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Respiratory effects of baclofen and 3-aminopropylphosphinic acid in guinea-pigs

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1 The effects of the $GABA_B$ receptor agonists, baclofen and 3-aminopropylphosphinic acid (3-APPi) given by the subcutaneous or intracerebroventricular (i.c.v.) route were examined on minute ventilation ($\dot{V}$), tidal volume (V_T) and respiratory rate (f) due to room air and carbon dioxide (CO_2)-enriched gas hyperventilation in conscious guinea-pigs.

2 Baclofen (0.3–10 mg kg^{-1}, s.c.) produced a dose-dependent inhibition of $\dot{V}$ and f due to room air and CO_2 inhalation. The maximum inhibition of room air breathing $\dot{V}$ was 85% ± 3 and f was 74% ± 3 at 10 mg kg^{-1}, s.c. The maximum effects on CO_2-induced hyperventilation were 68% ± 9 and 51% ± 6, for $\dot{V}$ and f respectively. Only the highest dose of baclofen studied (10 mg kg^{-1}) produced a significant inhibition of V_T due to room air breathing (46% ± 6) and CO_2 breathing (38% ± 11).

3 3-APPi (0.3–100 mg kg^{-1}, s.c.) did not affect $\dot{V}$, V_T or f due to room air breathing or CO_2 inhalation at any dose tested. Also, i.c.v. administration of 3-APPi (100 μg) did not affect ventilatory responses due to room air breathing or CO_2 inhalation.

4 Pretreatment with the $GABA_B$ antagonist, CGP 35348 3-aminopropyl-(diethoxymethyl) phosphinic acid (3–30 mg kg^{-1}, s.c.) blocked the respiratory depressant effects of baclofen (3 mg kg^{-1}, s.c.) in a dose-related fashion.

5 Intracerebroventricular (i.c.v.) administration of CGP 35348 (50 μg) blocked the respiratory depressant effects of baclofen. CGP 35348 given alone either i.c.v. or s.c. had no effects on respiration due to room air or CO_2 inhalation.

6 Pretreatment with either the $GABA_A$ antagonist bicuculline (30 mg kg^{-1}, s.c.) or the opioid antagonist, naloxone (1 mg kg^{-1}, s.c.) had no effect on the respiratory depressant action of baclofen (3 mg kg^{-1}, s.c.).

7 These results show that baclofen inhibits ventilation due to room air breathing, and attenuates the hyperventilation response to CO_2 inhalation. The peripherally acting $GABA_B$ agonist, 3-APPi had no effect on ventilation. These findings demonstrate that the respiratory depressant effects of baclofen are due to activation of CNS $GABA_B$ receptors and indicates that only $GABA_B$ receptor agonists that penetrate into the CNS may cause respiratory depression.

Keywords: 3-Aminopropylphosphinic acid; baclofen; CNS; $GABA_B$ receptors; minute volume; respiration

Introduction

Recent studies have found that the $GABA_B$ receptor agonists, baclofen and 3-aminopropylphosphinic acid (3-APPi) inhibit a variety of neurally-evoked responses in the airways including tachykinin-mediated and cholinergic bronchospasm, airway microvascular leakage and cough (Belvisi *et al.*, 1989; Chapman *et al.*, 1991; Danko *et al.*, 1992; Bolser *et al.*, 1993; Chapman *et al.*, 1993b). These effects are produced by inhibition of the release of neurotransmitters and/or neuromodulators from pulmonary nerves. On the other hand, baclofen inhibits cough primarily by activation of $GABA_B$ receptors in the central nervous system (CNS), while 3-APPi exerts predominantly a peripheral antitussive effect (Bolser *et al.*, 1994).

$GABA_B$ receptor agonists also produce respiratory depression by inhibiting activity of respiratory neurones in the CNS (Lalley, 1983; DeFeudis, 1984; Da Silva *et al.*, 1987; Schmid *et al.*, 1989; Wagner & Dekin, 1993). Therefore, highly lipophilic $GABA_B$ agonists, such as baclofen, that rapidly penetrate into the CNS produce respiratory depression when given parenterally in large doses (Lipscomb & Meredith, 1980; Lalley, 1983; DaSilva *et al.*, 1987). On the other hand, 3-APPi is a $GABA_B$ agonist that does not penetrate into the CNS (Hills & Howson, 1992) and would not be expected to depress respiratory function, although this has not been studied.

