muscimol and THIP, with baclofen there is a clear separation between doses required to produce analgesia and motor incoordination [50,83]. Analgesia is stereoselective for the L-isomer [4,82] and insensitive to bicuculline (Table 1) and is thought to be due to activation of central $GABA_B$ receptors. However, baclofen appears to activate a separate population of receptors in the spinal cord (see below), and a clear resolution of receptor types occupied by baclofen will require the use of specific antagonists.

Baclofen produces analgesia by actions at both spinal and supraspinal sites. Thus, intrathecal injection of 0.1–1 μg produces analgesia in the tail flick and hot plate tests [34, 81, 100]. Supraspinally, the ICV injection of baclofen 0.1–1 μg reduces the vocalization response to pinch but does not affect tail flick latency [52]. Microinjection of baclofen 1.5 μg into the caudal PAG and into lateral brainstem sites in and near the nucleus gigantocellularis (but not the nucleus raphe magnus) produces tail flick analgesia [51]. Unilateral microinjection of 2 μg baclofen in the lateral preoptic area produces analgesia in the hot plate test [53]. Although the sensitivity of spinal and supraspinal sites is comparable, spinal transection reduces baclofen analgesia in the rat tail flick test [71] and mouse tail immersion test [105] suggesting a predominant supraspinal action following systemic injection. However, the possibility of a significant interaction between these sites, as seen with morphine [101], remains to be investigated.

Baclofen analgesia is independent of opioid systems because it is not blocked by naloxone (2–50 mg/kg, Table 1) and cross-tolerance between baclofen and morphine does not occur [50,97]. Although there are some reports of bicuculline sensitive actions of baclofen [26, 37, 42] and baclofen can bind to $GABA_A$ receptors in high concentrations [64,98], neither bicuculline nor picrotoxin alter baclofen analgesia (Table 1). Central cholinergic mechanisms do not appear to be major mediators of baclofen analgesia because atropine only reduces analgesia when hot plate temperatures are used to 55°C where baclofen is a less potent analgesic than at 48°C [97]. In addition, blockade with atropine is non-competitive, in contrast to the competitive blockade observed with THIP [97].

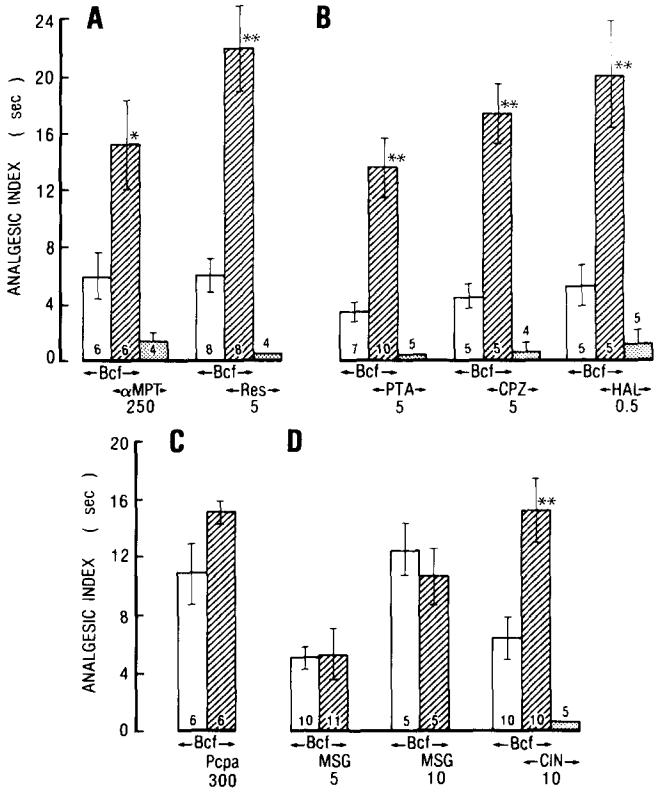

FIG. 1. Effect of monoamine depletors (A,C) and monoamine antagonists (B,D) on baclofen analgesia in the rat tail flick test. In all experiments, baclofen 10 mg/kg was injected after a baseline reading was made, and tail flick latencies determined 30, 60, 90 and 120 minutes later. The analgesic index is the sum of each of these readings minus the baseline value and represents an approximate area under the curve. Bcf=baclofen, αMPT=α-methyl-p-tyrosine, Res=reserpine, PTA=phentolamine, CPZ=chlorpromazine, HAL=haloperidol, Pcpa=p-chlorophenylalanine, MSG=methysergide, CIN=cinanserin. Values are mean±s.e.m. for number indicated in columns. Identical interactions were observed in the hot plate test which was run in tandem with the tail flick test. $*p<0.05$, $**p<0.01$ compared to corresponding control group by Student's t-test. Data derived from [75].

ROLE OF MONOAMINES IN BACLOFEN ANALGESIA

Effect of Antagonists and Depletors

Monoamines appear to play a critical role in baclofen analgesia. Analgeisa is potentiated by depletion of monoamines with reserpine or of catecholamines with α-methyl-p-tyrosine (αMPT) in the rat tail flick and hot plate tests (Fig. 1, Table 1, [75]). Systemic but not intrathecal baclofen is enhanced by these agents [75]. Pretreatment with the alpha receptor antagonists phentolamine [75] and ergotamine [5] and the dopamine receptor antagonists haloperidol and chlorpromazine also potentiates baclofen analgesia (Fig. 1, Table 1, [75, 94, 97]) suggesting both noradrenergic and dopaminergic systems are involved. Although serotoninergic mechanisms are involved in other forms of analgesia, they are not critical to the action of baclofen. Pretreatment with p-chlorophenylalanine, a tryptophan hydroxylase inhibitor which depletes serotonin levels, does not

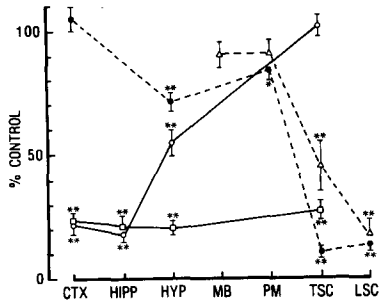

FIG. 2. Effect of lesions to ascending and descending noradrenergic pathways on NA levels in brain and spinal cord. NA was determined by HPLC [87]. CTX=cortex, HIPP=hippocampus, HYP=hypothalamus, MB=midbrain, PM=pons/medulla, TSC=thoracic spinal cord, LSC=lumbar spinal cord, extracted 12–16 days post lesion. (○) Dorsal bundle (DB) lesion (A +2.6 mm, L ±1.1 mm, V +3.7 mm from interaural line, incisor bar +5.0 mm), (□) locus coeruleus (LC) lesion (A −1.4 mm, L ±0.4 mm, V +1.0 mm), (●) medullary A1 lesion (A −6.0 mm, L ±2.0 mm, V −3.2 mm). In all cases, 6-OHDA 4 μg/2 μl was injected bilaterally in 0.2 mg/ml ascorbic acid. (△) Intrathecal (IT) injection of 20 μg/10 μl 6-OHDA. In all experiments, controls received identical injections of vehicle only. *$p<0.05$, **$p<0.01$ compared to corresponding controls. Data for DB, LC and IT lesion from [86]. Data for A1 lesion unpublished.