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The purpose of the present study was to determine the respiratory effects of baclofen and 3-APPi in guinea-pigs. We also studied the effect of a selective $GABA_B$ receptor antagonist, CGP 35348 3-aminopropyl-(diethoxymethyl) phosphinic acid (Olpe *et al.*, 1990) on the respiratory depressant effects of baclofen to verify the selectivity and site of action of this $GABA_B$ agonist.

Methods

Male Hartley guinea-pigs (Charles River Labs, Wilmington, MA, U.S.A.) ranging in weight from 350 to 450 g were used in these studies. The animals were given food and water *ad libitum*.

Pulmonary measurements

The guinea-pigs were placed in a head-out, whole body pressure plethysmograph (Penn-Century, Philadelphia, PA, U.S.A.). Copper gauze was placed inside the plethysmograph to keep the interior temperature of the plethysmograph constant. Latex collars were placed over the animals head to provide an airtight seal between the guinea-pig and the plethysmograph. A pressure transducer (Statham-Gould, San Juan, PR; Model P23XL) was connected to an outlet port in the plethysmograph wall and measured the pressure changes inside the plethysmograph. This pressure signal was visually

displayed on a chart recorder (Grass Instruments, Quincy, MA, U.S.A.; Model RPS 7C8) and used to measure tidal volume (V_T) and respiratory frequency (f). Minute ventilation ($\dot{V}$) was derived from these two measurements.

A transparent chamber was placed over the head of the guinea-pig to facilitate the inhalation of a hypercapnic gas mixture (10% CO_2, 21% O_2, 69% N_2), which was delivered from a compressed gas source (SOS Gases, Inc., Kearney, NJ, U.S.A.) at a constant flow of 40 ml s^{-1}. The guinea-pigs were exposed to the hypercapnic gas mixture for 10 min to achieve steady-state conditions and for ventilation to stabilize at an elevated level (Danko & Chapman, 1988).

Experimental protocol

The guinea-pigs were allowed to equilibrate in the plethysmograph for 15 min after which time measurements of V_T, f and $\dot{V}$ were obtained during room air breathing. The guinea-pigs were then exposed to the CO_2-enriched gas mixture and V_T, f and $\dot{V}$ were measured. Following exposure to the CO_2-enriched gas the guinea-pigs were taken out of the plethysmograph and injected subcutaneously with saline, baclofen or 3-APPi. Thirty minutes after the administration of these drugs, the guinea-pigs were placed into the plethysmograph and measurements of V_T, f and $\dot{V}$ were obtained during room air breathing and after exposure to the CO_2-enriched gas mixture. For studies involving antagonist, these compounds were given subcutaneously 10 min before baclofen.

In other experiments, CGP 35348 or 3-APPi was given into the CNS through chronically implanted intracerebroventricular cannulae (Mauser *et al.*, 1988; McLeod *et al.*, 1991). For implantation of the intracerebroventricular (i.c.v.) cannulae, guinea-pigs were anaesthetized with ketamine (30 mg kg^{-1}, i.m.) and placed in a rodent stereotaxic apparatus (David Kopf Instruments, Tujunga, CA, U.S.A.). A mid-line incision was made through the scalp to expose the skull and a small hole was drilled through the skull 0.5 mm anterior, and 2.0 mm lateral to the bregma. Through this hole a stainless steel cannula was inserted 4.5 mm below the skull surface to penetrate the lateral cerebral ventricle (McLeod *et al.*, 1991). Three anchoring screws were placed into the skull and the i.c.v. cannula was secured to the screws with dental acrylic cement. The scalp was sutured and the animals were left to recover for 1 week before study. All injections through the i.c.v. cannula were made with a microlitre syringe (Hamilton, Reno, NV, U.S.A.) and administered in artificial CSF in a volume of 10 µl.

Compounds

Compounds used in this study included (±)-baclofen, naloxone HCl (Research Biochemicals Inc., Natick MA, U.S.A.), bicuculline methiodide (Sigma Chemical Co., St. Louis, MO, U.S.A.), 3-APPi (Schering-Plough Research Institute, Kenilworth, NJ, U.S.A.) and CGP 35348 (Ciba-Geigy Corp., Basel, Switzerland).

Statistics

All data are represented as mean ± s.e.mean. Statistical differences between means were evaluated with Student's *t* test or one-way analysis of variance. Differences were considered significant if $P<0.05$.