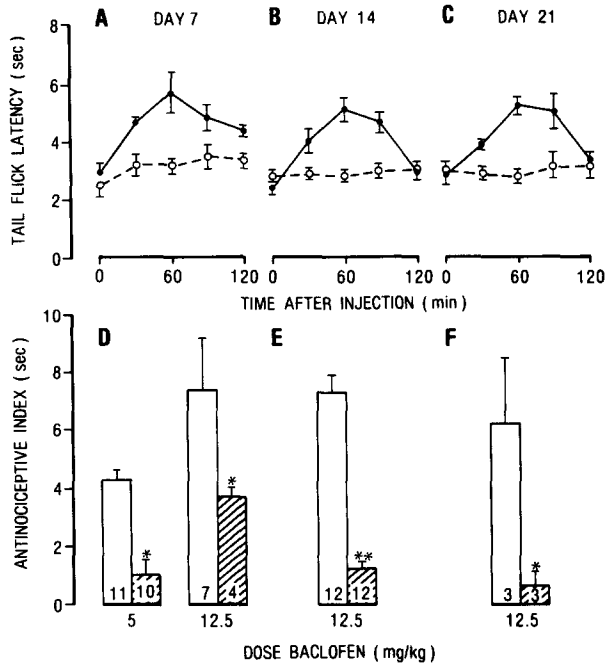

FIG. 3. Effect of dorsal bundle lesions on baclofen analgesia in the tail flick test on indicated days post-lesion. In A–C, baclofen 12.5 mg/kg injected after a baseline reading at t=0. (●) Vehicle-, (○) 6-OHDA-pretreated groups. Panels D–F represent data converted to an index score. Open columns vehicle-pretreated rats; hatched columns 6-OHDA-pretreated rats (mean±s.e.m. for number in columns). *$p<0.05$, **$p<0.01$. Figure from [86] with permission from publishers.

affect baclofen analgesia (Fig. 1, [75]). Results with serotonin antagonists are more equivocal because methysergide had no effect (Fig. 1, Table 1, [75]) or increases baclofen analgesia (Table 1, [5]) while cinanserin increases baclofen analgesia (Fig. 1). The depletion of spinal cord serotonin with 5, 6-dihydroxytryptamine produces a modest increase in baclofen analgesia in the tail flick but not the hot plate test [80]. In contrast, intrathecal methysergide reduces the effect of systemic baclofen in high doses (50–100 μg), an action perhaps due to an antagonist action at NA receptors at these doses [80]. The role of descending NA pathways in baclofen analgesia is considered below.

Role of Catecholamines in Baclofen Analgesia

Baclofen appears to interact with central noradrenergic pathways in a number of ways. It inhibits firing of neurons in the locus coeruleus [33], decreases the release of ^{3}H-noradrenaline (NA) from brain slices [9] and synaptosomes [25], and in high doses directly activates alpha-2 receptors [28]. Additional interactions at postsynaptic sites are possible because baclofen potentiates the increase in cyclic AMP produced by NA in brain slices [39,45]. The effects of baclofen on NA turnover are controversial. Thus, an increase in turnover in various brain regions is reported for 10–50 mg/kg using the α-MPT-induced depletion method [87] and in whole brain for 20 mg/kg by monitoring the accumulation of MOPEG, a major metabolite of NA [16]. However, an inhibition of metabolite production in cortex (but not hippocampus) has been reported for 10 mg/kg [95]. The observed increase in turnover is difficult to directly reconcile with inhibition of firing of neurons in the locus coeruleus and the antirelease effect of baclofen on NA, and suggests an indirect action of baclofen on noradrenergic neurons via other neurotransmitter systems rather than a direct action. A similar indirect action has been proposed for the effect of progabide, a mixed GABA$_A$ and GABA$_B$ agonist, on NA turnover [89].

Baclofen inhibits dopamine turnover in striatum using the αMPT method [2,87] and by measuring the accumulation of metabolites [16,30]. This action could be due to an inhibition of firing of cells in the substantia nigra [63] and the inhibition of ^{3}H-dopamine release from striatal slices [8]. The potentiating effect of haloperidol and chlorpromazine on baclofen analgesia noted above suggests this inhibition of function may contribute to analgesia. However, clonidine reverses the effect of baclofen on DA turnover in the striatum [87] but does not reverse the effect of a DA antagonist on baclofen analgesia [94].

Another class of compounds, the methylxanthines, also potentiate baclofen analgesia [76]. Although these agents have a number of pharmacolgical properties including adenosine receptor antagonism, phosphodiesterase inhibition, calcium mobilization and increasing NA turnover, it is likely that this latter action is responsible for the interaction with baclofen. Methylxanthines increase NA turnover [7,44] by stimulating the firing of neurons in the locus coeruleus [32]. Both the increase in NA turnover [29] and activation of neural firing [32] are blocked by clonidine, an alpha-2 agonist. Clonidine also reverses the potentiation of baclofen analgesia produced by theophylline and isobutylmethylxanthine [94]. Although clonidine reverses the effect of baclofen on NA turnover [87] it does not reverse baclofen analgesia [94], suggesting the critical effect of baclofen on NA systems

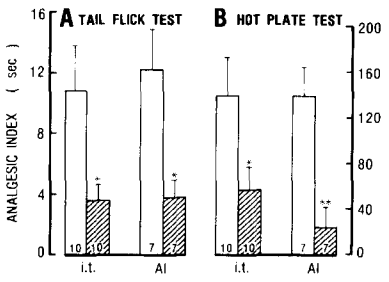

FIG. 4. Effect of lesioning descending NA pathways with 6-OHDA injected intrathecally (IT) via a chronic indwelling cannula or microinjected into the medullary A1 region on baclofen analgesia. Baclofen (8 mg/kg IT experiment, 12.5 mg/kg A1 experiment) administered 11–13 days post-lesion. Hollow columns vehicle-pretreated rats, hatched columns 6-OHDA-pretreated rats. $*p<0.05$, $**p<0.01$. Data compiled from [80] (IT experiment) and unpublished data (A1 experiment).

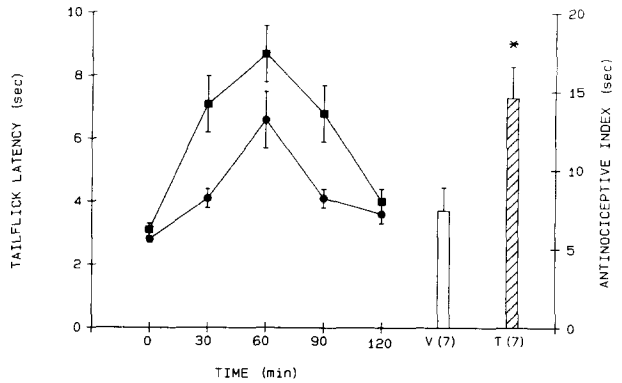

FIG. 5. Effect of locus coeruleus lesions on baclofen analgesia in the tail flick test. Baclofen 12.5 mg/kg injected after the baseline reading at t=0. (●) Vehicle- pretreated rats, (■) 6-OHDA-pretreated groups. Open columns vehicle-pretreated rats, hatched columns toxin-pretreated rats for indicated number of rats. $*p<0.05$. Data from [86].

relating to analgesia is more complicated than simply increasing NA turnover. Indeed, clonidine decreases NA turnover [1] while producing analgesia in a variety of test systems (see [24]).