Results

Table 1 shows the effect of baclofen and 3-APPi on V_T, f and $\dot{V}$ during room air breathing and after exposure to a carbon dioxide-enriched gas mixture. Baclofen (0.3–10 mg kg^{-1}, s.c.) produced a dose-dependent inhibition of f and $\dot{V}$ due to room air breathing and during CO_2 inhalation (Table 1). On the other hand, the inhibition of V_T by baclofen was seen only at 10 mg kg^{-1}. 3-APPi (0.3–100 mg kg^{-1}, s.c.) had no significant effect on V_T, f and $\dot{V}$ during room air breathing or during CO_2 inhalation (Table 1). Similarly, i.c.v 3-APPi did not alter normal ventilation responses to room air breathing or to CO_2-stimulated ventilation. In these studies, the changes of $\dot{V}$ after 3-APPi (100 µg, i.c.v.) were $-7 \pm 9\%$ for room air and $1 \pm 12\%$ for CO_2 hyperventilation ($n = 9$). In contrast, i.c.v. baclofen (3 µg; $n = 6$) significantly inhibited room air $\dot{V}$ ($-68 \pm 6\%$) and CO_2-stimulated $\dot{V}$ ($-60 \pm 8\%$).

Pretreatment with CGP 35348 (3–30 mg kg^{-1}, s.c.) reduced the respiratory depressant effects of baclofen (3 mg kg^{-1}, s.c.) during CO_2 inhalation (Figure 1). The dose of CGP 35348 that produced a 50% blockade of baclofen's inhibitory effect was 5.9 mg kg^{-1}. Intracerebroventricular administration of CGP 35348 (50 µg, i.c.v.) also reduced the respiratory depressant effects of baclofen (Figure 2). On the other hand, CGP 35348 (30 mg kg^{-1}, s.c.) had no significant effect on the respiratory depression caused by subcutaneous administration of pentobarbitone. In these studies, pentobarbitone (30 mg kg^{-1}, s.c.) caused a $64 \pm 2\%$ reduction in $\dot{V}$ during CO_2 inhalation. In the presence of CGP 35348 (30 mg kg^{-1}, s.c.), pentobarbitone reduced $\dot{V}$ by $71 \pm 6\%$.

Neither pretreatment with the $GABA_A$ antagonist, bicuculline (30 mg kg^{-1}, s.c.) nor with the opioid antagonist, naloxone (1 mg kg^{-1}, s.c.) inhibited the respiratory depressant effects of baclofen (3 mg kg^{-1}, s.c.) (Table 2). This dose of naloxone reverses the respiratory depressant effect of morphine (30 mg kg^{-1}, s.c.; data not shown).

Discussion

Previous studies in animals (Schmid *et al.*, 1989) and man (Lipscomb & Meredith, 1980) have demonstrated that baclofen is a powerful respiratory depressant. In conscious guinea-pigs, we found that subcutaneous administration of baclofen inhibited ventilation during room air breathing and attenuated the hyperventilatory response to carbon dioxide inhalation. The effects of baclofen were blocked by administration of the centrally acting $GABA_B$ antagonist, CGP 35348, but not by the $GABA_A$ antagonist, bicuculline, nor by naloxone, an opioid antagonist. On the other hand, 3-APPi, which is a peripherally acting $GABA_B$ agonist that does not penetrate into the CNS (Hills & Howson, 1992) had no effect on ventilation. These results demonstrate that the respiratory depressant effects of baclofen are specific to $GABA_B$ receptor activation and identify that only $GABA_B$ receptor agonists that penetrate into the CNS cause respiratory depression.

The respiratory depressant effects of baclofen were seen on both room air ventilation and on the ventilatory responses to carbon dioxide. Inhalation of carbon dioxide was used in these studies because it is a potent respiratory stimulant that causes consistently stable increases in f and $\dot{V}$ that are sensitive to drugs affecting the respiratory pattern (Danko & Chapman, 1988). Previous studies in anaesthetized cats reported that intravenous baclofen produces an apneustic breathing pattern with a large reduction in respiratory rate and an increase in tidal volume (Lalley, 1983; DaSilva *et al.*, 1987). In our study, baclofen produced the greatest reductions in respiratory rate and smaller reductions in tidal volume, but there was no evidence of apneustic breathing with doses as high as 10 mg kg^{-1}, s.c. This may reflect the fact that our studies were performed in the absence of general anaesthesia or possibly to a species difference. Involvement of CNS $GABA_B$ receptor activation was confirmed by blocking the respiratory depressant effects of baclofen with i.c.v. administration of the $GABA_B$ receptor antagonist CGP 35348 (Olpe *et al.*, 1990). These findings are in agreement with previously published results that show a central site of action of baclofen that inhibits respiration (Lalley, 1983; DaSilva *et al.*, 1987; Schmid *et al.*, 1989).