Effects of Lesions to Ascending and Descending NA Pathways on Baclofen Analgesia

Central NA pathways can be separated into distinct ascending and descending systems. The major ascending pathways are contained in the dorsal bundle (DB), the ventral bundle and the periventricular system [14,57]. The DB originates mainly in the locus coeruleus (LC) and projects to the cerebellum, cortex, hippocampus, hypothalamus and other forebrain structures, while the ventral bundle originates from more heterogenous cell groups in the pons and medulla and innervates the hypothalamus and forebrain structures but not the cortex or hippocampus. Descending NA pathways originate in the LC [61], as well as medullary structures [15]. We have examined the effect of lesioning ascending NA pathways (DB lesions), descending NA pathways (IT 6-OHDA and medullary A1 lesions) and both (LC lesions) by microinjection of 6-OHDA on baclofen analgesia to clarify the involvement of specific NA pathways in this action [86]. The effect of lesions on NA levels in brain and spinal cord are shown in Fig. 2. DB lesions deplete NA in brain but not spinal cord and almost completely eliminate the analgesic effect of baclofen in the tail flick test (Fig. 3), suggesting ascending NA pathways play a critical role in the action of baclofen. Intrathecal 6-OHDA selectively depletes spinal cord NA levels and inhibits baclofen analgesia (Fig. 4). Medullary A1 lesions profoundly deplete spinal cord NA levels (and reduce NA in the pons/medulla and hypothalamus to some extent) and also reduce the effect of baclofen in both tail flick hot plate tests (Fig. 4). These experiments suggest a critical role for descending NA pathways in baclofen analgesia, a conclusion supported by the observation that IT phentolamine inhibits analgesia produced by systemic baclofen [80]. Paradoxically, LC lesions which deplete NA in both ascending and descending pathways (Fig. 2) *potentiates* baclofen analgesia (Fig. 5). This result is consistent with that obtained using αMPT which depletes NA in both ascending and descending systems [87] and also potentiates the action of baclofen (Fig. 1). This curious observation suggests there is an important functional interaction between ascending and descending NA systems in relation to analgesic mechanisms.

SPINAL MECHANISMS OF BACLOFEN ANALGESIA

Receptor Types

The criteria required for a pharmacological effect of baclofen to be due to activation of $GABA_B$ receptors include stereoselectivity for the L-isomer, insensitivity to bicuculline and mimicry by GABA [8]. In peripheral tissues, blockade by δ-aminovaleric acid (δ-AV) [58] and 3-aminopropane sulfonic acid (APS) [31,47] has been demonstrated and such blockade also could be included in these criteria. Analgesia followed IT baclofen is clearly stereoselective for the L-isomer [81,100] and was originally thought to be due to $GABA_B$ receptor activation. However, the results of recent studies in this laboratory suggest that in the spinal cord, baclofen interacts with a receptor which is distinct from the $GABA_B$ receptor in producing spinal analgesia.

The above conclusion is based on the following observations. (1) GABA (up to 100 μg) does not produce analgesia following IT administration even in the presence of a GABA uptake inhibitor or a GABA-transaminase inhibitor [81]. L-Baclofen produces analgesia in the 0.1–1.0 μg range [81,100]. (2) The rank order of potency of baclofen (p-chlorophenyl GABA) analogs in producing analgesia differs from $GABA_B$ systems. Following IT injection, m-chlorophenyl GABA (mCPG) was both more potent (approximately 30 times) and more efficacious than o-chlorophenyphenyl GABA (oCPG) in producing analgesia [81]. This is in contrast to such $GABA_B$ systems as inhibition of ^{3}H-NA release from atria and binding studies where oCPG was 4–25 times more potent than mCPG [8]. In the mouse vas deferens, the two analogs were almost equipotent, but this still differs from the potency separation in analgesia tests by an order of magnitude. (3) The rank order of potency of

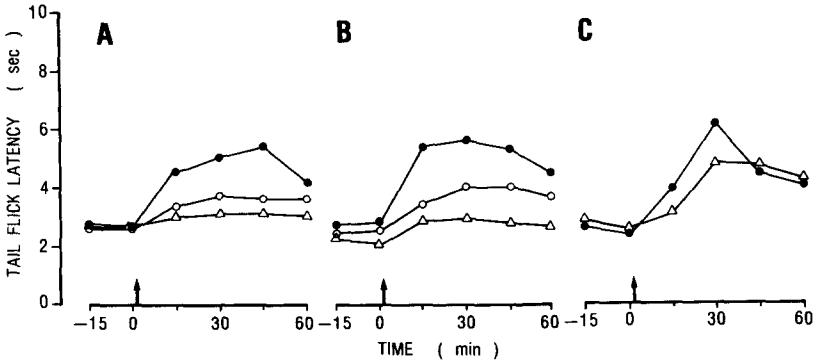

FIG. 6. Effect of (A) D-baclofen (D-Bcf), (B) δ-aminovaleric acid (δ-AV), and (C) 3-aminopropane sulphonic acid (APS) on the analgesic effect of L-baclofen in the tail flick test. Antagonists were administered after the initial baseline reading and L-baclofen 15 minutes later (at arrow) after a second tail flick reading. (●) Control response to L-baclofen 0.5 μg in all panels. (A) L-Bcf following (○) 5 μg D-Bcf, (△) 10 μg D-Bcf; (B) L-Bcf following (○) 5-AV 7.5 μg, (△) 5-AV 15 μg; (C) L-Bcf following (△) APS 15 μg. Values depict means (s.e.m.≤0.5 sec) for n=8, 8 and 4 in all groups in respective panels. Data from [78].

antagonists differs between the spinal cord analgesia system and a GABA$_B$ system. The analgesic effect of IT L-baclofen (0.1–0.5 μg) was blocked by pretreatment with D-baclofen (5–20 μg) [81]. At these doses, D-baclofen had intrinsic activity and the effect of L-baclofen was examined in the presence of a plateau level of activity. However, antagonism was confirmed using a repurified sample of D-baclofen (Fig. 6A, [78]). Pretreatment with δ-AV also blocked the effect of L-baclofen, but APS was ineffective as an antagonist (Fig. 6). D-baclofen was more potent than δ-AV. In an established GABA$_B$ system, the electrically stimulated longitudinal muscle-myenteric plexus preparation from the guinea pig ileum, both APS and δ-AV block the action of L-baclofen but D-baclofen is inactive [78]. Spinal analgesia by baclofen thus appears to be mediated by a distinct receptor. Table 2 lists criteria which could be used for determining which particular receptor type is involved in pharmacological effects of baclofen.

TABLE 2

CRITERIA FOR THE INVOLVEMENT OF GABA$_B$ OR BACLOFEN RECEPTORS IN A PHARMACOLOGICAL EFFECT OF BACLOFEN

		GABA$_B$	Baclofen
(1)		activated by baclofen	
(2)		stereoselective for the (−)-isomer	
(3)		insensitive to bicuculline	
(4)	agonists	oCPG>mCPG	mCPG>oCPG
(5)	antagonists	APS=δAV	D-Bcf>δAV

oCPG=o-chlorophenyl GABA, mCPG=m-chlorophenyl GABA, APS=3-aminopropane sulfonic acid, δAV=δ-aminovaleric acid, D-Bcf=D-Baclofen.

Electrophysiological Approaches

Electrophysiological studies indicate that systemic [36,67] and intrathecal baclofen [21] can selectively inhibit afferent input by small diameter fibres to the spinal cord. This inhibition is believed to be due to a presynaptic action of baclofen. GABA$_B$ receptors have been reported to be concentrated in the dorsal horn of the spinal cord [70]. This binding is reduced 40–50% by neonatal capsaicin treatment, suggesting about half of GABA$_B$ receptors are located on primary afferent nerve terminals [70]. In dorsal root ganglion cells, intracellular recordings from the cell soma indicate that baclofen decreases a voltage-dependent calcium current as well as decreasing action potential duration [20,23]. If this mechanism were to operate on afferent terminals, it could account for the presynaptic inhibitory effect of baclofen. Following iontophoretic administration baclofen inhibits both noxious and non-noxious evoked activity in the dorsal horn of the spinal cord [18], and produces both pre- and postsynaptic inhibitory effects [17].

Much of the recent electrophysiological literature on baclofen has assumed that the mechanisms of action of baclofen involves activation of GABA$_B$ receptors. However, in view of the possibility that spinal analgesia is mediated by a distinct receptor type, the relative involvement of GABA$_B$ receptors and baclofen receptors in demonstrated electrophysiological effects of baclofen remain to be determined. The use of D-baclofen as well as GABA$_B$ antagonists should prove useful in characterizing these effects.

Interactions With Substance P

Substance P (SP) is a putative primary afferent transmitter of noxious information [60], although this role has been questioned recently [99]. Baclofen inhibits the depolarizing effect of SP on spinal motoneurons in a relatively specific manner because the response to L-glutamate was much less affected [74]. The specificity of the blocking effect on SP has been questioned when baclofen is applied iontophoretically [38,69]. However, in *in vitro* experiments where bath concentrations can be maintained in the μM range, the selectivity of the blockade of SP has continued to be demonstrated [62,65]. Baclofen does not displace ^{3}H-SP fron binding sites [35], suggesting this action is not directly at receptor sites.

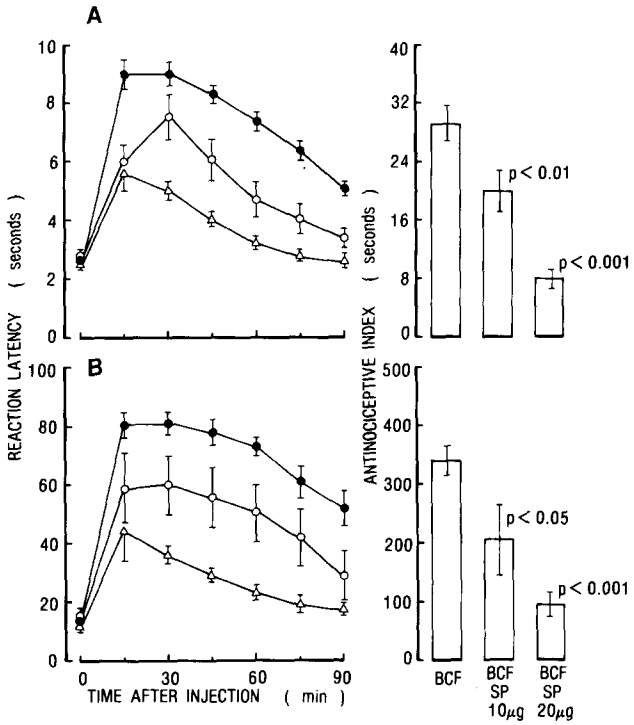

FIG. 7. Antagonism of the analgesic effect of intrathecal baclofen by substance P in the (A) tail flick and (B) hot plate tests. (●) Baclofen (BCF) 0.8 μg, (○) BCF + SP 10 μg, (△). BCF + SP 20 μg coadministered after the baseline reading at t=0 (n=5–10 rats per group). Figure from [84] with permission from publishers.

The results of a number of behavioural experiments also link SP to the spinal analgesic effect of baclofen. Baclofen blocks the biting, licking, scratching syndrome produced by central administration of SP [22]. This effect of SP is believed to reflect a direct postsynaptic activation of sensory pathways [66]. In addition, IT coadministration of SP blocks the antinociceptive effect of baclofen in the tail flick and hot plate tests (Fig. 7, [84]), suggesting an involvement of SP in this action of baclofen. Finally, SP receptors in the spinal cord can be desensitized following the repeated IT administration of SP [56]. Both the hyperalgesic response to SP and the biting, licking, scratching syndrome are reduced under these circumstances [56]. This effect is probably receptor mediated because peptides related to SP show cross-desensitization [56] but the hyperalgesic response to amine antagonists is much less or unaffected [88]. Pretreatment with desensitizing doses of SP potentiates the analgesic effect of baclofen when baclofen is injected immediately after the regimen [88], suggesting additivity between compromised SP action by baclofen and desensitization. However, a subsequent inhibition of the effect of baclofen occurs if baclofen is injected following a delay [88]. Whether this reflects a multiplicity of sites for SP action in the spinal cord is unclear (see discussion, [88]).

In contrast to the postsynaptic interaction with SP, baclofen does not inhibit SP release from spinal cord slices [82]. However, SP is present in only 20% of sensory neurons [41] and a variety of peptides other than SP are now implicated in pain pathways [41]. It remains to be seen whether the presynaptic inhibitory effects of baclofen implicated in electrophysiological studies are manifest in inhibition of release of other putative afferent transmitters.

MECHANISM OF ACTION OF GABA UPTAKE INHIBITORS AND GABA-TRANSAMINASE INHIBITORS

Both GABA uptake inhibitors and GABA-transaminase inhibitors are thought to produce analgesia by virtue of increasing GABA concentrations at central synapses. However, because analgesia produced by $GABA_A$ agonists is generally insensitive to bicuculline and $GABA_B$ antagonists which are effective following systemic administration have not been available, a direct implication of GABA receptor subtypes in analgesia produced by these agents has had to rely on less direct approaches. Thus, a comparison between the effects of various receptor antagonists on muscimol/THIP and baclofen analgesia can provide some grounds for interpretation (Tables 1 and 3).