Table 1 Effect of baclofen and 3-aminopropylphosphinic acid (3-APPi) on ventilation during breathing of room air and carbon dioxide

Compound[a]	Dose (mg kg^{-1}, s.c.)	n	% change[b] Room air breathing V_T	f	$\dot{V}$	Carbon dioxide breathing[c] V_T	f	$\dot{V}$
Saline	–	18	−4 ± 6	−5 ± 3	−9 ± 6	−1 ± 6	−5 ± 3	−8 ± 4
Baclofen	0.3	12	−15 ± 9	−6 ± 4	−18 ± 6	23 ± 17	−9 ± 4	−8 ± 7
	1	12	0 ± 8	−36 ± 5*	−38 ± 8*	−13 ± 11	−27 ± 4*	−26 ± 9*
	3	19	3 ± 8	−52 ± 5*	−54 ± 4*	−4 ± 7	−34 ± 5*	−56 ± 6*
	10	6	−46 ± 6*	−74 ± 3*	−85 ± 3*	−38 ± 11*	−51 ± 6*	−68 ± 9*
3-APPi	0.3	10	−8 ± 13	−8 ± 7	−4 ± 8	7 ± 7	−6 ± 3	1 ± 8
	3	8	0 ± 13	8 ± 5	−12 ± 3	−6 ± 10	0 ± 4	3 ± 10
	30	9	−8 ± 4	−3 ± 4	−13 ± 3	−20 ± 9	−4 ± 5	−5 ± 20
	100	9	−15 ± 3	1 ± 6	−14 ± 6	−3 ± 6	5 ± 7	−5 ± 14

[a]Compounds given 30 min before measurement. [b]Mean ± s.e.mean % change of room air and CO_2 enriched gas breathing ventilation due to compound, compared with respective baseline values. Baseline values for room air breathing are: $V_T = 2.5 \pm 0.2$, $f = 122 \pm 5$ and $\dot{V} = 309 \pm 20$. Baseline ventilation values due to carbon dioxide breathing are: $V_T = 7.5 \pm 0.4$, $f = 114 \pm 3$ and $\dot{V}$ 862 ± 55. [c]Exposure to 10% CO_2, 21% O_2, 69% N_2 for 10 min.
*$P < 0.05$ compared to values before administration of compound.

Table 2 Effect of selective antagonists on the respiratory depressant effects of baclofen during carbon dioxide breathing

First[a]	Treatment	Second[b]	n	Percent change[c] of minute volume
Saline		Baclofen	17	−46 ± 4*
Bicuculline	(30 mg kg^{-1})	Baclofen	6	−40 ± 13*
Bicuculline	(30 mg kg^{-1})	Saline	6	+1 ± 8
Naloxone	(1 mg kg^{-1})	Baclofen	12	−69 ± 7*
Naloxone	(1 mg kg^{-1})	Saline	6	−18 ± 10

[a]Compounds given 10 min before baclofen.
[b]Baclofen (3 mg kg^{-1}, s.c.) or saline given 30 min before measurement of minute volume during CO_2 inhalation.
[c]Mean ± s.e.mean percent change of minute volume due to baclofen or saline.
*$P < 0.05$ compared to values before administration of compounds.

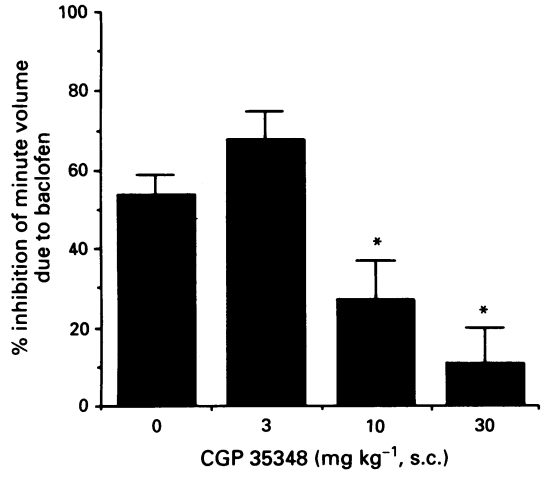

Figure 1 Blockade of baclofen-induced depression of CO_2 breathing with the $GABA_B$ antagonst, CGP 35348. Columns represent mean ± s.e.mean percentage inhibition of minute volume due to baclofen (3 mg kg^{-1}, s.c.) ($n = 6-12$); *$P < 0.05$, represents statistically significant differences from ventilation responses in animals that received baclofen alone.