Analgesia produced by the uptake inhibitor SKF 89976A is reduced by spinal transection [105], suggesting a predominantly supraspinal action. However, ICV administration of nipecotic acid ethyl ester (NAee) to rats does not produce analgesia in the tail flick test [104] in a dose which was sufficient to block opiate-induced analgesia. Chronic THIP reduces NAee analgesia [3], but it is difficult to interpret this observation because chronic THIP also reduces analgesia produced by baclofen and morphine which act via distinct receptors and mechanisms. NAee analgesia is insensitive to

TABLE 3

EFFECT OF VARIOUS RECEPTOR ANTAGONISTS ON ANALGESIC EFFECT OF GABA UPTAKE INHIBITORS AND GABA-TRANSAMINASE INHIBITORS

Treatment	Dose (mg/kg)	GABA Uptake Inhibitors	GABA-Transaminase Inhibitors	Reference
naloxone	2	⟷* MTI† SKF100330A		[106]
	5	⟷ MTI NAee	⟷ MTI GVG	[46]
bicuculline	0.5		↓ RVT GVG	[10]
	1	⟷ MTI SKF100330A		[106]
	2		⟷RTF ↑ RHP GAG	[79]
	2		↑ MHP,RHP AOAA	[5]
atropine	5	↓ MTI NAee	↓ MTI GVG	[46]
scopolamine	1	↓ MTI SKF100330A		[106]
chlorpromazine	5		↑ RTF,RHP GAG	[79]
haloperidol	0.5		↑ RTF,RHP GAG	[79]
phentolamine	10		⟷ RTF,RHP GAG	[79]
yohimbine	5	↓ MTI NAee		[12]
methysergide	10		⟷ RTF,RHP GAG	[79]
theophylline	50		↓ RTF,RHP GAG ↓ RTF,GVG	[79]

*†Explanations as in Table 1. Abbreviations: NAee, nipecotic acid ethyl ester; GAG, γ-acetylenic GABA; GVG, γ-vinyl GABA.

naloxone but blocked by atropine [46] and yohimbine [12], indicating a similarity between the influence of these agents on THIP and NAee analgesia. Nevertheless, it has been emphasized that GABA uptake inhibitors produce a greater degree of analgesia than a THIP and baclofen combination as well as a GABA-transaminase inhibitor [106] introducing the possibility that these agents can produce analgesia by mechanisms independent of GABA systems.

Analgesia produced by the GABA-transaminase inhibitor AAOA is blocked by spinal transection [93], suggesting a predominant supraspinal action. GAG has been reported to increase tail flick latencies following intrathecal injection [81], suggesting an action at spinal sites as well. The effect of GVG in the vocalization test was blocked by bicuculline [10] but the effect of GAG in the tail flick test was not [79]. The potentiating effect of bicuculline on GAG analgesia in the hot plate test is probably due to intrinsic activity in this test [79]. The pharmacological profile of GABA-transaminase inhibitors does not parallel that of either GABA$_A$ agonists or baclofen (Tables 1 and 3). Thus, although atropine inhibits GVG and THIP analgesia [46], haloperidol potentiates GAG [79] but not THIP [97]. On the other hand, although chlorpromazine and haloperidol potentiate GAG [79] and baclofen analgesia [75,97], reserpine and phentolamine do not alter GAG [79] while potentiating baclofen analgesia [75]. Finally, theophylline inhibits GAG and GVG analgesia [79] while potentiating the action of baclofen [75]. The possibility that GABA-transaminase inhibitors produce analgesia by mechanisms unrelated to GABA should be considered.

ACKNOWLEDGEMENTS

I would like to thank Hanni Taylor, Charles Dickson and Allison Reid for technical assistance during the course of many of these studies. Work conducted in this laboratory has been supported by the Medical Research Council of Canada.

REFERENCES

1. Andén, N. E., M. Grabowska and U. Strömbom. Different alpha-adrenoreceptors in the central nervous system mediating biochemical and functional effects of clonidine and receptor binding agents. *Naunyn Schmiedebergs Arch Pharmacol* **292:** 43–52, 1976.
2. Andén, N. E. and H. Wachtel. Biochemical effects of baclofen (β-parachlorophenyl-GABA) on the dopamine and noradrenaline in the rat brain. *Acta Pharmacol Toxicol (Copenh)* **40:** 310–320, 1977.
3. Andree, T., D. A. Kendall and S. J. Enna. THIP analgesia: Cross tolerance with morphine. *Life Sci* **32:** 2265–2272, 1983.
4. Bartolini, A., R. Bartolini, A. Biscini, A. Giotti and P. Malmberg. Investigations into baclofen analgesia: effect of naloxone, bicuculline, atropine and ergotamine. *Br J Pharmacol* **72:** 156–157P, 1981.
5. Bartolini, A., P. Malmberg, R. Bartolini and A. Giotti. Effect of antimigrane drugs on nonopioid analgesia. In: *Advances in Neurology*, vol 33, edited by M. Critchley, A. P. Friedman, S. Gorini and F. Sicuteri. New York: Raven Press, 1982, pp. 89–97.
6. Baumeister, A. A. and G. D. Frye. Involvement of the midbrain reticular formation in self injurious behaviour, stereotyped behaviour, and analgesia induced by intranigral microinjection of muscimol. *Brain Res* **369:** 231–242, 1986.
7. Berkowitz, B. A., J. H. Tarver and S. Spector. Release of norepinephrine in the central nervous system by theophylline and caffeine. *Eur J Pharmacol* **10:** 64–71, 1970.
8. Bowery, N. G. Baclofen: 10 years on. *Trends Pharmacol Sci* **3:** 400–403, 1982.
9. Bowery, N. G., D. R. Hill, A. L. Hudson, A. Doble, D. N. Middlemiss, J. Shaw and M. Turnbull. (−)-Baclofen decreases neurotransmitter release in the mammalian CNS by an action at a novel GABA receptor. *Nature* **283:** 92–94, 1980.
10. Buckett, W. R. Irreversible inhibitors of GABA transaminase induce antinociceptive effects and potentiate morphine. *Neuropharmacology* **19:** 715–722, 1980.
11. Christensen, A. V. and J. J. Larsen. Antinociceptive and anticonvulsant effect of THIP, a pure GABA agonist. *Pol J Pharmacol Pharm* **34:** 127–134, 1982.
12. Costa, L. G. and S. D. Murphy. Antinociceptive effect of the GABA mimetic 4,5,6,7-tetrahydroisoxazolo (5,4-c) pyridin-3-ol (THIP): chronic treatment increases alpha-2 adrenoceptors in the mouse brain. *J Pharmacol Exp Ther* **229:** 386–390, 1984.
13. Cutting, D. A. and C. C. Jordan. Alternative approaches to analgesia: baclofen as a model compound. *Br J Pharmacol* **54:** 171–179, 1975.
14. Dahlström, A. and K. Fuxe. Evidence for the existence of monoamine neurons in the central nervous system. I. Demonstration of monoaminese in the cell bodies of brain stem neurons. *Acta Physiol Scand* **64:** Suppl 232, 1–55, 1964.
15. Dahlström, A. and K. Fuxe. Evidence for the existence of monoamine nerves in the central nervous system. II. Experimentally induced changes in the intraneuronal amine levels of bulbo-spinal neuron systems. *Acta Physiol Scand* **64:** Suppl 247, 1–36, 1965.
16. DaPrada, M. and H. H. Keller. Baclofen and γ-hydroxybutyrate: similar effects on cerebral dopamine neurons. *Life Sci* **19:** 1253–1264, 1976.
17. Davies, J. Selective depression of synaptic excitation in cat spinal neurons by baclofen: an iontophoretic study. *Br J Pharmacol* **72:** 373–384, 1981.
18. Davies, J. and S. E. Johnston. Selective antinociceptive effects of tizanidine (DS 103-282), a centrally acting muscle relaxant, on dorsal horn neurons in the feline spinal cord. *Br J Pharmacol* **82:** 409–421, 1984.
19. DeFeudis, F. V. GABAergic analgesia—a naloxone insensitive system. *Pharmacol Res Commun* **14:** 383–390, 1982.
20. Désarmenien, M., P. Felz, G. Occhipinti, F. Santangelo and R. Schlichter. Coexistence of $GABA_A$ and $GABA_B$ receptors on AS and C primary afferents. *Br J Pharmacol* **81:** 327–333, 1984.
21. Dickenson, A. H., C. M. Brewer and N. A. Hayes. Effects of topical baclofen on C fibre-evoked neuronal activity in the rat dorsal horn. *Neuroscience* **14:** 557–562, 1985.
22. Dobry, P. J. K., M. F. Piercey and L. A. Schroeder. Pharmacological characterization of scratching behaviour induced by intracranial injection of substance P and somotostatin. *Neuropharmacology* **20:** 267–272, 1981.
23. Dunlap, K. Functional and pharmacological differences between two types of GABA receptor on embryonic chick sensory neurons. *Neurosci Lett* **47:** 265–270, 1984.
24. Fielding, S., T. C. Spaulding and H. Lal. Antinociceptive actions of clonidine. In: *Progress in Clinical and Biological Research. Psychopharmacology of Clonidine*. New York: A. R. Liss, 1981, pp. 225–242.
25. Fillenz, M. and S. C. Fung. Effect of GABA on (^{3}H)noradrenaline release from rat hippocampal synaptosomes. *J Physiol* **339:** 39P–40P, 1983.
26. Fox, S., K. Krnjević, M. E. Morris, E. Puil and R. Werman. Action of baclofen on mammalian synaptic transmission. *Neuroscience* **3:** 495–515, 1978.
27. Fromm, G. H., C. F. Terrence and A. S. Chattha. Baclofen in the treatment of trigeminal neuralgia: Double blind study and long-term follow up. *Ann Neurol* **15:** 240–244, 1984.
28. Fung, S. C., M. J. Swarbrick and M. Fillenz. Effect of baclofen on *in vitro* noradrenaline release from rat hippocampus and cerebellum: an action at an α_2-adrenoceptor. *Neurochem Int* **7:** 155–163, 1985.
29. Galloway, M. P. and R. H. Roth. Clonidine prevents methylaxanthine stimulation of norepinephrine metabolism in rat brain. *J Neurochem* **40:** 246–251, 1983.
30. Gianutsos, G. and K. E. Moore. Tolerance to the effects of baclofen and γ-buytrolactone on locomotor activity and dopaminergic neurons in the mouse. *J Pharmacol Exp Ther* **207:** 859–869, 1978.
31. Giotti, A., S. Luzzi, S. Spagnesi and L. Zilletti. Homotaurine: a $GABA_B$ antagonist in guinea pig ileum. *Br J Pharmacol* **79:** 855–862, 1983.
32. Grant, S. J. and D. E. Redmond. Methylxanthine activation of noradrenergic unit activity and reversal by clonidine. *Eur J Pharmacol* **85:** 105–109, 1982.
33. Guyenet, P. G. and G. K. Aghajanian. ACh, substance P and met-enkephalin in the locus coeruleus: Pharmacological evidence for independent sites of action. *Eur J Pharmacol* **53:** 319–328, 1979.
34. Hammond, D. L. and E. J. Drower. Effects of intrathecally administered THIP, baclofen and muscimol on nociceptive threshold. *Eur J Pharmacol* **103:** 121–125, 1984.
35. Hanley, M. R., B. E. B. Sandberg, C. M. Lee, L. L. Iversen, D. E. Brudish and R. Wade. Specific binding of ^{3}H-substance P to rat brain membranes. *Nature* **286:** 810–812, 1980.
36. Henry, J. L. Effects of intravenously administered enantioners of baclofen on functionally identified units in lumbar dorsal horn of the spinal cat. *Neuropharmacology* **21:** 1073–1083, 1982.
37. Henry, J. L. Pharmacological studies on the prolonged depressant effects of baclofen on lumbar dorsal horn units in the cat. *Neuropharmacology* **21:** 1085–1093, 1982.
38. Henry, J. L. and Y. Ben-Ari. Actions of the p-chorphenyl derivative of GABA, Lioresal, on nociceptive and non-nociceptive units in the spinal cord of the cat. *Brain Res* **117:** 540–544, 1976.
39. Hill, D. R. and A. C. Dolphin. Modulation of adenylate cyclase activity by $GABA_B$ receptors. *Neuropharmacology* **23:** 829–830, 1984.
40. Hill, R. C., R. Maurer, H. H. Buescher and D. Roemer. Analgesic properties of the GABA-mimetic THIP. *Eur J Pharmacol* **69:** 221–224, 1981.