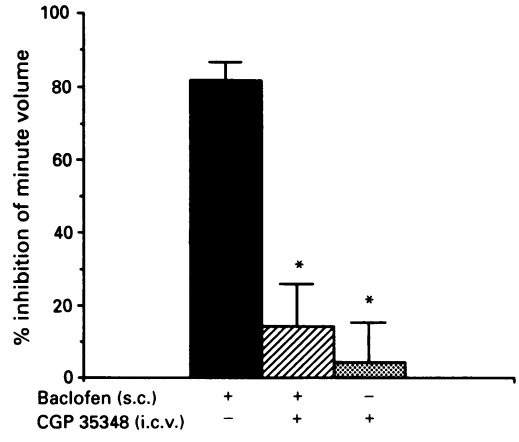

Figure 2 Effect of intracerebroventricular (i.c.v.) CGP 35348 on baclofen-induced depression of CO_2 breathing in the guinea-pig. Solid column is baclofen (3 mg kg^{-1}, s.c.) + i.c.v. artificial CSF; cross-hatched column is baclofen (3 mg kg^{-1}, s.c.) + i.c.v. CGP 35348 (50 μg). Stippled column is saline (s.c.) + i.c.v. CGP 35348 (50 μg). Values represent mean ± s.e.mean. *$P < 0.05$, compared to animals that received baclofen alone. Note that CGP 35348 given alone did not affect ventilation responses to CO_2 breathing.

We previously found that baclofen and 3-APPi are potent antitussive agents that inhibit the cough reflex by acting at central and peripheral $GABA_B$ receptors respectively. In the present study, baclofen inhibited ventilation at doses of 1 mg kg^{-1}, s.c. and higher. This is greater than the dose of baclofen ($ED_{30} = 0.04$ mg kg^{-1}, s.c.) required to inhibit cough in guinea-pigs (Bolser *et al.*, 1993). Therefore, the antitussive activity of baclofen can be dissociated from its ability to exert central respiratory depressant effects. This may reflect either a peripheral site of action of baclofen that inhibits cough or an effect on components of the central cough generation system that may be more sensitive and/or automatically separate from neurones controlling ventilation and ventilatory responses to CO_2.

3-APPi is a potent $GABA_B$ receptor agonist that inhibits neurally-evoked tachykinin and cholinergic mediated bronchospasm (Chapman *et al.*, 1993a) and cough (Bolser *et al.*, 1994) in guinea-pigs. This compound demonstrates antitussive activity in guinea-pigs with an ED_{30} of 0.36 mg kg^{-1}, subcutaneously (Bolser *et al.*, 1993). In our study, 3-APPi had no effect on ventilation or on the ventilatory responses to CO_2 inhalation at doses as high as 100 mg kg^{-1}, s.c.; this dose is 278 fold greater than its antitussive ED_{30} (Bolser *et al.*, 1993). Although a possible explanation for this result is that 3-APPi does not cross the blood-brain barrier (Hills & Howson, 1992), the present finding that centrally administered 3-APPi does not inhibit ventilation argues against this mechanism playing a significant role in the lack of respiratory depressant effects of 3-APPi. Taken together, the most likely explanation for these results is that baclofen inhibits ventilation by activation of 3-APPi-insensitive $GABA_B$ receptors in the CNS.

We have previously reported that $GABA_B$ receptor agonists inhibit a variety of neural responses in the airways that may be important in the pathogenesis of airway obstructive diseases, like asthma (Chapman *et al.*, 1993b). Therefore, $GABA_B$ agonists may be therapeutically useful for the treatment of these airway disorders. The results from this study indicate that CNS penetration could limit the therapeutic usefulness of a $GABA_B$ receptor agonist due to respiratory depression. However, $GABA_B$ agonists that preferentially activate peripheral $GABA_B$ receptors and do not penetrate the blood-brain barrier may be devoid of the liability to this potential side effect.

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