41. Hökfelt, T., L. Skirboll, J. M. Lundberg, C. J. Dalsgaard, O. Johansson, B. Pernow and G. Jansćo. Neuropeptides and pain pathways. In: *Advances in Pain Research and Therapy*, Vol 5, edited by J. J. Bonica, U. Lindbloom and A. Iggio. New York: Raven Press, 1983, pp. 227–246.
42. Inoue, M., T. Matsuo and N. Ogata. Characterization of pre- and post-synaptic actions of (−)-baclofen in the guinea-pig hippocampus *in vitro*. *Br J Pharmacol* **84:** 843–851, 1985.
43. Iwamoto, E. T. and E. L. Way. Opiate actions and catecholamines. In: *Neurochemical Mechanisms of Opiates and Endorphins*, edited by H. H. Loh and D. H. Ross. *Advances in Biochemical Psychopharmacology*, Vol 20. New York: Raven Press, 1979, pp. 357–407.
44. Karasawa, T., K. Furukawa, K. Yoshida and M. Shimzu. Effect of theophylline on monoamine metabolism in the rat brain. *Eur J Pharmacol* **37:** 97–104, 1976.
45. Karbon, E. W., R. S. Duman and S. J. Enna. GABA$_B$ receptors and norepinephrine-stimulated cAMP production in rat brain cortex. *Brain Res* **306:** 237–332, 1984.
46. Kendall, D. A., M. Browner and S. J. Enna. Comparison of the antinociceptive effect of γ-aminobutyric acid (GABA) agonists: Evidence for a cholinergic involvement. *J Pharmacol Exp Ther* **220:** 482–487, 1982.
47. Kerr, D. I. B. and J. Ong. Evidence that ethylendiamine acts in the isolated ileum of the guinea-pig by releasing endogenous GABA. *Br J Pharmacol* **83:** 169–177, 1984.
48. Kjaer, M. and H. Nielson. The analgesic effect of the GABA-agonist THIP in patients with chronic pain of malignant origin. A phase-1-2 study. *Br J Clin Pharmacol* **16:** 477–485, 1983.
49. Kuriyama, K. and Y. Yoneda. Morphine induced alterations of γ-aminobutyric acid and taurine contents and L-glutamate decarboxylase activity in rat spinal cord and thalamus: possible correlations with analgesic action of morphine. *Brain Res* **148:** 163–179, 1978.
50. Levy, R. A. and H. K. Proudfit. The analgesic action of baclofen β-(4-p-chlorophenyl)-γ-aminobutyric acid. *J Pharmacol Exp Ther* **202:** 437–445, 1977.
51. Levy, R. A. and H. K. Proudfit. Analgesia produced by microinjection of baclofen and morphine at brain stem sites. *Eur J Pharmacol* **57:** 43–55, 1979.
52. Liebman, J. M. and G. Pastor. Antinociceptive effects of baclofen and muscimol upon intraventricular administration. *Eur J Pharmacol* **61:** 225–230, 1980.
53. Lim, C. R., D. G. Garant and K. Gale. GABA agonist induced analgesia elicited from the lateral preoptic area in the rat. *Eur J Pharmacol* **107:** 91–94, 1985.
54. Lindeburg, T., S. Folsgard, H. Sillesen, E. Jacobsen and H. Kehlet. Analgesic, respiratory and endocrine responses in normal man to THIP, a GABA-agonist. *Acta Anaesthesiol Scand* **27:** 10–12, 1983.
55. Lloyd, K. G., S. Arbilla, K. Beaumont, M. Briley, G. de Montis, B. Scatton, S. Z. Langer and G. Bartolini. γ-Aminobutyric acid (GABA) receptor stimulation. II. Specificity of progabide (SL 76002) and SL 75102 for the GABA receptor. *J Pharmacol Exp Ther* **220:** 672–677, 1982.
56. Moochhala, S. M. and J. Sawynok. Hyperalgesia produced by intrathecal substance P and related peptides: desensitization and cross desensitization. *Br J Pharmacol* **82:** 381–388, 1984.
57. Moore, R. Y. and F. E. Bloom. Central catecholamine neurone systems: anatomy and physiology of the norepinephrine and epinephrine systems. *Annu Rev Neurosci* **2:** 113–168, 1979.
58. Muhyaddin, M., P. J. Roberts and G. N. Woodruff. Presynaptic γ-aminobutyric acid receptors in the rat anococcygeus muscle and their antagonism by 5-aminovaleric acid. *Br J Pharmacol* **77:** 163–168, 1982.
59. Newberry, N. R. and R. A. Nicoll. Comparison of the action of baclofen with γ-aminobutyric acid on rat hippocampal pyramidal cells *in vitro*. *J Physiol* **360:** 161–185, 1985.
60. Nicoll, R. A., C. Schenker and S. E. Leeman. Substance P as a transmittercandidate. *Annu Rev Neurosci* **3:** 227–268, 1980.
61. Nygren, L. G. and L. Olson. A new major projection from locus coeruleus: The main source of noradrenergic nerve terminals in the ventral and dorsal columns of the spinal cord. *Brain Res* **132:** 85–93, 1977.
62. Ogata, N. and H. Abe. Further supports for the postsynaptic action of substance P and its blockade with baclofen in neurons of the guinea-pig hypothalamus *in vitro*. *Experientia* **37:** 759–761, 1981.
63. Olpe, H. R., H. Demieville, V. Baltzer, W. L. Bencze, W. R. Koella, P. Wolf and H. L. Haas. The biological activity of d- and l-baclofen (Lioresal®). *Eur J Pharmacol* **52:** 133–136, 1978.
64. Olsen, R. W., M. K. Ticker, P. C. Van Ness and D. Greenlee. Effects of drugs on γ-aminobutyric acid receptors, uptake, release and synthesis *in vitro*. *Brain Res* **139:** 277–294, 1978.
65. Otsuka, M. and M. Yanagisawa. The effects of substance P and baclofen on motoneurones of isolated spinal cord of the newborn rat. *J Exp Biol* **89:** 201–214, 1980.
66. Piercey, M. F., P. J. K. Dobry, L. A. Schroeder and F. J. Einspahr. Behavioural evidence that substance P may be a spinal cord sensory neurotransmitter. *Brain Res* **210:** 407–412, 1981.
67. Piercey, M. F. and R. P. Hollister. Effects of intravenous baclofen on dorsal horn neurons of spinal cats. *Eur J Pharmacol* **53:** 379–382, 1979.
68. Pinto, O. de S., M. Polikar and G. Debono. Results of international clinical trials with Lioresal. *Postgrad Med J* 48 Suppl **5:** 18–23, 1972.
69. Phillis, J. W. Is β (4-chlorophenyl)-GABA a specific antagonist of substance P on cerebral cortical neurons? *Experientia* **32:** 493–594, 1976.
70. Price, G. W., G. P. Wilkin, M. J. Turnbull and N. G. Bowery. Are Baclofen-sensitive GABA$_B$ receptors present on primary afferent terminals of the spinal cord? *Nature* **307:** 71–74, 1984.
71. Proudfit, H. K. and R. A. Levy. Delimitation of the neuronal substrates necessary for the analgesic action of baclofen and morphine. *Eur J Pharmacol* **47:** 159–166, 1978.
72. Retz, K. C. and L. Holaday. Microinjection of THIP, a GABAergic agonist, into the ventrolateral periaqueductal gray matter of the rat: Effects on analgesia. *Soc Neurosci Abstr* **10:** 107, 1984.
73. Romandini, S. and R. Samanin. Muscimol injections in the nucleus raphé dorsalis block the antinociceptive effect of morphine in rats: apparent lack of 5-hydroxytryptamine involvement in muscimol's effect. *Br J Pharmacol* **81:** 25–29, 1984.
74. Saito, K., S. Konishi and M. Otsuka. Antagonism between lioresal and substance P in rat spinal cord. *Brain Res* **97:** 177–180, 1975.
75. Sawynok, J. Monoamines as mediators of the antinociceptive effect of baclofen. *Naunyn Schmiedebergs Arch Pharmacol* **323:** 54–57, 1983.
76. Sawynok, J. Theophylline-induced potentiation of the antinociceptive action of baclofen. *Br J Pharmacol* **78:** 353–357, 1983.
77. Sawynok, J. GABAergic mechanisms in antinociception. *Prog Neuropsychopharmacol Biol Psychiatry* **8:** 581–586, 1984.
78. Sawynok, J. Baclofen activates two distinct receptors in the rat spinal cord and guinea pig ileum. *Neuropharmacology* **25:** 795–798, 1986.
79. Sawynok, J. and C. Dickson. Involvement of GABA in the antinociceptive effect of γ-acetylenic GABA (GAG), an inhibitor of GABA-transaminase. *Gen Pharmacol* **14:** 603–607, 1983.
80. Sawynok, J. and C. Dickson. Evidence for the involvement of descending noradrenergic pathways in the antinociceptive effect of baclofen. *Brain Res* **335:** 89–97, 1985.
81. Sawynok, J. and C. Dickson. D-Baclofen is an antagonist at baclofen receptors mediating antinociception in the spinal cord. *Pharmacology* **31:** 248–259, 1985.

82. Sawynok, J., N. Kato, V. Havlicek and F. S. LaBella. Lack of effect of baclofen on substance P and somatostatin release from rat spinal cord in vitro. *Naunyn Schmiedebergs Arch Pharmacol* **319:** 78–81, 1982.
83. Sawynok, J. and F. S. LaBella. On the involvement of GABA in the analgesia produced by baclofen, muscimol and morphine. *Neuropharmacology* **21:** 397–404, 1982.
84. Sawynok, J., S. M. Moochhala and D. J. Pillay. Intrathecal substance P antagonizes the spinal antinociceptive effect of morphine, baclofen and noradrenaline. *Neuropharmacology* **23:** 741–747, 1984.
85. Sawynok, J., C. Pinsky and F. S. LaBella. Minireview. On the specificity of naloxone as an opiate antagonist. *Life Sci* **25:** 483–493, 1979.
86. Sawynok, J. and A. Reid. Role of ascending and descending noradrenergic pathways in the antinociceptive effect of baclofen and clonidine. *Brain Res* **386:** 341–350, 1986.
87. Sawynok, J. and A. Reid. Clonidine reverses baclofen-induced increase in noradrenaline turnover in rat brain. *Neurochem Res* **11:** 723–731, 1986.
88. Sawynok, J. and G. Robertson. Desensitization to substance P following intrathecal injection: A technique for investigating the role of substance P in nociception. *Naunyn Schmiedebergs Arch Pharmacol* **331:** 152–158, 1985.
89. Scatton, B., B. Zivkovic, J. Dedek, K. G. Lloyd, J. Constantinidis, R. Tissot and G. Bartholini. γ-Aminobutyric acid (GABA) receptor stimulation. III. Effect of progabide (SL76002) on norepinephrine, dopamine and 5-hydroxytryptamine turnover in rat brain areas. *J Pharmacol Exp Ther* **220:** 678–688, 1982.
90. Sivam, S. P., T. Nabeshima and I. K. Ho. An analysis of GABA receptor changes in the discrete regions of mouse brain after acute and chronic treatments with morphine. *J Neurochem* **39:** 933–939, 1982.
91. Sivam, S. P. and I. K. Ho. GABAergic drugs, morphine and morphine tolerance: A study in relation to nociception and gastrointestinal transit in mice. *Neuropharmacology* **22:** 767–774, 1983.
92. Sivam, S. P. and I. K. Ho. Minireview. GABA in morphine analgesia and tolerance. *Life Sci* **37:** 199–208, 1985.
93. Spaulding, T. C., J. Little, K. McCormack and S. Fielding. The antinociceptive effects of GABA agonists and antagonists in mice. *Brain Res Bull* **4:** 711, 1979.
94. Steardo, L. and J. Sawynok. Clonidine reverses methylxanthine-induced potentiation of baclofen antinociception. *Pharmacol Biochem Behav* **22:** 905–907, 1985.
95. Suzdak, P. D. and G. Gianutsos. Differential coupling of $GABA_A$ and $GABA_B$ receptors to the noradrenergic system. *J Neural Transm* **62:** 77–89, 1985.
96. Suzdak, P. D. and G. Gianutsos. Parallel changes in the sensitivity of γ-aminobutyric acid and noradrenergic receptors following chronic administration of antidepressant and GABAergic drugs. A possible role in affective disorders. *Neuropharmacology* **24:** 217–222, 1985.
97. Vaught, J. L., K. Pelley, L. G. Costa, P. Setler and S. J. Enna. A comparison of the antinociceptive responses to the GABA-receptor agonists THIP and baclofen. *Neuropharmacology* **24:** 211–216, 1985.
98. Waddington, J. L. and A. J. Cross. Baclofen and muscimol: Behavioural and neurochemical sequelae of unilateral intranigral administration and effects on ^{3}H-GABA receptor binding. *Naunyn Schmiedebergs Arch Pharmacol* **306:** 275–280, 1979.
99. Wall, P. D. and M. Fitzgerald. If substance P fails to fulfill the criteria as a neurotransmitter in somatosensory afferents, what might be its function? In: *Substance P in the Nervous System, Ciba Foundation Symposium No. 91.* London: Pitman, 1982, pp. 249–261.
100. Wilson, P. R. and T. L. Yaksh. Baclofen is antinociceptive in the spinal intrathecal space of animals. *Eur J Pharmacol* **51:** 323–330, 1978.
101. Yeung, J. C. and T. A. Rudy. Multiplicative interaction between narcotic agonisms expressed at spinal and supraspinal sites of antinociceptive action as revealed by concurrent intrathecal and intracerebroventricular injections of morphine. *J Pharmacol Exp Ther* **215:** 633–642, 1980.
102. Young, R. R. and P. J. Delwaide. Drug Therapy: Spasticity. *N Engl J Med* **304:** 96–99, 1981.
103. Zambotti, F., N. Zonta, M. Parenti, R. Tommasi, L. Vicentini, F. Conti and P. Mantegazza. Periaqueductal gray matter involvement in the muscimol-induced decrease of morphine antinociception. *Naunyn Schmiedebergs Arch Pharmacol* **318:** 368–369, 1982.
104. Zonta, N., F. Zambotti, L. Vicentini, R. Tammiso and P. Mantagazza. Effects of some GABA mimetic drugs on the antinociceptive activity of morphine and β-endorphin in rats. *Naunyn Schmiedebergs Arch Pharmacol* **316:** 231–234, 1981.
105. Zorn, S. H. and S. J. Enna. The effect of mouse spinal cord transection on the antinociceptive response to the γ-aminobutyric acid agonists THIP (4,5,6,7-tetrahydroisoxazolo (5,4-C) pyridine-3-ol) and baclofen. *Brain Res* **338:** 380–383, 1985.
106. Zorn, S. H. and S. J. Enna. GABA uptake inhibitors produce a greater antinociceptive response in the mouse tail-immersion assay than other types of GABAergic drugs. *Life Sci* **37:** 1901–1912, 1985.